Grain Growth and Endosperm Cell Size Under High Night Temperatures in Rice (Oryza sativa L.)

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Background and Aims: High night temperatures are more harmful to grain weight than high day temperatures. Grain growth rate and growth duration were investigated to determine which was the cause of the decrease in final grain weight under high night temperatures. Endosperm cell number and cell sizes were also examined to determine which might cause the decrease in final grain weight.

Methods: Rice plants were grown outdoors in plastic pots and moved at heading time to three temperature-controlled glasshouses under high night temperature (HNT; 22/34°C), high day temperature (HDT; 34/22°C) and control conditions (CONT; 22/22°C). Grains were sampled periodically, and the time-course of grain growth was divided into rate and duration by logistic regression analysis. Endosperm cell numbers and cell sizes were analysed by digitalized hand-tracing images of endosperm cross-sections.

Key Results: The duration of grain growth was reduced by high temperature both day and night. However, the rate of grain growth was lower in HNT than in HDT. The number of cells in endosperm cross-sections in HNT was similar to that in HDT, and higher than that in CONT. The average cell area was smaller in HNT than in either CONT or HDT. The differences in average cell areas between HNT and HDT were greater at distances 60–80% from the central point of endosperm towards the endosperm surface.

Conclusions: The results show that HNT compared with HDT reduced the final grain weight by a reduction in grain growth rate in the early or middle stages of grain filling, and also reduced cell size midway between the central point and the surface of endosperm.

Key words: Cell size, cell number, digitalized image analysis, endosperm, grain growth duration, grain growth rate, grain weight, high night temperature, logistic regression, Oryza sativa L., rice, vascular bundle.
growth rate or a decrease in the grain growth duration. A second objective was to determine whether the endosperm cell numbers and/or cell sizes decrease in rice under high-night temperatures. Singh and Jenner (1982) developed a method to determine endosperm cell numbers by counting the suspension nuclei using cellulase and alpha-amylase, which macerate the cell wall and starch granules, respectively. However, their method did not allow for an analysis of the cell size as it relates to cell sites in the endosperm. To enable such an analysis, we developed a method of counting cells and determining cell areas using digitalized hand-tracing of images of endosperm cross-sections.

MATERIALS AND METHODS

Experiment 1

Plant material and growth conditions. Experiments were performed at Fukuyama (34°30’N, 133°23’E), Japan. Two-week-old rice seedlings (Oryza sativa L. ‘Kinuhikari’) were transplanted into plastic pots (diameter 16 cm, length 20 cm) containing black volcanic ash soil on 21 June, 1996. Ten plants per pot were prepared, and tillers were removed periodically to restrict each plant to its main culm. Plants were given a basal dressing of 5 g per pot (commercial fertilizer, N : P : K = 14 : 14 : 14) at sowing, and a top-dressing of 1.2 g per pot (commercial fertilizer, N : P : K = 17 : 0 : 17) on 25 July (16 d before heading). Pots were placed in a concrete-covered water bath outdoors until the flowering stage. The water level was continuously maintained at about 3–5 cm above the soil.

Treatments and sampling. Plants with 13 emerging leaves and a heading date of 10 August were selected for sampling. Eight grains per panicle (4th and 5th grains from the top on the 4th to 7th rachis-branch in each panicle with a flowering date of 11 August) were sampled periodically, at 6, 10, 14, 18, 22, 26, 31 and 42 d after flowering (DAF). Between six and fourteen panicles (nine on average) per treatment were used for replicates at each sampling time. Plants were subjected to temperature variations from 13 August to 22 September (maturing stage). The temperature variations were conducted in three temperature-controlled glasshouses (natural irradiance) under a high night temperature (22/34°C: day/night, HNT), a high day temperature (34/22°C, HDT) and control conditions (22/22°C, CONT) as an optimum temperature. Days and nights ranged between 0800 h–1800 h and 2000 h–0600 h, respectively. Temperature changes in the day were provided linearly at a rate of 6°C h⁻¹. Plants were supplied with tapwater three times a day (morning, early afternoon and evening) to ensure that water stress did not occur. Photosynthetically active radiation from 1000 h to 1400 h in the glasshouses was 713 μmol m⁻² s⁻¹ on average (1430 μmol m⁻² s⁻¹ on a clear day) during the 30 d after treatments began, and the relative humidity was 80 ± 15%.

Measurements and statistical analysis. Samples were dehulled with forceps and dried at 130°C for 20 h in a ventilated oven, and weighed in 0.1 mg units. The data for grain dry weight were fitted to a logistic regression curve as

\[ G = A/(1 + \exp[-(\lambda + \kappa t)]) \]  

(1)

where \( A \) is the estimated maximum grain weight, \( G \) the average grain weight, \( \kappa \) the relative grain growth rate, \( t \) the day after flowering, and \( \lambda \) a constant. These parameter values and the contribution of the regression equation were estimated by minimizing the residual sum of squares (least-squares method), using the curve-fit procedure of Delta Graph Pro 4.0-4 (Delta Point Inc., USA). The \( t \) at which \( G \) was 0.95A was considered as the indicator of the duration of grain growth (days after flowering to maturity).

The time-courses of the grain growth rate were estimated by differentiating eqn (1):

\[ R = A\kappa \exp[-(\lambda + \kappa t)]/[1 + \exp[-(\lambda + \kappa t)]]^2 \]  

(2)

where \( R \) is the grain growth rate, and \( A, \kappa, \lambda \) and \( t \) are the same as in eqn (1).

These methods were similar to those described by Darroch and Baker (1990).

Experiment 2

To investigate the effect of high temperature on endosperm cell numbers and cell areas in grain cross-sections, rice plants were grown and temperature treatments were applied in 1997. Plant material and growth conditions were the same as those in experiment 1, except that the transplanting date was 11 July.

Treatments and sampling. Plants with 13 emerging leaves and a heading date of 27 August were selected for sampling. They were given the same temperature treatments as in experiment 1 from 31 August to 8 October (maturing stage). Four grains per panicle (4th and 5th grains from the top on the 5th and 6th rachis-branch in each panicle) were sampled periodically, at 15, 18, 21, 24, 27, 30 and 40 DAF, for grain dimension measurements of 3–5 panicles at each sampling time. Photosynthetically active radiation from 1000 h to 1400 h in the glasshouses was 713 μmol m⁻² s⁻¹ on average (1430 μmol m⁻² s⁻¹ on a clear day) during the 30 d after treatments began, and the relative humidity was 80 ± 15%.

Endosperm cell numbers and cell areas in grain cross-sections. Samples were dehulled with forceps and 3-dimensional measurements (length, width, thickness) were taken of grains, which were then fixed and stored in FAA (formalin/acetic acid/70% ethanol, 5 : 5 : 90) for more than 1 month. The samples taken at 24 DAF in HNT and HDT, and 30 DAF in CONT were used for endosperm cell analysis. After rinsing with water, cross-sections about 20 μm thick were sliced from the central region of the grain with a Microslicer (DTK-1000, D. S. K., Japan) and stained with toluidine blue. The cross-sections were photographed through a microscope at 40× magnification with a 35-mm camera. The images of endosperm without an aleurone layer were prepared by tracing cell contours on a
The maximum rate of grain growth under HNT was shorter than that of CONT (27 d, which was 22 % of the estimated maximum grain weight of HNT eqn (1). The duration of grain growth calculated by the course of grain growth, with a contribution rate above than that of both HDT and CONT (Fig. 2A). The time-course of grain dry weight increases (1 d).

The grain dry weight at maturity (42 DAF) of HNT was 21.2 mg grain−1, which was significantly (9–10 %) lower than that of both HDT and CONT (21.5 d), and shorter than that of CONT (27.0 d) (Fig. 2B). The maximum rate of grain growth under HNT was 1.57 mg grain−1 d−1, which was lower than that of HDT (1.73 mg grain−1 d−1), but higher than that of CONT (1.40 mg grain−1 d−1) (Fig. 2B). The DAF needed to bring the grain growth rate to its maximum in HNT was 12 DAF, which was similar to that in HDT, but earlier than that in CONT (15 DAF) (Fig. 2B). There was a slight difference between HNT and HDT in their grain growth rate from the early ripening stage (6–10 DAF), with that difference reaching its maximum at 11 DAF (Fig. 2B).

### Grain dimensions

Grain length, width and thickness peaked at 24 DAF in both HNT and HDT, and at 30 DAF in CONT. Table 1 shows the three dimensions of the grain. Grain lengths did...
not differ between the three treatments, and grain widths were smaller in HNT than in CONT. Although the dorsal radius was smaller in HNT (1.51 mg) than in control (1.80 mg), the ventral radius did not differ between the three treatments. Grain thicknesses were smaller in HNT than in HDT.

**Endosperm cell numbers and cell sizes on grain cross-sections**

The areas of endosperm cross-sections in HNT were significantly smaller than those in both the control and HDT (Table 2). The number of cells in endosperm cross-sections in HNT was similar to that in HDT, and both were significantly higher than that in the control. In contrast, the average cell area was smaller in HNT than in both the control and HDT. The average cell area in the control was largest among the three temperature treatments (Table 2). The largest average cell areas appeared at distances 50–60% from the central point of endosperm towards the endosperm surface in all treatments (Fig. 3B). Furthermore, the cell areas were smaller near the central point and surface of the endosperm. The differences in average cell areas between HNT and HDT were greater at distances of 30–80%, and were significant ($P < 0.05$) at distances of 60–80%. In the control, the average cell areas were significantly greater than both HDT and HNT at most distances. As shown in Fig. 4B, the change of difference in cell areas due to the angle of direction towards the vascular bundle from the central point was not as great as that due to the distance from the central point. However, the cell areas were larger in the direction near the vascular bundle (0–30°) compared with other directions in the control. In contrast, in HNT and HDT, the cell areas were smaller in that direction (0°). Thus, the differences in cell areas between the control and both HDT and HNT were greater in that direction. The differences in cell areas between HNT and HDT were static for all distances, with other directions in the control. In contrast, in HNT and HDT, the cell areas were smaller in that direction (0°). Consequently, many extra cells. Irrespective of the distance from the central point, controls had fewer cells than both HNT and HDT. There were no significant differences at any distance between HNT and HDT. Regarding the direction toward the central point, cell numbers were lowest at 90° from the vascular bundle (i.e. the direction of the lateral radius), and higher close to the vascular bundle or 180° away from it (i.e. the direction of the ventral radius) in all treatments (Fig. 4A). Moreover, the highest cell numbers appeared in the direction towards the vascular bundle. Cell numbers at the direction 120–180° from the vascular bundle were lower in controls than in HNT and/or HDT. There were no significant differences at any angle between HNT and HDT.

**DISCUSSION**

**Smaller rates and similar durations of grain filling under high night temperatures vs. high day temperatures**

The experimental conditions for HNT (22/34°C), in which the night temperature was 12°C higher than the day temperature, is unnatural. However, it is meaningful as a model experiment condition to compare the effect of high...
night temperature with high daytime temperature (34/22 °C) since those treatments yield the same average temperature (28 °C).

The grain dry weight under high night temperature decreased, with a reduction in the rate but not in the duration of grain growth (Fig. 2) compared with those under high day temperatures. In both HNT and HDT, the duration of grain growth was shorter than in the control. Previous work has revealed that an increase in grain growth rate is induced by high temperature, but this fails to compensate for the reduction in the duration of grain growth, so that grain dry weight ultimately decreases (Chowdhury and Wardlaw, 1978; Tashiro and Wardlaw, 1989; Wilhelm et al., 1999). The results in this study suggest that the duration of grain growth decreases with an increase in the daily average temperature regardless of the period (day or night) of high temperature.

The determination of the grain growth duration has been considered to be related to senescence of source and/or sink organs i.e. reduced leaf area duration (Spiertz et al., 1971), photosynthetic decline (Chowdhury and Wardlaw, 1978) and the duration of enzyme activity in the endosperm (Chevalier and Lingle, 1983). It is necessary, therefore, to ascertain whether such events related to senescence can be accelerated in rice under high temperature regardless of its timing.

Smaller average cell areas and similar cell numbers of the endosperm under high night temperatures vs. high day temperatures

The production of lower grain dry weights under high night temperatures than under high day temperatures was based not on a difference in grain length, but on differences in grain width and thickness (Table 1), and also endosperm cross-sectional area (Table 2). The area of the endosperm cross-sections is the product of endosperm cell numbers and average cell areas. The number of wheat endosperm cells was recognized as determining the potential grain size (Brooklehurst, 1977). In maize (Jones et al., 1985; Commuri and Jones, 1999; Engelen-Eigles et al., 2000) and wheat (Nicolas et al., 1984), grain dry weight loss was highly correlated with the number of endosperm cells being reduced by high temperature and/or drought. In contrast, Hoshikawa (1962) reported that cell numbers of wheat endosperm were similar when high temperatures persisted throughout the period of endosperm cell development. Hoshikawa (1962) also reported that endosperm cell sizes in wheat were reduced under high temperatures. However, no equivalent data have been reported for rice in previous studies.

In this study, the number of endosperm cells in rice increased by around 20 % during both high night and high day temperatures compared with controls (Table 2). The reduction in the average cell area under high day temperature offset the increase in cell numbers. However, the reduced cell area under high night temperatures exceeded the gain in cell numbers. This result revealed that neither the lower rate of grain growth nor the lower final grain weight in rice under high night temperatures was caused by reduced cell division.

The region within the endosperm influencing cell area reduction under high temperatures

Endosperm cells between the 2nd and 11th cell layers from their central point to their surface enlarged rapidly between 5 DAF and 12 (ventral side) to 15 DAF (dorsal side and lateral side; Hoshikawa, 1967a, b, c). In this study, no progress of cell enlargement was observed. However, the above observations suggest some relationship between the region of inferior cell enlargement in the endosperm under high night temperatures compared with that under high day temperatures (Figs 3B and 4B) and the DAF by which the difference in grain growth rate between the two temperatures was recognized (Fig. 2). Thus, the high night (rather than high day) temperatures should affect the grain growth rate in the early or middle stages of grain filling through a reduction in cell enlargement in the region at which the cells enlarge at those stages.

Furthermore, cell sizes on the dorsal side (0–60° around the vascular bundle) were affected by both high night and high day temperatures in experiment 2 (Fig. 4B). These regions are considered to enlarge at the end of grain filling, since the endosperm is generally formed in the order of longitudinal, ventral, dorsal and lateral radius. Moreover, Hoshikawa (1968) concluded that the transport pathway of
photoassimilates into the endosperm is finally limited to a direction from the vascular bundle as grain-filling progress. Thus, the regions (i.e., those around the vascular bundle) in which the cell size was reduced under both high day and high night temperatures were responsible for the reduction in the grain growth rate at the later stages of grain filling, as well as for the shortness of grain growth duration compared with the control.

The reduction of the enzyme activity involved in starch metabolism in the endosperm has been considered a factor in the decrease in both the grain growth rate and final grain weight in some cereals under high temperatures (Hawker and Jenner, 1993; Jenner et al., 1993; Keeling et al., 1993; Wilhelm et al., 1999). However, no studies to date have examined whether the reduction of enzyme activity in endosperm is greater at high night- than at high day-temperatures. Further research into enzyme activity under high night temperature will be required.

In addition, lower assimilate supply may have caused the lower rate of grain growth and final grain weight under high night temperatures. We do not consider a 22°C temperature to be more harmful for photosynthesis than a 34°C temperature (Vong and Murata, 1977). We do not know, however, whether a 22°C day temperature after a 34°C night temperature is harmful or not. Further investigation of this will be required.

Kobata and Uemuki (2004) have recently suggested that the potential rate of increase of grain dry matter in rice is not reduced under high temperature, and that any grain yield reduction under such conditions is likely to be due to a failure of the assimilate supply to meet the requirements of the accelerated rate of increase of grain dry matter. It is speculated that high night temperatures produce a condition in which the potential rate of increase of grain dry matter is accelerated and the assimilate supply to the grain is insufficient, since photosynthesis is dormant during the night. Therefore, such a situation may induce a reduction in endosperm cell enlargement and final grain weight under high night temperatures.

CONCLUSIONS

The results presented in experiment 1 indicate that, although the duration of grain growth in rice is reduced by high temperatures both day and night, the rate of grain growth is lower under high night than under high day temperatures; however, both high temperature conditions induce a higher growth rate than in controls. Consequently, only high night temperatures reduce the final grain weight, which becomes similar to that in controls under high day temperatures. In experiment 2, the image analyses of cell sizes and numbers in the cross-sections of endosperm suggest that high night, compared with high day, temperatures affect the grain growth rate in the early or middle stages of grain filling, and also affect the cell enlargement midway between the central point and the surface of the endosperm, where the cells ordinarily enlarge at those stages. It was also suggested that the reductions in cell sizes on the dorsal side around the vascular bundle under both high night and high day temperatures were closely related to the reduction in the grain growth rate in the later stages of grain filling, as well as for the short duration of grain growth compared with the control. Such an image analysis of endosperm cells should be useful in determining the grain filling process under any environmental stress or genetic variation.

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LITERATURE CITED


