Rice Milling Quality, Grain Dimensions, and Starch Branching as Affected by High Night Temperatures

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ABSTRACT

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Important rice grain quality characteristics such as percentage of chalky rice kernels are affected by both high and low night temperatures and by different day and day/night temperature combinations. High nighttime temperatures have also been suspected of reducing rice milling quality including head rice yields. Experiments to confirm or refute this have not been reported. A controlled climate experiment was conducted. Conditions in the chambers were identical except between 2400 and 0500 hours (midnight and 5 am). For those times, two temperature treatments were imposed: 1) 18°C (low temperature treatment) and 2) 24°C (high temperature treatment). Two cultivars were tested: LaGrue and Cypress. The high temperature treatment reduced head rice yields compared with the low temperature treatment. Grain widths were reduced for the high temperature treatment compared with the low temperature treatment. There was no effect of temperature on grain length or thickness. Amylopectin chain lengths 13–24 were increased by the high temperature treatment by ≈1%. Future research will focus on determining whether genetic variability exists among cultivars in their head rice yield response to high temperatures. After identifying a source of resistance to high temperature effects, this characteristic can be incorporated into rice cultivars. In addition, ways to reduce this effect, including biotechnological remedies, have the potential for increasing rice yield and quality.

Whole grain milling yields are known to be controlled by both genotype and environment effects (Jodari and Linscombe 1996). For several years, rice growing regions in Arkansas have experienced high night temperatures and reduced head rice yields. Consequently, the hypothesis has been made that head rice yields have been reduced by high night temperatures. For example, Cypress has had uniformly high head rice yields during this time while LaGrue has had variable head rice yields (K. A. K. Moldenhauer, *personal communication*). There has been some question as to whether the variable head rice yields of LaGrue are partially due to high temperatures. Downey and Wells (1975) found a positive correlation between Starbonnet rough rice yields and the number of hours below 70°F during the period between 40 and 110 days after emergence (with 50% heading for Starbonnet occurring at ≈91 days). The most definitive study to date is that of Peng et al (2004), who determined that rice yields decreased with increasing temperatures and particularly with higher night temperatures. Controlled studies examining the effects of high night temperatures on milling quality have not been performed.

In addition to the reports on associations between growing temperatures and milling yields and traits that influence it, temperatures during grain maturation affect certain physiological and genetic processes. Eight enzymes (most, if not all, in multiple isoforms) accomplish the conversion of sucrose into fully branched starch molecules in the developing rice (and other cereal) grain. These steps are taken by sucrose synthase, UDP glucose pyrophosphorylase, ADP glucose pyrophosphorylase, starch synthase, granule-bound starch synthase, starch branching enzyme, starch debranching enzymes, and D-enzyme. Starch synthase adds glucose units to starch chains. The starch synthesis enzymes in wheat (*Triticum aestivum*, L.) and maize (*Zea mays,* L.), particularly starch synthase, are sensitive to high temperature (Keeling et al 1994). Singletary et al (1994) found responses of ADP-glucose pyrophosphorylase and starch synthase enzyme activity were best correlated to reductions in grain weight and starch productions at high temperatures. In addition, the starch synthase temperature optimum for maize and wheat is significantly lower than for other enzymes associated with starch production (Keeling et al 1994). There are at least four isoforms of starch synthase (Myers et al 2000). Tetlow et al (2004) noted that there is some good evidence for the occurrence of starch synthase and starch branching enzymes in complexes that accomplish much of the branching work in forming amylopectin. The waxy gene codes for granule bound starch synthase. Polymorphisms in the waxy gene explain much of the variation in apparent amylose content across a wide range of genetic backgrounds (Ayres et al 1997; Bergman et al 2001). The expression of this gene and amylose levels are affected by the temperature during kernel maturation (Hirano and Sano 1998). Other enzymes and genes related to the formation of starch may also be influenced by temperatures during grain filling.

None of the excellent studies mentioned above have examined the effect of high temperature on milling and head rice yields using controlled climate. The objectives for this study were to determine head rice yield, dimensions, and starch properties of Cypress and LaGrue in response to two night temperatures in an experiment designed to exclude other possible explanations for those responses.

MATERIALS AND METHODS

Controlled climate night temperature experiments were conducted at the University of Arkansas Rice Research and Extension Center and the Dale Bumpers National Rice Research Center. Rice was grown in a circular arrangement with 13 plants/pot in 16-L unvented pots in the greenhouse. Pots were filled with equal parts Cornell mix and Stuttgart silt loam soil. An excess number of plants were grown in the greenhouse until the plants were at the R3 growth stage (Counce et al 2000). At that stage, plants were paired for similarity in size and projected yield (based on ranking the plants for tiller and panicle numbers) and assigned randomly to either of two treatments (18 or 24°C). Four pots were assigned to each treatment and placed together in the growth chamber. Irradiance was taken from a low of 0 at 0500 hours to a high of 1200 μmol m^{-2} sec^{-1} from 1200 to1400 hours. Irradiance was decreased to zero again by 1900 hours. Temperatures were

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TABLE I
Milling Yields and Amylopectin Chain Length Distribution of Rice Starches[a]

Cultivar	Temperature Treatment (T)[b]	Milled Rice (%)	Head Rice (% of amylopectin)	Amylopectin Chain Length Distribution		
				DP 6–12	DP 13–24	DP 25–36
Cypress	Low	71.2	62.4	20.60	51.65	14.15
Cypress	High	70.0	54.9	20.08	52.55	13.80
LaGrue	Low	67.9	50.7	18.83	52.75	14.58
LaGrue	High	69.0	46.6	18.58	53.78	14.38
Cultivar (C)		<0.01	<0.01	<0.05	<0.01	<0.05
Temperature (T)		ns	<0.01	ns	<0.01	ns
C × T interaction		<0.01	ns	ns	ns	ns

[a] Probability that the effect is due to random variation: <0.05, <0.01; ns, effect is not significant.
[b] Between 2400 and 0500 hours, temperatures were 18°C for the low temperature treatment and 24°C for the high temperature treatment.

TABLE II
Mean Individual Rough Rice Grain Weights and Dimensions[a]

Cultivar	Temperature Treatment (T)[b]	Individual Grain Wt (mg/grain)	Hull Wt (mm)	Dimension		
				Length	Width	Thickness
Cypress	Low	22.3	4.0	8.54	2.49	2.20
Cypress	High	21.4	3.9	8.54	2.44	2.13
LaGrue	Low	22.6	2.8	8.49	2.52	2.23
LaGrue	High	23.1	3.6	8.52	2.44	2.23
Cultivar (C)		ns	ns	ns	<0.05	<0.05
Temperature (T)		ns	ns	ns	<0.01	ns
C × T interaction		ns	ns	ns	ns	ns

[a] Probability that the effect is due to random variation: <0.05, <0.01; ns, effect is not significant.
[b] Between 2400 and 0500 hours, temperatures were 18°C for the low temperature treatment and 24°C for the high temperature treatment.

increased gradually from 18 or 24°C at 500 hours to 35°C at 1400 then decreased to 24°C by 1900 hours. These pots were transferred to the controlled climate conditions in growth chambers. Photoperiods were 14 hr. The temperature regime in each growth chamber was identical except for the period from 2400 hours (midnight) and 0500 hours. Between 2400 and 0500, temperatures were 18°C for the low temperature treatment and 24°C for the high temperature treatment. Relative humidity was maintained at ≥70% during darkness and at ≥65% for the day. As the plants reached the R9 growth stage, panicles were harvested and placed in a room at 22°C and 50% rh until all panicles had been harvested and the grain had equilibrated to 12.5% moisture. Moisture was determined before removing the panicles from the room and before subsequent analysis of samples. Milling procedures are described in Siebenmorgen and Jindal (1986) modified as follows: a rough rice sample of 15–28 g (rather than 125 g) was milled in a polisher (Kett, Tokyo, Japan) rather than a McGill No. 2 mill.

Grains (100) from each treatment were weighed on an analytical balance (Ainsworth AA-250, Denver Instrument Co.). We measured individual kernel dimensions (length, width, and thickness) of the samples using a rice image analyzer (Satake Co., Japan). Rice kernels were placed on the feeding mechanism that individually delivers the kernels on an imaging screen. Two CCD cameras captured kernel images in binary form. A software program (v. 1.0A, RIA System) digitized captured images and automatically converted values into a text file and recorded them on computer. One camera captured images of rice kernels from the top view that measures the length and width. A second camera captured images of rice kernels from the side view that provides measurement for the thickness.

The chain-length distribution of amylopectin was characterized by high-performance anion-exchange chromatography equipped with a pulsed amperometric detector (HPAEC-PAD) according to the method of Kasemsuwan et al (1995) with minor modifications. The HPAEC-PAD (Dionex DX500) system consisted of a GP50 gradient pump, LC20-1 chromatography organizer, ED40 electrochemical detector, 450-mm CarboPac PA1 guard column, 4250-mm CarboPac PA1 analytical column, and AS40 automated sampler. Sugars with DP 1–7 were used to identify the chromatographic peaks. The assignment for the chromatographic peaks with DP >7 was based on the assumption that each successive peak represented a saccharide that was 1 DP longer than that of the previous peak. Defatted starch (20 mg) was added with 3.2 mL of deionized water and gelatinized in a boiling water bath for 1 hr. After cooling to room temperature, 30 L of isoamylase (3,100 enzyme units, Hayashibara Biochemical Laboratories, Okayama, Japan) and 0.4 mL of acetate buffer (pH 3.5) were added to the starch sample and the mixture was incubated at 40°C for 48 hr. The enzyme was inactivated in a boiling water bath for 20 min. A mixed bed exchange resin (IONAC NM-60, J.T. Baker) was added for 1 min to the debranched solutions to eliminate the interference from the buffer. The mixture was filtered through a 0.45-μm filter and placed into sample vials before injection.

RESULTS AND DISCUSSION

For milling yields, there was a significant temperature by cultivar interaction (Table I). The effect of temperature on milling yield was different with cultivar. The high temperature treatment reduced total milled rice yields (≈1%) for Cypress but increased milled rice yields (≈1%) for LaGrue (Table I). Head rice yields were lower for LaGrue than for Cypress. The high temperature treatment reduced head rice yields for both Cypress and LaGrue.

Individual rough rice grain and hull weights and rough rice lengths were not different between LaGrue and Cypress or between high temperature and low temperature treatments (Table II). LaGrue had greater rough rice grain widths than Cypress (the high temperature treatments were within rounding errors for LaGrue and Cypress and the coefficient of variation was very low). The low temperature treatment resulted in greater rough rice grain widths than the high temperature treatment. Rough rice grains of LaGrue were thicker than those of Cypress. Grain thickness was unaffected by temperature treatment. There was no interaction of cultivar and temperature treatment for any rough rice grain weight or dimension response.

TABLE III
Mean Individual Brown Rice Grain Weights and Dimensions[a]

Cultivar	Temperature Treatment (T)[b]	Individual Grain Wt (mg/grain)	Dimension		
			Length	Width	Thickness
Cypress	Low	18.4	6.63	2.09	2.01
Cypress	High	17.6	6.61	2.07	1.93
LaGrue	Low	19.3	6.59	2.07	1.99
LaGrue	High	19.5	6.64	2.04	2.02
Cultivar (C)		<0.01	ns	<0.01	ns
Temperature (T)		ns	ns	<0.05	ns
C × T interaction		ns	ns	ns	ns

[a] Probability that the effect is due to random variation: <0.05, <0.01; ns, effect is not significant.
[b] Between 2400 and 0500 hours, temperatures were 18°C for the low temperature treatment and 24°C for the high temperature treatment.

Individual brown rice weights and dimensions were affected somewhat by the temperature treatments (Table III). Individual brown rice grain weights were greater for LaGrue than for Cypress but unaffected by temperature. Brown rice lengths did not differ for cultivar or temperature treatment. Cypress had greater brown rice grain widths than LaGrue. The low temperature treatment resulted in greater brown rice grain widths than the high temperature treatment. Grain thickness for brown rice did not differ between LaGrue and Cypress or between the temperature treatments. There was no interaction of cultivar and temperature treatment for any brown rice grain weight or dimension response.

Starch was composed of 24.4% amylose and 75.6% amylopectin (averaged across treatments). There was no significant response of apparent (or amylose equivalent) amylose or amylopectin content to cultivar, temperature or the interaction (data not shown). Average chain length of amylopectin was 34.5 (glucosyl units/chain) and did not differ between cultivars or between temperature regimes (data not shown). Chain lengths 6–12 units composed a greater percentage of the amylopectin for Cypress than for LaGrue (Table I). Amylopectin chain lengths 13–24 composed a greater percentage for LaGrue than for Cypress. Amylopectin chain lengths 13–24 were increased by the high temperature treatment by ≈1%. Amylopectin chain lengths from 25 to 36 composed a greater percentage of the amylopectin for LaGrue than for Cypress. Percentage of chain lengths >36 units did not differ between Cypress and LaGrue and were unaffected by temperature (data not shown). Inouchi et al (2000) reported that rice grown under lower temperatures had increased amounts of short amylopectin chains and decreased amounts of long chains relative to rice grown at higher temperatures.

In summary, amylopectin chain lengths 13–24 were increased by higher night temperatures. Chain lengths 6–12 units composed more of the Cypress amylopectin than LaGrue amylopectin. Chain lengths 13–36 composed a greater percentage of the amylopectin for LaGrue than for Cypress. Head rice yields were lower for LaGrue than for Cypress. Grain widths were greater for the low temperature treatment than for the high temperature treatment. Head rice yields were reduced by the high temperature treatment compared with the low temperature treatment for both cultivars. This is the first definitive report of this response. As noted by the breeders and millers, LaGrue had lower milled rice and head rice yields than Cypress. Some of the cultivar responses may also be linked to the differences in amylopectin chain lengths for the two cultivars.

CONCLUSIONS

Rice producers, millers, and others have suspected a link between high night temperatures and reduced rice milling quality. This research confirms that link and suggests that research into breeding rice that resists the negative effects of high night temperatures could be beneficial to rice producers. We hope to identify the reason for the reduced yields, find a rice line resistant to the high night temperatures, and incorporate that characteristic into rice cultivars. The head rice yield reductions at high temperatures may be partially related to the chain length differences between temperature treatments. Further tests are needed to determine this. Also needed are sensitive methods for determining differences in how the starch is laid down in rice grains.

Overall, this study increases our understanding of grain filling in rice and head rice yield responses. In our study, high night temperatures were associated with reduced head rice yields, smaller grain widths, and greater amounts of intermediate amylopectin chain lengths. It should be noted that differences in grain dimensions and starch chain lengths, although statistically significant, are not economically significant. Moreover, the differences in chain lengths would doubtless have little effect on functionality. More exploration into this phenomenon is planned.

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