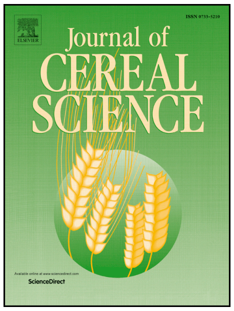

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INFLUENCE OF HIGH DAYTIME TEMPERATURE DURING THE GRAIN FILLING STAGE ON FISSURE FORMATION IN RICE

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ABSTRACT

High daytime temperatures during the grain filling stage in rice have negative impacts on milling quality traits. In this study, we used growth chambers to evaluate the influence of high daytime temperature (33°C) during grain filling, together with grain moisture content at harvest (26%, 18% and 15%), on grain fissure formation. Varietal susceptibility to fissure formation was also evaluated by exposing grains to high temperature at different grain filling stages (milky, dough, maturing). Two fissure resistant varieties: Cypress (long-grain) and Reiziq (medium-grain) and susceptible varieties: YC53-00-7 (long-grain) and Baru (medium-grain) were compared. The average HRY of Cypress declined from 62.7% at 25°C to 53.5% at 33°C, while Reiziq declined from 56.2% (25°C) to 47.4% (33°C). Both were significantly higher than the HRY of YC53-00-7 (39.2% and 24.9%) and Baru (39.3% and 31.7%) at 25°C and 33°C, respectively. When grains were drier at harvest (15% cf. 26%) there was a greater reduction in HRY. When the four varieties were exposed to high temperature, the highest average reduction of HRY was recorded at 21 DAH. It is important to choose an optimal sowing date to avoid coincidence of the final grain-filling stage with high temperatures, in order to minimize milling quality losses.

Keywords: breakage, fissuring, milling, *Oryza sativa*.

INTRODUCTION

Rice is the staple food for more than half of the world's population (Khush, 2005). Rice is processed in two steps namely de-hulling and milling and milled rice is consumed as whole grains. Therefore, broken grains produced during the milling process reduce the economic return to growers and millers. Broken grains are only half the price of whole grain or head rice (Banaszek & Siebenmorgen, 1990). Whole grain has a kernel length more than 75% of the original brown rice length. Head rice yield (HRY) is defined as the weight ratio of whole grains to the initial weight of rough rice, after complete milling (Aquerreta *et al.*, 2007). Grain fissure formation and chalkiness have been identified as the major causes of rice grain breakage during milling (Fitzgerald & Resurreccion, 2009).

Pre- and post-harvest environmental conditions, such as high temperature during grain filling, grain moisture content (GMC) at harvesting and the rate of drying influence grain fissure formation and chalkiness (Bautista *et al.*, 2004). The number of wetting-drying cycles during final grain filling stage and storage also influence fissure formation. Rice production and grain quality traits have been impacted due to global warming as high temperature during grain filling shortens the grain filling period and results in decreased grain weight and size (Peng *et al.*, 2004; Tashiro & Wardlaw, 1991a). In addition, high temperature also increases the amount of chalky and fissured grains, thus reducing HRY (Wei *et al.*, 2009; Yamakawa *et al.*, 2007).

Previous studies have shown that there are varietal differences to high temperature during the grain filling stage for chalk and fissure formation (Cao *et al.*, 2009; Lisle *et al.*, 2000; Tashiro & Wardlaw, 1991a). While chalky grain formation at high temperature has been well studied (Fitzgerald *et al.*, 2009; Lisle, *et al.*, 2000), little information is available on grain fissure formation at high daytime temperatures during the grain filling stage.

Grain fissuring occurs as a result of moisture imbalance between the centre and the surface of the grain. During rapid moisture adsorption and desorption, a higher gradient of moisture is created between the centre and the surface of the grain (Kamst *et al.*, 1999). The higher the moisture gradient, the greater chance that fissure formation will occur. Most rice growing regions around the world have high daytime temperatures (more than 33°C) during the grain filling stage (Krishnan *et al.*, 2011). Identification of possible causes and tolerance mechanisms are vital to develop rice varieties resistant to fissure formation, especially at high temperatures. Further knowledge on critical temperature levels during different grain filling stages is important to decide on optimal sowing times to minimise exposure to high temperatures.

GMC at harvesting has a great impact on milling quality traits. The GMC is used as an indicator for determination of the optimal time for harvesting. In many situations, harvesting at 24% - 26% GMC has given higher HRY than harvesting at lower GMC (Jodari & Linscombe, 1996). It is reported that when grains were dried to below 18% GMC in the field but were then exposed to rain or dew before harvesting, fissuring increased significantly (Lan & Kunze, 1996).

As noted, high temperature during the grain filling stage and GMC at harvesting are critical factors in the fissuring of rice grains. This study had the following objectives: 1) to determine the influence of high daytime temperature during the grain filling stage on fissure formation in resistant and susceptible rice varieties, 2) to determine the most susceptible stage of grain filling to high temperature: milky (14-15 days after heading - DAH), dough (20-23 DAH) and maturing (28-30 DAH) and 3) to determine the optimal GMC at harvesting to minimize grain fissuring.

MATERIALS AND METHODS

1. Influence of high daytime temperature and harvest GMC on fissure formation.

Two long-grain (Cypress and YC53-00-7) and two medium-grain (Reiziq and Baru) varieties were used in the study. Cypress (Blanche & Linscombe, 2009; Counce *et al.*, 2005) and Reiziq (unpublished data) are known to be fissure resistant, while YC53-00-7 and Baru (unpublished data) are fissure susceptible.

Treatments:

Four rice varieties (Cypress, YC53-00-7, Reiziq and Baru), two light period (daytime) temperatures (25°C and 33°C), three harvesting times at different GMCs (26%, 18% and 15%) and four replicates were used. The trial was conducted twice (run-1 and run-2) and in each run there were two replicates.

Plant growth phases:

During the vegetative phase from sowing to flowering (heading), plants were grown inside a temperature controlled glasshouse (28°/22°C, day/night). Each variety per replicate consisted of nine pots (see Figure S1, showing the layout of tubs in the glasshouse with pots, with the same layout used in the growth chambers). Each pot (15 cm diameter) was filled with clay-loam soil and eight pre-germinated seeds were placed in each pot. Sowing dates of varieties were staggered to ensure synchronized flowering. After establishment, plants were thinned to leave four uniform seedlings. Pots were kept in 1.5 m x 1.5 m fibreglass tubs, 75 cm in height. During the growth period, recommended management practices, especially control of water and application of fertilizer were adopted. A nitrogen rate of 125 kg/ha (applied as urea), phosphorus (triple superphosphate) at 30 kg/ha and potassium (muriate of potash) at 38 kg/ha were applied. A split application of nitrogen and potassium fertilizer was carried out one week before panicle initiation to ensure better grain filling. The water level was maintained just above the pot height. The number of days to 50% flowering in each pot was recorded. There were no pest or disease incidences during the growth period and uniform crop growth was observed. At heading, plants were transferred into the control growth chambers. While the plants for run-1 were growing in the growth chambers, the plants needed for run-2 were grown as above in the glasshouse.

During the grain filling stage from flowering to harvesting, plants were grown inside controlled growth chambers. A split-split plot design was used with two replications (per run), where the main plot was temperature, sub-plot was variety and sub-sub plot was harvesting time. For run-2 the same controlled chambers were used, but the chamber that in run-1 was set to 25°C was set to 33°C and vice versa; this was done to minimize any random effects that might exist due to chamber effects. In all treatment units, varieties were allocated randomly.

Controlled growth chamber parameters:

Controlled growth chambers were used for the grain filling phase of crop growth. Both chambers were run at 60% relative humidity and light with photosynthetically active radiation (PAR) of 700±10 μmol $m^{-2}s^{-1}$ for 10 hour period, with a period of 3 hrs for ramping-up from zero to 700 μmol $m^{-2}s^{-1}$ and 2 hrs for ramping-down from 700 μmol $m^{-2}s^{-1}$ to zero. The daytime temperatures of the two chambers were 25±1°C and 33°±1°C. In both chambers the dark period temperature was 8ºC lower than the light period temperature.

Time of harvesting:

Harvesting was done at three time intervals after maturity, based on GMC at 1) 26% (physiological maturity), 2) 18% (on average 5-6 days later than physiological maturity) and 3) 15% (on average 10-14 days later than physiological maturity). GMC was determined by taking the average of three values from the top, middle and bottom of the panicles using a grain moisture tester (Riceter J-series, Kett, Japan). When each experimental unit was harvested, seeds from the 12 plants were bulked and 20 g of filled grains were used for milling. After harvesting, the GMC was gradually reduced to 14%, in a humidity-controlled (15%) and temperature-controlled (20°C) room, over 45 days.

Milling:

Milling was performed in two steps, de-hulling and polishing. Each sample (bulked from 12 plants in an experimental unit) of 20 g paddy rice was de-hulled (Rice Machine THU, Satake Engineering Co., Tokyo, Japan). The total amount of brown rice was then milled using a laboratory-type brush mill (Rice Machine THU, Satake Engineering Co., Tokyo, Japan). After milling, whole grains and broken grains present in the white rice sample were separated and weights were recorded. Fissured grains from the whole grain sample were identified using a Grainscope (TX-200, Kett Electric Laboratory, Japan) and then separated and their weight recorded.

Chalky grains present in the sample of whole grains (fissured and non-fissured) were separated and weights were recorded. A visual rating was used to identify chalkiness (Cruz & Khush, 2000). If a single grain had chalkiness of more than 10% of the total area, then it was considered as a chalky grain.

Data analysis:

Random effects of the growth chambers were estimated by analysing the means of run-1 and run-2, separately. Treatment effects with non-significant chamber effects were analysed by combining run-1 and run-2 (total of four replicates) using the general linear model procedure from the SAS statistical software package (v6.12, SAS Institute, Inc. Cary, NC, USA, 1996). Means of HRY, percentage of fissured and chalky grains at the two temperatures were compared with the Tukey significance test at a 0.05 probability level.

2. Determination of the grain filling stage sensitive to high temperature.

In addition to the above experiment (Figure S1) an additional 32 pots (as grown per run) were used to determine the most sensitive stage of grain filling to high temperature.

A total of eight pots per variety were initially kept in a 25°C controlled chamber at heading. A single pot represented an experimental unit with two replicates (per run). Two pots were transferred at a time to a 33ºC temperature controlled chamber at 14 (milky), 21 (dough) and 28 (maturity) DAH. The remaining two pots were kept in the 25°C chamber as controls.

The experiment was repeated (run-2), under identical conditions. As in the previous experiment, temperature inside the chambers was interchanged to minimize random effects of the chambers. All these plants were harvested at physiological maturity, 26% GMC. The total data set from four replicates (run-1 and run-2) was used for analysis.

RESULTS

1. Influence of high daytime temperature and harvest GMC on fissure formation.

Plant growth duration from heading to different harvest-times where rice GMC had reached 26, 18 or 15% was recorded at the two daytime temperatures, 25°C and 33°C (Table 1). HRY, percentages of chalky and fissured grains were recorded after complete milling.

Head Rice Yield (HRY)

At both daytime temperatures of 25°C and 33°C during grain filling, Cypress (long grain, resistant) and Reiziq (medium grain, resistant) produced a significantly higher HRY than YC53-00-7 (long grain, susceptible) and Baru (medium grain, susceptible) (Figure 1a, b). The average HRY of the susceptible varieties was 20.2% less than the resistant varieties at 25°C, and 22.2% less at 33°C (Table 2). The average reduction of HRY for resistant and susceptible varieties was 9.2 and 10.9%, respectively, when daytime temperature during grain filling was increased (from 25°C to 33°C) (Table 2). Thus the inherent differences in HRY between resistant and susceptible varieties were greater than the differences associated with temperature treatments.

The harvest GMC had a significant effect on HRY. GMC at harvesting had a greater effect on HRY in susceptible varieties than did the high daytime temperature treatment. Harvesting at 15% GMC compared to 26%, resulted in a reduction of HRY at both temperature levels during grain filling. When grain filling occurred at 25°C, the average reduction of HRY of the susceptible varieties was 19.9%, while the reduction was 9.8% in resistant varieties when harvested at 15% GMC compared to 26%. At a daytime temperature of 33°C, YC53-00-7 showed the highest reduction in HRY (24.1%) when harvesting was delayed (harvested at 15% GMC), while the resistant varieties showed an average of 14.1% HRY reduction with delayed harvesting (Figure 1 a & b).

Chalky grains

The percentage of chalky grains was significantly higher at a daytime temperature of 33°C (Figure 1 c & d). The highest percentage of chalky grains was observed in Reiziq under both temperature conditions, while Cypress had the lowest percentage of chalky grains (Table 3). Further, GMC at harvesting had no effect on the percentage of chalky grains.

Fissured grains

Cypress showed the lowest percentage of fissured grains at both grain filling temperature treatments (Table 4). Under stress conditions (daytime temperature, 33°C and 15% GMC), all four varities showed the lowest percentage of fissured grains (Figure 1e & 1f), compared to the optimum temperature (25°C) and GMC (26%), indicating most of the fissured grains had already broken during milling and contributed to the lower HRY at 33°C and 15% GMC.

2. Determination of the grain filling stage sensitive to high temperature

When the plants were exposed to a daytime temperature of 33°C during different grain filling stages, the resistant and susceptible varieties had different responses for the measured grain quality traits (HRY, percentages of chalky and fissured grains).

Head Rice Yield (HRY)

The HRY was significantly reduced when plants were exposed to high temperature at 21 DAH, followed by 28 DAH and 14 DAH (Figure 2a). The percentage reduction of whole grains was higher in susceptible varieties than in resistant varieties. Cypress had the highest HRY of 52.7%, while YC53-00-7 and Baru had the lowest (16.7% and 16.9%, respectively) with exposure to high temperature during the dough stage.

Chalky grains

The percentage of chalky grains was greatest when plants were exposed to high temperature at 21 DAH (Figure 2b). Cypress had the lowest percentage of chalky grains, while Reiziq and Baru had the highest percentage chalkiness after high temperature exposure at 21 DAH. Under the high temperature treatment at 21 DAH, the increase in chalky grains in Baru, YC53-00-7, Reiziq and Cypress was 22.2, 14.2, 15.7 and 10.4%, respectively.

Fissured grains

YC53-00-7 and Baru were sensitive to high temperature at 28 DAH. The percent increase of fissured grains (compared to the control) was 9.4 and 2.1% in YC53-00-7 and Baru, respectively. However, the resistant varieties showed a non-significant (P = 0.1199) slight increased percentage of fissured grains, when exposed to high temperature 28 DAH (Figure 2c).

DISCUSSION

1. Influence of high daytime temperature and harvest GMC on fissure formation.

When exposed to the high daytime temperature treatment (33°C) grain filling took, on average for the four varieties, bsix days less than at 25°C (Table 1). Previous reports indicate that high temperatures during the reproductive phase reduce the grain filling duration, resulting in low grain weight and size (Kim *et al.*, 2011; Tashiro & Wardlaw, 1991b). In addition, high temperatures have been shown to have adverse effects on cooking and eating quality traits (Krishnan et al. 2011).

High temperature had a significant negative effect on HRY (Figure 1a and 1b). A similar finding has been reported by Counce *et al.* (2005) for varieties LaGrue and Cypress. Further, increased nighttime temperature, caused a significant decrease in grain width and length, which influenced HRY (Cooper *et al.*, 2008). Moreover, at high nighttime temperature, some chemical properties were changed and induced breaking forces (Cooper *et al.*, 2008). In our study, it was observed that the susceptible varieties were much more sensitive to high daytime temperature. At high daytime temperature, starch structure is altered (without change in grain dimensions) in susceptible varieties due to an increased rate of translocations. The percentage of fissured grains and loosely packed starch granules were significantly increased at high daytime temperature. The average HRY of Cypress (62.73% and 53.53%) and Reiziq (56.04% and 47.39%) were significantly higher than that of YC53-00-7 (39.15% and 24.91%) and Baru (39.32% and 31.71%) at 25°C and 33°C, respectively. At high temperature, the rate of dry matter accumulation in the grain increases. This produces loosely packed starch granules in the endosperm and these loosely packed regions appear opaque in mature grains of rice (after complete milling) and are known as chalky (Yamakawa *et al.*, 2008). The increase in percentages of chalky grain were 11.2% (Cypress), 9.8% (YC53-00-7), 15.8% (Reiziq) and 15.5% (Baru) at 33°C compared to 25°C (Table 3). On the other hand, the amount of fissured grains was low at high daytime temperature (33°C) compared to the optimum daytime temperature (25°C), indicating most of the fissured grains had already broken and contributed to the lower HRY at 33°C.

GMC is a good indicator of physiological maturity. At 25°C, grains reach 24-26% moisture content within an average of 30 DAH, which is the optimum GMC for harvesting (Jodari & Linscombe, 1996). Harvesting at low moisture contents (18 or 15%) can have negative effects on grain milling quality traits. HRY was greatly reduced if harvested at 15% GMC. The reduction in HRY was greater in susceptible varieties (YC53-00-7 and Baru) than in resistant varieties (Cypress and Reiziq) at 15% GMC. The reduction of HRY in susceptible varieties was approximately half that of the resistant varieties, if harvested at 15% over 26%

GMC at both 25°C and 33°C. In contrast, GMC at harvesting had no significant effect on the percentage of chalky grains at both temperatures during grain filling. The percentage of fissured grains decreased slightly with reduction of GMC. Most of these fissured grains had been broken during de-hulling and milling.

Among the two stress factors, high temperature and low harvest GMC, the influence of temperature was comparatively less on HRY than harvest GMC. When temperature increased from 25°C to 33°C, a 5.8 and 9.5% reduction in HRY was recorded in resistant and susceptible varieties respectively, at 26% harvest GMC. However, the average reduction in HRY due to the decreased GMC (from 26 to 15%) at harvest, was 11.9 and 18.9% respectively in resistant and susceptible varieties at each temperature level (Figure 1a, b). Therefore, when high temperatures are unavoidable, harvesting at 26% GMC will minimize the reduction of HRY.

2. Determination of the grain filling stage sensitive to high temperature

High daytime temperature during different grain filling stages (milky, dough and maturing) had varying effects on milling quality traits. When developing grains were exposed to high daytime temperature at the dough stage (21 DAH), there was a significant reduction of HRY found in all four varieties. YC53-00-7 and Baru were highly sensitive to high temperature, with a significant decrease in HRY at 21 DAH. With high daytime temperature (33°C) exposure at 21 DAH, the average reduction of HRY in susceptible varieties was 31.6%, while it was 16.6% in resistant varieties, compared to the control (25°C).

Water status of the developing rice grain has a direct association with various biochemical and physiological changes, such as accumulation of dry matter in the endosperm (Kano *et al.*, 1990). At maturity, grains hold free water until 22 DAH under the optimum temperature condition (25°C) and water mobility then decreases with the hardening of the endosperm (Funaba *et al.*, 2006). Under optimum temperature, the endosperm slowly changes from fluid to doughy with a decrease of water content and accumulation of assimilates taking place. Any rapid decrease of grain free water content at the dough stage alters dry matter accumulation. Brooks *et al.* (1982) reported that rapid decrease of grain water content (e. g. due to exposure to high temperature), affected starch synthesis and other metabolic reactions in wheat and barley. The assimilate demand from the grain was greatly reduced (Savin & Nicolas, 1996) and translocation of assimilates was also reduced under rapid decrease of GMC in cereals (Sultana *et al.*, 1999). The impaired accumulation of dry matter results in poor milling quality traits. However, high temperature exposure at the early grain filling stage (e.g. 14 DAH) until harvesting had less influence on HRY, as dry matter accumulation was completed at a rapid rate prior to the decrease of grain water content below a critical level (change from fluid to dough). At high temperature, grain water content decreases below a critical level, one week earlier than at optimal temperature (Funaba, *et al.*, 2006).

Similarly, exposure of grains to high temperature at 21 DAH was correlated with the formation of chalky grains (Figure 2b). The total percentage of chalky grains in Baru, YC53-00-7 and Reiziq was significantly higher (average 34.4%) at 21 DAH, than in Cypress (15.7%), indicating tolerance to formation of chalk in Cypress when exposed to high temperature during grain filling. The highest amount of chalky grains were observed when rice plants were exposed to high temperature at the early grain filling stages (Tashiro & Wardlaw, 1991a).

When plants were exposed to high temperature at maturing stage (28 DAH), the formation of fissured grain was significantly increased. YC53-00-7 and Baru were highly sensitive to high temperature, forming grain fissures when they were exposed to high temperature at the final stage of grain filling. The average increase of fissured grains in susceptible varieties was 5.75%, compared to the control. However, Cypress and Reiziq showed tolerance to fissure formation during all grain filling stages at high temperature (Figure 2c). The average increase of fissured grains was 2.6% in resistant varieties at 28 DAH under high temperature. Faster moisture desorption was observed in mature grains of susceptible varieties at high temperatures, suggesting that the susceptible varieties have a more permeable hull (unpublished data). At a rapid rate of moisture desorption, a greater moisture gradient was created between the centre and the surface of the grain (Kunze, 1983), resulting in fissure formation in susceptible varieties.

The later part of the grain filling (from 21 DAH to harvesting) was more critical for formation of chalky and fissured grains at high temperature. If the plants were exposed to high temperature during this period, the milling quality characteristics were greatly reduced. Therefore, it is important to determine the optimum sowing date in order to avoid the likelihood of high temperature conditions during final stages of grain filling according to the particular variety.

CONCLUSION

High daytime temperature during grain filling stage (e. g. more than 33°C) and low GMC (15%) at harvesting had significant negative impacts on milling quality traits. GMC at harvesting had a greater effect on HRY in susceptible varieties than did the high temperature treatment.

High temperature exposure at 21 DAH, resulted in significantly decreased HRY in susceptible varieties. Further, plants exposed to high temperature at the final stages of grain filling (from 21 DAH to harvesting) also resulted in increased percentages of chalky and fissured grains. Therefore, it is important to decide the date of sowing which best avoids the the occurrence of high temperature during the highly sensitive stage of grain filling, to maximise milling quality traits.

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Table 1. Average plant growth (days to heading, in glasshouse) and grain filling duration (in growth chambers) of 12 plants at optimum (25°C) and high (33°C) temperatures.

Varieties	Days to heading	Grain-filling duration (days)					
		25°C			33°C		
		26%	18%	15%	26%	18%	15%
Cypress	102.3 (2.6)	31.7 (3.1)	36.4 (2.7)	43.6 (2.2)	25.2 (2.6)	31.6 (2.0)	35.7 (3.1)
YC53-00-7	84.6 (1.2)	29.0 (1.5)	32.3 (1.1)	38.1 (1.4)	24.8 (2.2)	28.0 (2.4)	32.4 (1.0)
Reiziq	103.2 (1.8)	32.5 (1.8)	37.4 (2)	42.2 (1.8)	25.7 (0.8)	30.3 (1.6)	35.8 (2.0)
Baru	105.5 (3.4)	30.5 (2.7)	34.4 (2.9)	40.8 (3.4)	26.4 (3.1)	30.6 (2.7)	34.2 (3.3)

Standard deviation of the mean is in parentheses.

Table 2. HRY (%) of the four rice varieties at optimum (25°C) and high (33°C) temperatures during grain filling (average of 26, 18 and 15% GMC).

Temperature	Varieties			
	Cypress	YC53-00-7	Reiziq	Baru
25°C	62.7^{aA}	39.2^{aC}	56.1^{aB}	39.3^{aC}
33°C	53.5^{bA}	24.9^{bD}	47.4^{bB}	31.7^{bC}

Different letters represent significance levels of HRY at *P*=0.05 using Tukey significance test; lowercase letters represent differences between temperatures for a given variety (columns); uppercase represent differences between varieties for a given temperature (rows).

Table 3. The percentage of chalky grains of the four rice varieties at optimum (25°C) and high (33°C) temperatures during grain filling.

Temperature	Varieties			
	Cypress	YC53-00-7	Reiziq	Baru
25°C	6.2^{bC}	10.1^{bBC}	21.6^{bA}	14.5^{bB}
33°C	17.4^{aC}	19.9^{aC}	37.4^{aA}	29.9^{aB}

Different letters represent significance levels of chalky grains at *P*=0.05 using the Tukey significance test; lowercase letters represent differences between temperatures for a given variety (columns); uppercase represent differences between varieties for a given temperature (rows).

Table 4. The influence of temperature during grain filling on the percentage of fissured grains of the four rice varieties.

Temperature	Varieties			
	Cypress	YC53-00-7	Reiziq	Baru
25°C	1.9^{aC}	5.3^{aB}	4.4^{aB}	7.3^{aA}
33°C	2.4^{aC}	2.8^{bBC}	4.2^{aAB}	5.7^{aA}

Different letters represent significance levels of fissured grains at *P*=0.05 using Tukey significance test; lowercase letters represent differences between temperatures for a given variety (columns); uppercase represent differences between varieties for a given temperature (rows).

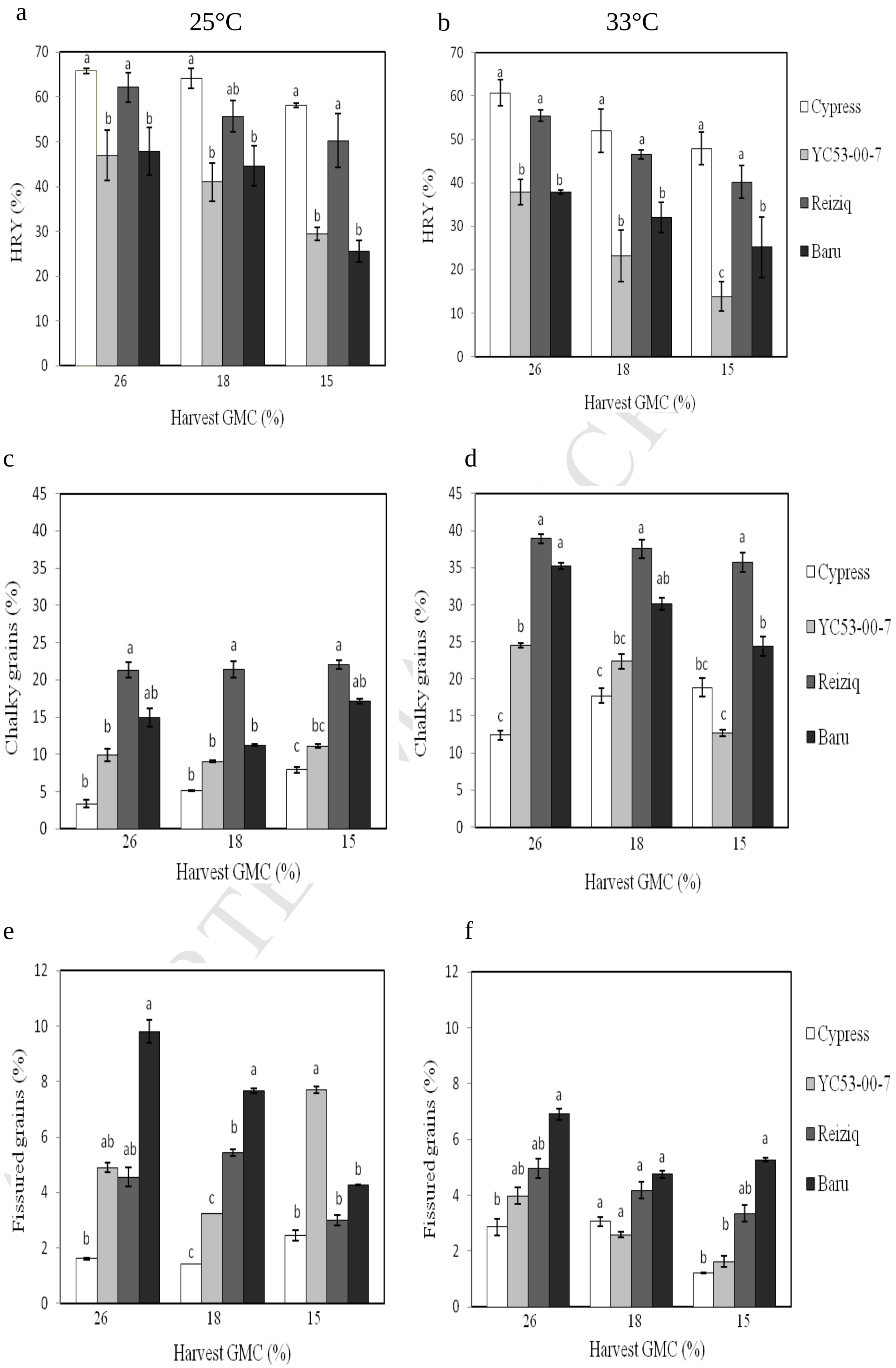
a
25°C
b
33°C
HRY (%)
Harvest GMC (%)
26
18
15
□ Cypress
▨ YC53-00-7
▨ Reiziq
■ Baru
c
d
Chalky grains (%)
e
f
Fissured grains (%)
a
b
ab
bc
c

Figure 1. Milling quality traits of Cypress, YC53-00-7, Reiziq and Baru at 25°C and 33°C (left and right hand side histograms, respectively) during the grain filling stage and harvest at different GMC (26, 18 and 15%). (**a and b)** HRY, (**c and d**) percentage of chalky grains and (**e and f**) percentage of fissured grains. Means within each variety group with the same letter were not significantly different at 0.05 level probability. Error bars represent standard error.

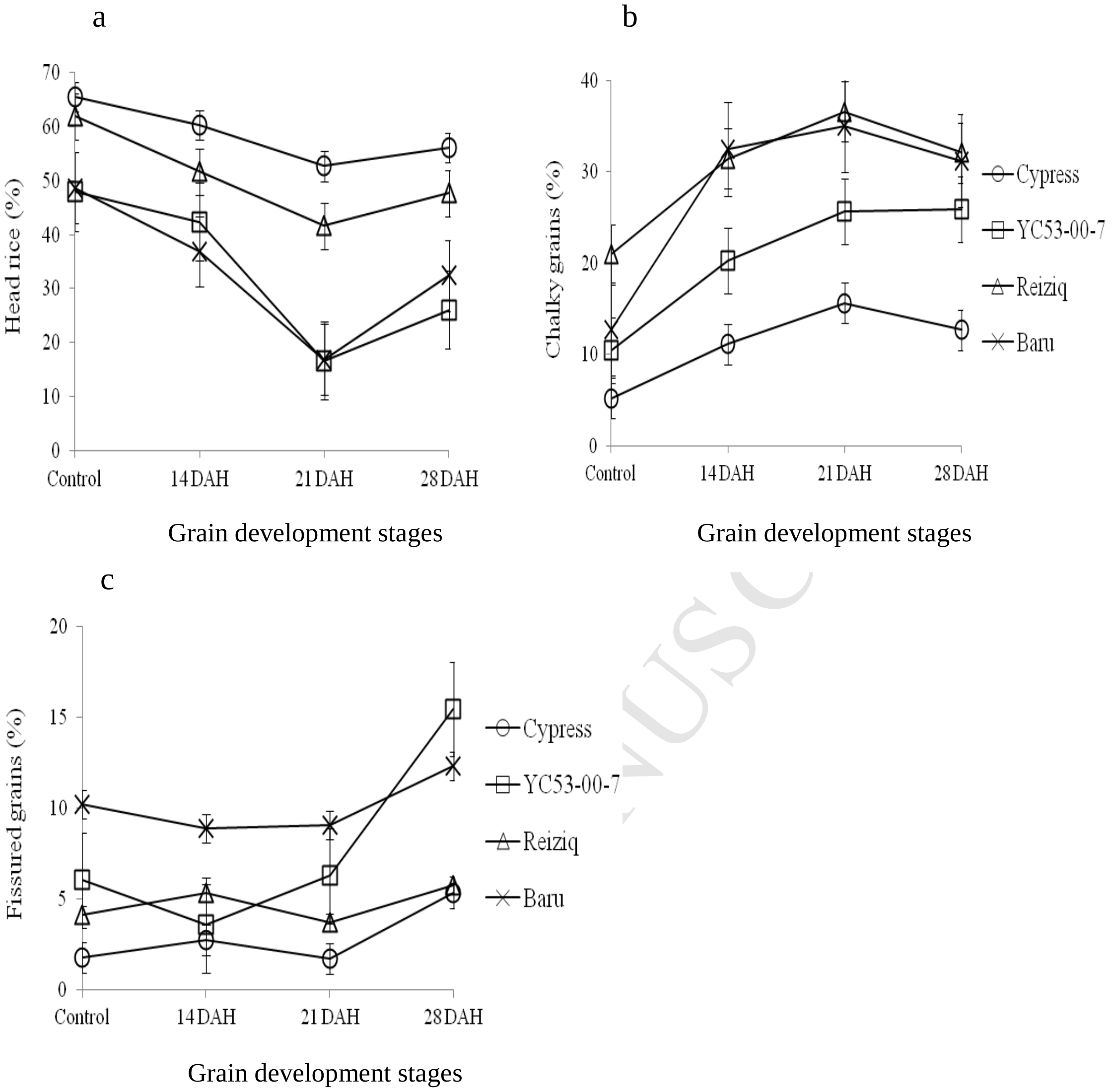

Figure 2. Milling quality traits of the four rice varieties, exposed to high temperature (33°C) at different grain development stages (14, 21 and 28 DAH). (a) percentage of head rice, (b) percentage of chalky grains and (c) percentage of fissured grains. Error bars represent the standard error.

Supplementary information

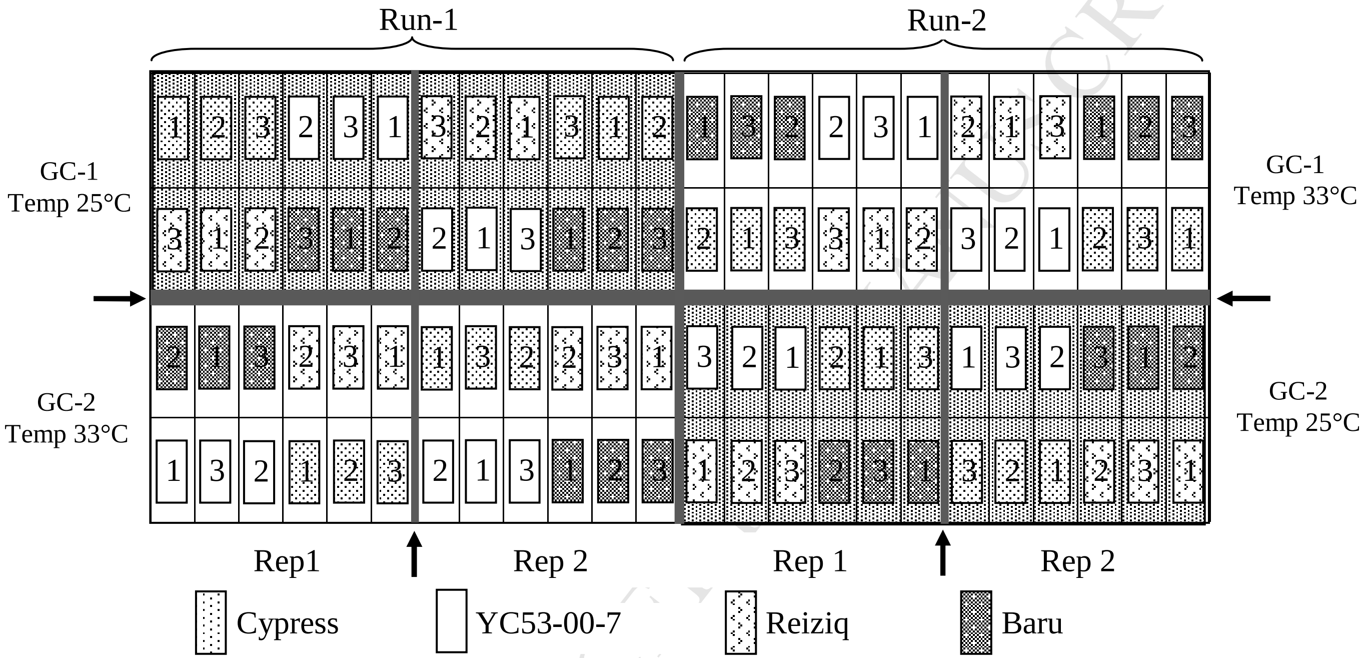

Figure S1. Schematic of randomization (split-split plot design) and time of harvesting of the four rice varieties. Temperature was used as the main plot, while varieties were used as sub-plots and harvesting time was used as sub-sub-plots. Harvesting time is represented by 1 (26% GMC), 2 (18% GMC) and 3 (15% GMC). There were three pots, each with four plants, in each experimental unit (smallest rectangles). GC=growth chamber and Temp=temperature.

Highlights

- High temperature during grain filling and low GMC at harvesting, reduced HRY
- Effect of GMC at harvesting is greater than high temperature on HRY
- High temperature exposure at 21 DAH, resulted the lowest HRY
- Temperature sensitive grain filling stage is used deciding optimum sowing date