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Efficacy of sowing date adjustment as a management strategy to cope with rice (*Oryza sativa* L.) seed quality deterioration due to elevated temperature

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Abstract

The objective of this study was to evaluate the effect of high temperature on rice grain quality, and determine the existence of genotypic variation plus efficacy of sowing time adjustment as a management strategy to avoid heat stress. A field trial was conducted during 2009 on a set of nine rice genotypes collected from various parts of China, and which represent both *indica* and *japonica* ecotypes. Different temperatures were imposed by sowing at three different dates; with earlier sowing (SD1) resulting in comparatively greater temperature followed by mid (SD2) and late sowing (SD3), respectively. Milling degree, recovery and dimensions of milled kernel such as grain length (GL), and width (GW) were negatively affected by SD1 for most of the genotypes, followed by SD2 when compared with SD3. Moreover, SD1 increased the protein content, percentage of grains with chalkiness (PGWC), percent area of chalky endosperm (PACE) and percent degree of endosperm chalkiness (PDEC) than the mid and late sowing. SD2 showed intermediate results between SD1 and SD3 for most of the traits. In terms of genotypes, the effect of temperature treatments on these traits was cultivar dependant. Although, the results of this trial confirm the deleterious effects of high temperature on rice grain quality, the findings also prove the existence of genotypic variation and efficacy of sowing time adjustment as a useful management strategy to cope with temperature stress in warmer ecosystems.

Keywords: Genotypic variation; grain quality; high temperature; sowing date.
Abbreviations: HRY_head rice yield; MDP_milling degree percentage; MRP_milling recovery percentage; SD_sowing date; PGWC_percentage of grains with chalkiness; PACE_percent area of chalky endosperm; PDEC_percent degree of endosperm chalkiness.

Introduction

Global warming is expected to be the major driver for changes in agricultural systems during this century (Gaiser et al., 2011) and is causing more frequent and severe extreme temperature events leading to reduction in crop yields (Sun and Huang, 2011). The more frequent occurrence of heat stress especially in warmer climates has already started affecting susceptible crops like rice in terms of not only yield but also quality. Research on different strategies to somehow enable rice crop to cope with this menace of increasing temperature requires unprecedented focus from the scientific community. In China, the mean temperature has increased by 1.2^{o}C since 1960 (Piao et al., 2010). As substantial spatiotemporal and seasonal variability exists in the warming trend (Rehmani et al., 2011), both larger spatial scale and regional studies are needed for adaptation of agriculture to climate changes (Eitzinger et al., 2010). Direct studies aimed to assess the impact of global warming on crop growth and yield may provide more accurate information about its effects on crops (Peng et al., 2004). High temperature is an important environmental factor that affects the productivity and grain quality of rice (Li et al., 2011). Thus, a rise in mean temperature or episodes of high temperature stresses during sensitive stages will significantly affect rice crop (Krishnan et al., 2011). The physiological and biochemical changes associated with elevated temperature (Shah et al., 2011) are also accompanied by deterioration of the quality of rice kernels. The major components of rice quality are its appearance, cooking quality, eating quality and nutritional quality (Zheng et al., 2008). Environmental temperature during kernel development plays an integral role in causing the observed, unexplained fluctuations in rice grain quality (Cooper et al., 2006). For instance, Counce et al. (2005) found an association of the high nighttime temperature during kernel development with decrease in head rice yield (HRY). Similarly, high-temperature stress during grain ripening facilitates the formation of chalky grains (Ishimaru et al., 2009), low amylose content and lower grain size (Yamakawa et al., 2007; Cooper et al., 2008; Counce et al., 2005). Moreover, high temperatures during grain filling can modify flour and bread quality and other physico-chemical properties of grain crops (Perrotta et al., 1998), including changes in protein content of the flour (Wardlaw et al., 2002). Access to a suite of varieties which can avoid or escape environmental stresses at critical periods is an important strategy to cope with climate change (Ceccarelli et al., 2010). Fortunately, significant variations in response to high temperatures among rice genotypes have been reported earlier (Prasad et al., 2006; Yamakawa et al., 2007). Sowing time can play a critical role in determining the environmental factors like temperature during crop growth. In several studies, sowing at different times has already been used as a strategy to study the response of rice under different temperature regimes (Krishnan and Rao, 2005; Nagarajan et al., 2010). Efficient exploitation of the already existing genotypic variation in rice and adjustment of the sowing time can mitigate, at least in part, the negative impacts on yield associated with elevated temperatures (Shah et al., 2011b; Krishnan et al., 2007). But there is paucity of literature regarding the possible role of these two strategies on the qualitative traits of rice. Thus to get a deeper insight into the effect of different high temperatures on rice grain quality, we designed this trial with the following main objectives (1) to quantify the effect of high temperature on rice quality; (2) to evaluate the genotypic variation in response of different rice genotypes covering both *indica* and *japonica* ecotypes; (3) To determine the efficacy of sowing time adjustment as a management strategy to cope with elevated temperatures.

Results

Rice milling or processing qualities

Rice milling or processing qualities which include milling degree and milling recovery were affected by sowing at different dates. Milling recovery percentage (MRP) was less under SD1 when compared with SD2 and SD3 (Fig. 2a) and the effect was relatively more pronounced compared with that on milling degree percentage (MDP). Both minimum (69.1%) and maximum (74.8%) values of MRP were found under SD1 in a *japonica* cultivar ZH8 and an *indica* variety J87-304, respectively. Head rice yield was also less under SD1 compared with the other two sowing dates but the effect was not significant (data not shown). MDP of all genotypes was negatively affected by high temperature associated with earlier sowing (Fig. 2b) except a *japonica* cultivar (J87-304). But the reduction in SD1 was only significant for five varieties when compared with SD3. The MDP of J87-304 was higher under earlier sowing than both mid and late sowing. No significant relationship of milling degree, recovery and head rice percentage was observed with mean, maximum or minimum temperatures of 30 days period after flowering (data not shown).

Grain dimensions

Some of the genotypes showed significant variation in response to sowing dates in terms of grain length (Fig. 3a). The GL of DTWX was significantly more under SD1 than the other sowing dates while for CNSJ, ZH8 and J87-304 it was significantly less under early sowing. In contrast, GW of most varieties was not significantly affected by different sowing dates (Fig. 3b). Only two genotypes i.e. DTWX and J87-304 showed significant variation. The length to width ratio (LWR) of most of the genotypes was highest under late sowing compared to early and mid sowing (Fig. 3c). LWR of the tested cultivars did not show any significant relationship with mean, maximum or minimum temperatures of 30 days period after flowering (data not shown).

Grain chalkiness and protein content

Milled grain chalkiness was sensitive to sowing at different dates as it was significantly affected by sowing dates. Earlier sowing resulted in increased chalkiness, as maximum values of PGWC for all genotypes (except a *japonica* cultivar J87-304) was found under SD1, followed by SD2 and SD3, respectively (Fig. 4a). *Japonica* type varieties had lower percentages of chalky rice than *indica* under most of the treatments but the relative increase under SD1 treatment compared to SD3 implies that both ecotypes were equally susceptible to sowing related temperature increases. In addition, within each ecotype the tested varieties differed significantly. Among the *indica* type genotypes, XWX1 and DTWX showed maximum values of PGWC, while in case of *japonica* type cultivars; DY5, JWR221 and ZH8 had greater PGWC than J87-304. Similarly, the PACE was also greater under SD1 in all varieties compared with SD3 but the difference was significant for only five genotypes (Fig. 4b). Like PGWC, the PACE value was less in *japonica* varieties than *indica*. Percent degree of endosperm chalkiness (PDEC) of all varieties was also significantly higher under SD1 than SD3 (except J87-304) while, the effect of SD2 treatment was intermediate between these two extremes (Fig. 4c). As PDEC is a trait dependant on PGWC and PACE, it understandably, also showed the same trend and was more in *indica* than *japonica* ecotype. All these three chalkiness related traits were significantly correlated with mean, maximum and minimum diel temperatures of the 30 days period after flowering (Table 3). The tested chalkiness related traits i.e. PGWC, PACE and PDEC showed stronger relationships with maximum diel temperatures rather than mean or minimum diel temperature of 30 DAF. Significant variations in protein content were observed for most of the genotypes (Table 4) as only ZH8 was not significantly affected by different sowing dates. Maximum protein content was found under early sowing and followed by mid sowing (except for DTWX and J87-304). Among the cultivars, maximum protein content was recorded for SKC under SD1 and SD2 while minimum value was found for J87-304 under SD1 and SD3. Generally no difference between *indica* and *japonica* groups was observed and when combined for all the tested genotypes, protein content did not show significant relationship with mean, maximum or minimum temperatures of 30 DAF

Discussion

Along with genetic factors, rice grain quality is also influenced by the environment. The degree of milling is one of the key determinants of rice grain quality. The reduction in the MRP and MDP of majority of the genotypes under SD1 compared to SD2 and SD3 (Fig. 2a and 2b) can be attributed to the relatively higher temperature found after 30 DAF under SD1. Mostly, the difference between SD1 and SD2 was more pronounced than that found between SD2 and SD3, which can be ascribed to the relatively greater difference of diel temperature between the former two (Fig. 1). Likewise, great variations existed among the tested genotypes within both ecotypes for these two traits. Previously, a similar significant temperature by cultivar interaction has been reported in terms of milling related traits (Counce et al., 2005). A reduction in rice grain dimensions in response to high night temperature has been documented in many earlier studies (Counce et al., 2005; Cooper et al., 2008; Yamakawa et al., 2007). GLWR is an important trait which determines the shape of the rice grain. In this trial, the LWR of the grains contributed more to the variation in grain dimensions than only length or width (Fig. 3a, 3b and 3c). No marked variation in response to different sowing dates was observed for the tested genotypes, especially in case of GW. Grain length of the genotypes used in this study, seemed to be inversely related to the width as it gradually decreased when width was increased and vice versa. Grains

Table 1. A brief description of the cultivars used in this study including their species ecotype, place where widely cultivated, climate and grain type.

Cultivar full name	Ecotype group	Origin	Climatic zone	Grain type
XiangWanXian1	*Indica*	Hunan	Subtropical-Monsoon-Humid	Medium
SanKeCun	*Indica*	Sichuan	Subtropical-humid	Long
DongTingWanXian	*Indica*	Hubei	Subtropical	Medium
ZhenXian232	*Indica*	Jiangsu	Temperate + Subtropical	Long
ChengNongShuiJing	*Indica*	Sichuan	Subtropical-humid	Long
DangYu5	*Japonica*	Anhui	Warm-temperate	Long
JWR221	*Japonica*	Jiangsu	Temperate + Subtropical	Long
ZhongHua8	*Japonica*	Beijng	Subtropical	Long
Jing87-304	*Japonica*	Hunan	Monsoon-Humid	Short

of an *indica* cultivar CNSJ had the maximum length and minimum width, while in contrast, a *japonica* cultivar J87-304 has the minimum length and maximum width among all varieties (Fig. 3a and 3b). Similarly, both maximum and minimum LWR of the milled grains were also observed for these two varieties (CNSJ and J87-304, respectively), irrespective of the sowing time. Rice grain chalkiness is directly influenced by elevated temperature beyond a certain optimum level. Wakamatsu et al. (2007) found that chalky grains are frequently observed when the average temperature during the 20 day period after heading is above 26-27°C. In contrast to their findings, in this trial a mean temperature of less than 26^{o}C (except for *indica* ecotype under SD1 for which temperature was more than 27^{o}C) caused chalkiness. The reason might be the fact that we used the temperature for a period of 30 DAF which was understandably lower than for a period of 20 DAF as a declining trend of temperature with time was observed after flowering. The significant increases in chalkiness related traits of most of the rice genotypes under SD1 (followed by SD2) treatment further confirm that the elevated temperature than optimum is a major cause contributing to chalkiness. Previously, it has been shown that high-temperature stress during grain ripening facilitates the formation of chalky grains (Ishimaru et al., 2009), while its greatest influence on chalkiness occurs at the milky stage of grain filling (Tashiro and Wardlaw, 1991). Similar results have been also reported by Yamakawa and Hakata (2010) who found an increase in the chalky endosperm of rice grains in response to high temperature. The probable reason for increased chalkiness under SD1 could be the fact that less assimilates might have partitioned to the grains due to increased temperature. A reduction is assimilates partitioning to the grain usually leads to increase in chalkiness. Although, all varieties (except J87-304) showed greater chalkiness under SD1, there was a visible genotypic variation in the degree of response among these varieties. In comparison with SD3, the percentage of chalky rice under SD1 increased by 28.9%, 94.3%, 197.8%, 231.3%, 94.6%, 87.7% and 103.4% for varieties XWX1, DTWX, ZX232, CNSJ (*indica*), DY5, JWR-221, ZH8 and J87-304 (*japonica*), respectively. Similarly under SD1, chalkiness increased by 100%, 598%, 1170%, 341.7%, 698%, 261.5% and 157.6% for varieties XWX1, DTWX, ZX232, CNSJ, DY5, JWR-221, ZH8 and J87-304, respectively, when compared with SD2. Moreover, the increase in chalkiness was more obvious for *indica* ecotype than *japonica* under SD1 and SD2 compared with SD3. Such findings are in accordance to Zhong et al. (2005) who stated that the inferior quality of *indica* varieties than *japonica* can be ascribed to their poor response to high temperature when grown in early season. But in addition the relatively more pronounced increase of grain

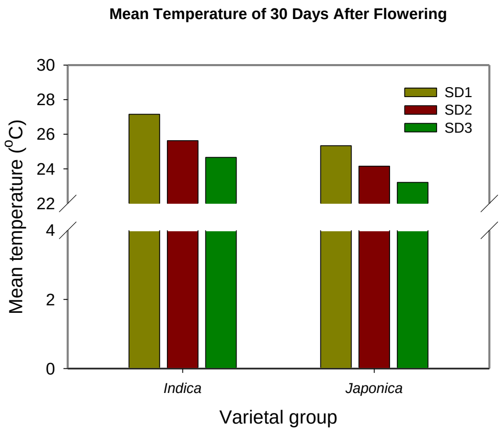

Fig 1. Mean temperature of 30 days after flowering. SD1, SD2 and SD3 represent early, mid and late sowings, respectively. Temperature values are the averages of 30 days period after flowering calculated for all tested varieties of a particular ecotype.

chalkiness in *indica* cultivars can be also partially ascribed to the greater temperature than *japonica* varieties (Fig. 1). Protein is the one of the main chemical components and is crucial to rice grain quality and nutritional value (Lin et al., 2005). Protein content of rice increases with an increase in temperature. In terms of protein content rice is different from some other crops like corn etc as if the protein content increases it deteriorates the eating quality of rice. While in other crops scientists are trying to increase the protein content to improve their nutritional value. Earlier sowing increased the percent protein content of the genotypes than SD2 and SD3, confirming that high temperature increases the protein content in rice. There were marked variations among the response of different cultivars. Earlier studies also showed that the extent of variation in response to high temperature varies with the genotype (Jin et al., 2005). Like rice, high temperatures during grain filling can also modify the physico-chemical properties of other grain crops such as wheat (Perrotta et al., 1998), including changes in protein content of the flour (Wardlaw et al., 2002). Most of the varieties used in this study had generally higher protein content. The results of this study strongly suggest the existence of considerable variation among the genotypes plus their trait dependency in response to high temperature, as no single cultivar was identified to be completely tolerant. Instead, based on the studied traits different genotypes responded with a different level. The findings further imply the need of breeding for an ideotype which can accumulate the desired traits of different genotypes.

Table 2. Mean diel temperature variation of 30 days after flowering (DAF) for each genotype and sowing date.

Varieties	Sowing date	$Temp_{Avg}$ (^{o}C)	$Temp_{Max}$ (^{o}C)	$Temp_{Min}$ (^{o}C)	$Temp_{Dif}$ (^{o}C)
	SD1	27.37	33.32	23.23	10.10
XWX1	SD2	25.87	31.68	21.80	9.88
	SD3	24.72	31.24	20.35	10.90
	SD1	27.22	33.14	23.02	10.12
SKC	SD2	25.63	31.57	21.50	10.07
	SD3	24.72	31.24	20.35	10.90
	SD1	27.37	33.32	23.23	10.10
DTWX	SD2	25.87	31.68	21.80	9.88
	SD3	24.72	31.24	20.35	10.90
	SD1	26.90	32.86	22.69	10.17
ZX232	SD2	25.39	31.43	21.19	10.24
	SD3	24.58	31.27	20.35	10.92
	SD1	26.90	32.86	22.69	10.17
CNSJ	SD2	25.39	31.43	21.19	10.24
	SD3	24.58	31.27	20.35	10.92
	SD1	27.22	33.14	23.02	10.12
DY5	SD2	25.63	31.57	21.50	10.07
	SD3	24.72	31.24	20.35	10.90
	SD1	26.58	32.57	22.41	10.16
JWR221	SD2	24.87	31.22	20.58	10.64
	SD3	24.29	31.67	19.64	12.04
	SD1	22.96	30.92	18.10	12.82
ZH8	SD2	21.81	30.47	16.71	13.76
	SD3	20.67	30.02	15.32	14.70
	SD1	24.58	31.27	20.35	10.92
J87-304	SD2	24.29	31.67	19.64	12.04
	SD3	23.18	30.97	18.13	12.85

The abbreviations in first column stand for varieties XiangWanXian1, SanKeCun, DongTingWanXian, ZhenXian232, DangYu5, JWR221, ZhongHua8 and Jing87-304, respectively. While, $Temp_{Avg}$, $Temp_{Max}$, $Temp_{Avg}$ and $Temp_{Dif}$ stand for average, maximum, minimum and difference between maximum and minimum diel temperatures, respectively.

In addition, the findings provide the evidence for the efficacy of sowing time adjustment as a tool to cope with elevated temperature.

Materials and methods

Plant materials

A group of nine rice (*Oryza sativa* L.) genotypes representing a range of *indica* and *japonica* ecotypes were tested in this trial. Among these genotypes, five were *indica,* while the remaining four were *japonica,* whose details are given in (Table 1). These cultivars were collected from different ecosystems and showed considerable variation in terms of place of origin and seed morphology. As an *indica* variety SKC was found to be completely chalky variety, thus this variety was not included in some analysis like chalkiness and grain dimensions related traits.

Crop husbandry

The trial was carried out at the research field of Dajin Township, Wuxue County, Hubei province, China (29°59' N 115°36' E) during 2009 under irrigated field conditions. Field preparation and crop management were performed according to the standard practices suited to this region. Certified seeds of all these cultivars (already germinated) were sown in nursery field on 16th May, 26th May and 4th June for SD1, SD2 and SD3, respectively. The seedlings were then transplanted to the field on 11th, 18th and 25th June, respectively. Plant to plant distance of 13.3 and row to row distance of 26.7 cm were maintained. Phosphorus at the rate of 40 Kg ha^{-1}, potassium chloride at the rate of 100 Kg ha^{-1} and Zn as Zinc sulphate at the rate of 5 Kg ha^{-1} were split applied (1:1) at transplantation and 45 days after

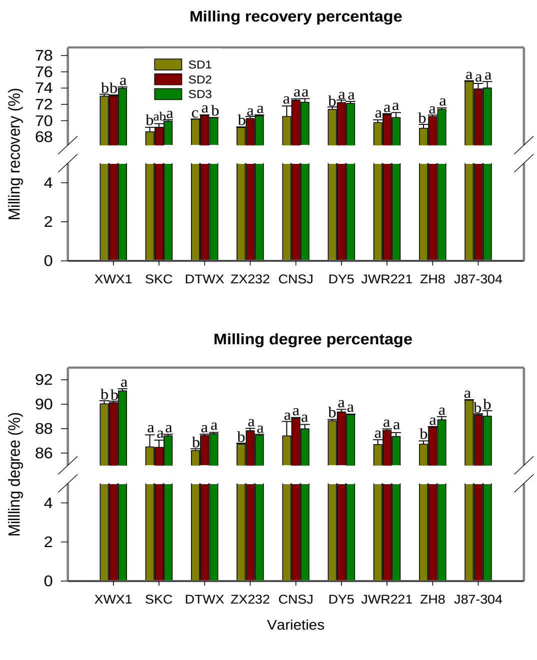

Fig 2. Milling recovery percentage (a) and milling degree percentage (b) of all tested varieties under different sowing dates. SD1, SD2 and SD3 represent early, mid and late sowings, respectively while the abbreviations on X-axis stand for varieties XiangWanXian1, SanKeCun, DongTingWanXian, ZhenXian232, DangYu5, JWR221, ZhongHua8 and Jing87-304, respectively. Values followed by different letters for a variety are significantly different at a probability level of 0.05 under the three sowing dates.

Table 3. The relationships of 30 DAF diel temperatures with grain quality related traits.

Diel temperature of 30 days after flowering period	Percentage of grains with chalkiness PGWC (%)	Percent area of chalky endosperm PACE (%)	Percent degree of endosperm chalkiness PDEC (%)	Protein content (%)
Mean	0.57**	0.54**	0.60**	ns
Maximum	0.63**	0.56**	0.66**	ns
Minimum	0.54**	0.53**	0.57**	ns
Difference (Max minus min) diel temperature	-0.41*	-0.44*	-0.44*	ns

DAF represents days after flowering while ns stands for non-significant, while * and ** denote significant differences at 0.05 and 0.01 probability level, respectively.

transplantation. Nitrogen fertilizer at the rate of 180 Kg N ha^{-1} was also applied in three-way split (4:3:3) at transplantation, mid tillering and panicle initiation stages, respectively. Weeds were uprooted manually, while pests were controlled intensively by spraying pesticides.

Experimental design and temperature treatments

The trial was conducted in randomized complete block design (RCBD) with split plot arrangements and replicated three times; in which sowing dates were randomly assigned to main-plots while varieties to subplots, respectively. Temperature data were recorded using Hobos temperature data loggers. Data regarding mean, maximum and minimum diel temperatures along with the difference between maximum and minimum are presented in Table 2. While the mean diel temperature data of 30 DAF (days after flowering) for all genotypes under different sowing dates are given in Fig. 1. It is clear from Table 2 that temperature of the first month after flowering for each variety was the highest under early sowing, followed by mid and late sowings, respectively. Moreover, temperature for *indica* varieties was higher than *japonica* under each sowing as shown in Fig. 1.

Grain qualitative traits

Various quality related parameters like milling degree, milling recovery, grain length, width, chalkiness, amylose and protein content of all the tested genotypes were evaluated. To measure these traits, 25 grams of rice kernel samples were dehulled by a laboratory dehuller and weighed to determine milling degree. Then the bran was removed in a laboratory polisher to calculate milling recovery. After that head and broken rice were separated through a grain separator. Hundred grains were then scanned and GL, GW, PGWC, and PACE were determined by image analyzer (IA) software. These data were then used to calculate GLWR and PDEC for the tested treatments. Protein content was determined in polished milled rice through spectroscopic method by an *Infratec TM model 1241 Grain analyser (FOSS Tecator).*

Data analysis

To test the significance of various treatments, data was statistically analyzed using analysis of variance (one way ANOVA; SAS statistical analysis package version 9.2; SAS Institute, Cary, NC, USA). The means were separated using Least Significance Difference (LSD) at a probability level of 0.05. If there was not significant difference then the values were averaged and presented with standard errors as error bars.

Conclusion

In conclusion high temperature stress can deteriorate the quality of rice genotypes, though significant variation exists among the genotypes. Sowing time plays a vital role in

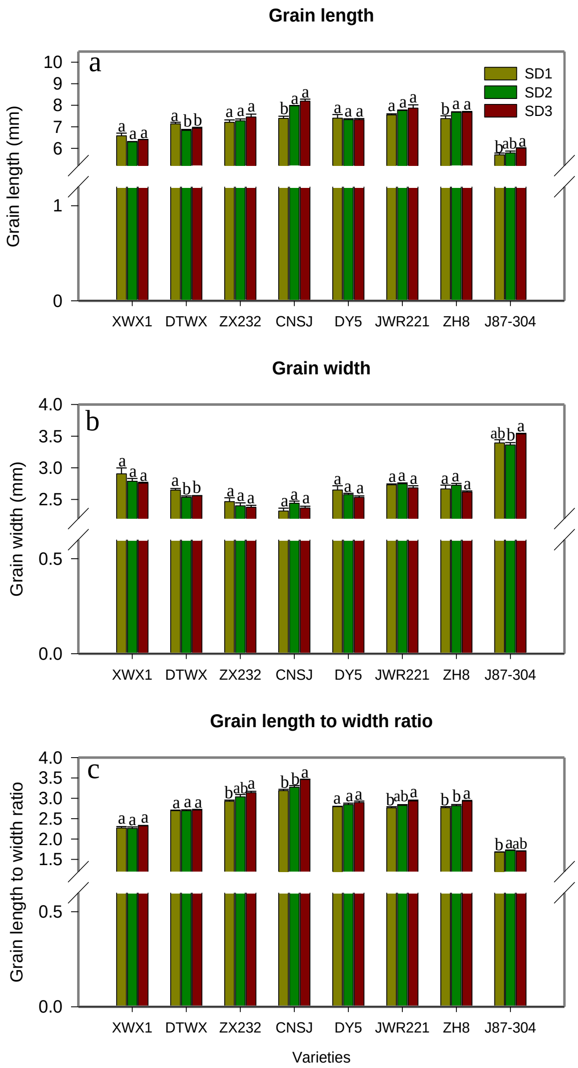

Fig. 3. Milled grain length (a), milled grain width (b) and milled grain length to width ratio (c), of the tested varieties under different sowing dates. SD1, SD2 and SD3 represent early, mid and late sowings, respectively. While the abbreviations on X-axis stand for varieties XiangWanXian1, DongTingWanXian, ZhenXian232, DangYu5, JWR221 and ZhongHua8, respectively, among which the first five belong to *indica* ecotype while the rest belong to *japonica*. Values followed by different letters for a variety are significantly different at a probability level of 0.05 under the three sowing dates.

Table 4. Protein content (%) in polished grains of different varieties under three sowing dates.

Varieties	SD1	SD2	SD3
XWX1	9.2 a	9.3 a	7.0 b
SKC	11.6 a	11.7 a	10.5 b
DTWX	9.7 b	9.2 b	11.5 a
ZX232	9.1 a	8.3 c	8.9 b
CNSJ	9.7 a	9.6 ab	8.5 b
DY5	9.7 a	8.9 c	9.5 b
JWR221	10.5 a	8.3 b	9.3 ab
ZH8	10.7 a	9.6 a	9.9 a

The abbreviations in first column stand for varieties XiangWanXian1, SanKeCun, DongTingWanXian, ZhenXian232, DangYu5, JWR221 and ZhongHua8, respectively. While, means followed by different letters within a row are significantly different at 0.05 probability level according to least significant difference (LSD) test.

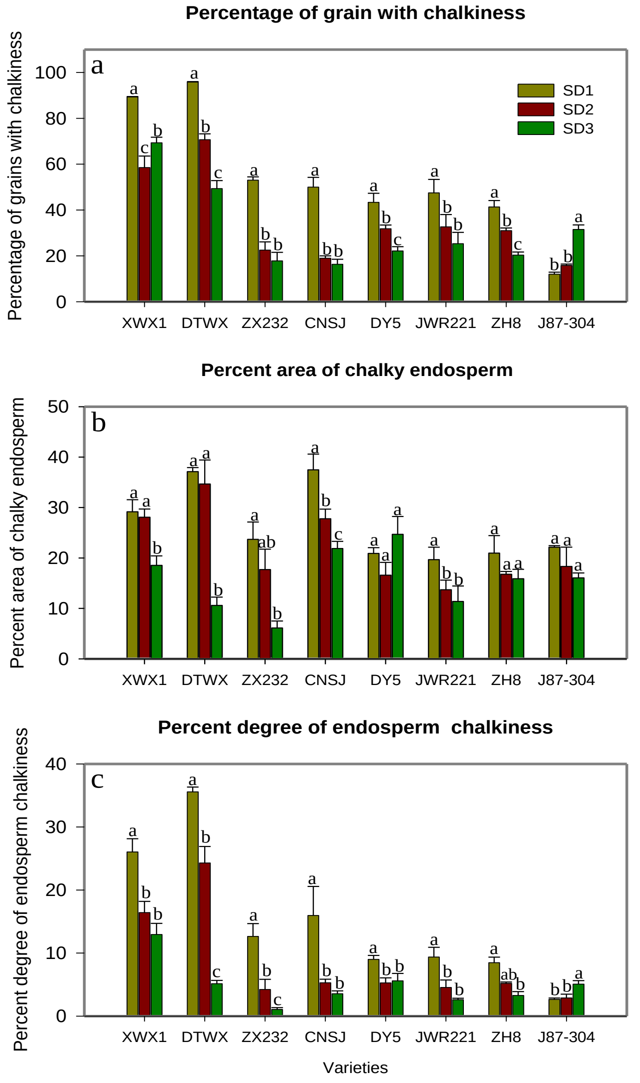

Fig 4. Percentage of grains with chalkiness (a), percent area of chalky endosperm (b) and percent degree of endosperm chalkiness (c) of all tested varieties under different sowing dates. SD1, SD2 and SD3 represent early, mid and late sowings, respectively. While the abbreviations on X-axis stand for varieties XiangWanXian1, DongTingWanXian, ZhenXian232, DangYu5, JWR221 and ZhongHua8, respectively, among which the first five belong to *indica* ecotype while the rest belong to *japonica*. Values followed by different letters for a variety are significantly different at a probability level of 0.05 under the three sowing dates.

determining the quality of rice by controlling the environmental factors. This suggests that if properly managed, adjustment of the sowing dates can greatly help the crops to avoid heat stress and the negative effects of global warming can be ameliorated to some extent, if not fully by wide adaptation of this strategy. But it may not be as simple, as sowing time greatly depends on the preceding crop and the farmers have to reconsider their cropping pattern of the whole year to accommodate such adjustments. Future efforts to exploit the genetic diversity and identify the physiological pathways underlying the variation in response of various genotypes under high temperature stress plus evaluation of management options will maintain a sustainable rice production system in warmer climates.

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