

# Effect of Sowing Date on Grain Endosperm Chalkiness of Different Rice Varieties

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**Abstract:** Grain chalkiness is a major cause of low quality in many rice-producing areas of the world. Though, little is known about the relationship between different sowing dates and the characters of grain chalkiness for grain quality improvement in rice. In this study, the grain chalkiness characters of twenty-one and seven rice varieties were investigated across four and six sowing dates in two years. The results showed a wide range variation of grain chalkiness in diverse varieties across sowing dates, and a positive correlation between chalkiness rate (CR) and the temperature during grain filling phase for all of the varieties. Slight correlation between grain chalkiness area (CA) and the temperature during grain filling phase was also observed for the test varieties in 2015. The chalkiness characters of some varieties were dramatically sensitive to heading dates, while other varieties had a low level of CA and CR across different sowing dates. Improvement of grain chalkiness could be realized by selecting optimum sowing dates in rice production and varieties with low and stable chalkiness could be considered as the elite parental lines in the artificial breeding of the new rice varieties. Therefore, our results have paved the way for the improvement of grain chalkiness in rice.

**Keywords:** Rice Variety, Grain Chalkiness, Appearance Quality, Sowing Date

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## 1. Introduction

Rice (*Oryza sativa* L.) is one of the most important crops and the staple food for more than 3 billion people in the world [1]. With the improvement of people's living standards, the demand for high quality crops is also increasing [2-5]. Though, grain quality is a complex trait which mainly includes the appearance quality, milling quality, nutritional quality, cooking and eating quality [6-9]. The grain appearance quality is directly determined by grain type, transparency and

chalkiness characters [10, 11]. Chalkiness is one of the most important quality traits in rice grains, and the formation of grain endosperm chalkiness is due to the limits of development and enrichment of the endosperm starch granules and protein bodies during grain filling phase, leading to starch granules arrange loosely and irregularly, and finally inflated to form a white opaque portion [5, 12, 13]. Previous studies have shown that grain chalkiness was related to the content and structure of starch [12, 14], compared with non-chalky grains, chalky grains' starch granules usually arranged loosely, amylase and protein content decreased,

milling quality decline, head milled rate and transparency depressed, eventually lead to eating quality and yield decrease [14-19]. Thus, grain chalkiness not only affects the appearance quality of rice, but also affects the other quality traits and final grain yield [14, 20-23]. Despite the diverse consumers' preference for grain taste in rice-producing areas of the world, non-chalky grain still the first choice for most purchaser. Therefore, grain chalkiness is closely related to the grain quality and price worldwide [24, 25].

With the growing of the world population, demand for food crops is also increasing year by year [3]. In this regard, a large number of rice breeders focused on rice yield and as results high quality rice varieties is relatively scarce in the market. Similarly, the excellent rice varieties are still fairly limited in many rice-producing areas of South China, due especially to their high chalkiness rate (CR) and degree of endosperm chalkiness, which makes their grain quality improvement an urgent matter [26]. Grain chalkiness is a complex trait controlled by polygenes and/or quantitative trait loci (QTLs) [27]. At present, many QTLs controlling grain chalkiness characters have been detected; some genes affecting chalkiness have also been cloned, i.e. *GW2* [28], *Chalk5* [29], *OsPPDKB* [30], *SSIIIa* [31, 32], *GIF1* [33], *OsRab5a* [34], *flo2* [35], *GL7* [36]. Grain chalkiness is readily influenced simultaneously by environmental factors, such as field management practices, small farmland environments, soil conditions, high temperature induction, temperature difference between day and night, the daily mean temperature, the length of sunshine, ... etc [9, 20, 22, 23, 37]. Among these factors, the climatic conditions, especially temperatures during grain filling phase, are the most significant to grain chalkiness. Previous studies have indicated that the high temperature affects seriously the growth and development of grain endosperm cells during grain filling phase, which resulted in the increase of grain chalkiness and quality decrease [38, 39, 40]. Because of the actual global warming situation worldwide, the rising temperature during the grain filling period which led to the increase of grain chalkiness will become a trend in the future [16, 37, 41]. This becomes a global problem in rice cultivation and it may have a significant impact on its production and grain quality [15, 24]. Thus, reducing grain chalkiness and improving grain quality are the important goal in rice breeding program for the improvement new varieties.

Even though there are many current studies on grain chalkiness, most of the research focused on the physiological mechanism of chalkiness formation under certain temperature conditions and the genetic basis of grain chalkiness using different populations [4, 9, 42-44]. These studies investigated chalkiness phenotype by different methods and often used a fewer rice varieties under specific environmental conditions (like room temperature). In this study, twenty-one and seven different rice varieties with a wide range of genetic background were investigated for chalkiness rate (CR) and grain chalkiness area (CA) across four and six sowing dates in the field trial in 2014 and 2015, respectively. The objectives of this study were to determine the effects of different sowing

dates corresponding by different grain filling stages' temperatures on grain chalkiness and the varietal differences in grain chalkiness using diverse rice varieties, and to further determine the appropriate period of grain filling sensitive to temperature for the occurrence of rice grain chalkiness. Thus, a systematic analysis of the relationship between the sowing date and grain endosperm chalkiness could pave the way for reducing grain chalkiness and might provide an important basis for optimizing the improvement of the grain quality in rice breeding.

## 2. Materials and Methods

### 2.1. Rice Variety and Grain Filling Phase

All of the rice varieties were planted on the experimental farm of Xinyang Academy of Agricultural Science, Xingyang, Henan province, China, during the rice-growing season from March to October in 2014 and 2015. The twenty-one different rice varieties planted in 2014, were divided into four sowing dates. The twenty-one varieties and their sources were as follows: there were nine varieties (Nongxianggeng 4, Xiangnuo 1862, Xiangbao 1, Xiangbao 2, Xianggeng 33, Xinchanggeng, Aiganxiangdaowan, Nongxianggeng and Zhengdao 18) from Henan Province, four varieties (Wuyungeng 23, Wuxianggeng 23, Nangeng 44 and Xu 68 you 201) from Jiangsu Province, two varieties (9 you 418 and Longyou 1875) from Liaoning Province, Heixiangdao 193 from Jilin Province, Baixianggeng from Heilongjiang Province, Yilatai 104 from France, Huangjinjing from Japan, Mors from America and Zhenggeng 88 from Zhengjiang Province. The four sowing dates used in 2014 were April 10<sup>th</sup>, April 20<sup>th</sup>, May 2<sup>nd</sup> and May 10<sup>th</sup>, respectively. In addition, the twenty-one rice varieties were arranged in three replications within each sowing date.

In 2015, six sowing dates were used for seven rice varieties arranged in three replications within each sowing date, i.e. the sowing dates were March 20<sup>th</sup>, April 1<sup>st</sup>, April 10<sup>th</sup>, April 20<sup>th</sup>, May 2<sup>nd</sup> and May 10<sup>th</sup>, respectively. Three varieties (Wuyungeng 23, Nangeng 44 and Xu 68 you 201) were from Jiangsu Province, two varieties (9 you 418 and Longyou 1875) were from Liaoning Province, and Zhengdao 18 was from Henan Province.

Data for all materials are based on a field experiment using a randomized complete block design with at least three replications in each sowing, and the planting spacing was 16.5 × 26.4 cm. Conventional cultivation and field management from sowing to maturity were carried out, and at harvest, matured rice seeds were tested after threshed, air dried and room temperature for 3 months.

The air temperature was obtained from the temperature data recorder. And average value of daily minimum and maximum temperature was calculated as the daily mean temperature.

### 2.2. Determination of Chalkiness Traits

CR and CA were measured and analyzed when the grains were air-dried and stored at room temperature for at least 3

months [10, 25, 45]. i.e. randomly 100 full and complete milling rice grains in each variety were selected, and counted the numbers of rice grains with chalkiness (repeated three times), and the average value was taken as the CR. For CA, randomly selected 10 rice grains with chalkiness in each variety, estimated the ratio of the CA to the whole kernel square for each grain by visual assessment (repeated three times), and the average area is taken as the CA. SPSS software 17.0 was used to analyze the variance of the data measured.

### 3. Results

#### 3.1. Average Temperature Change in Two Years

The daily mean temperature variations were recorded

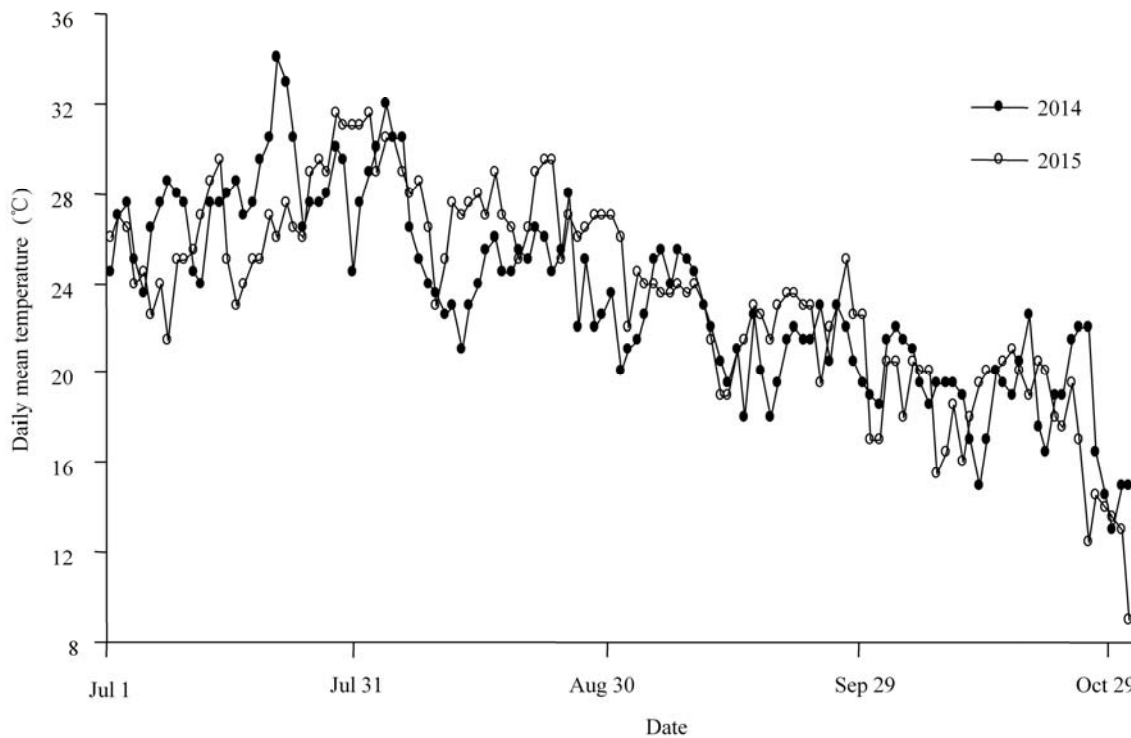


Figure 1. Daily mean air temperature from July to October in 2014 and 2015.

#### 3.2. Field Performance of Chalkiness in Different Rice Varieties in Two Years

The CR and CA of the twenty-one rice varieties were investigated across four sowing dates in 2014; the results showed that there was a great disparity in chalkiness characters among the rice varieties. For the same rice variety, the variations of some CR and CA reached a significant level, while other varieties had no significant variation across the four sowing dates. Among the twenty-one varieties, the maximum CR ranged from 4.1% for Mors and Xianggeng 33 to 100% for Heixiangdao 193, while the minimum CR ranged from 2.8% for Mors to 95.1% for Heixiangdao 193. Moreover,

during the rice growth seasons from June to October in 2014 and 2015, respectively. Overall, the variation of daily mean temperature was not significant between 2014 and 2015, yet the daily mean temperature in 2014 was still slightly lower than that in 2015 (Figure 1). The monthly mean temperature of July in 2014 was higher than that in 2015, while the monthly mean temperature in August was lower in 2014 than that in 2015. The temperature variations during September and October were little between 2014 and 2015. The heading dates of the twenty-one tested varieties were mainly between July 7<sup>th</sup> and September 28<sup>th</sup>, in the meantime, the temperature variations during grain filling phase may have an important influence on the formation of grain endosperm chalkiness.

the average CR across the four sowing dates ranged from 3.3% for Mors to 97.1% for Heixiangdao 193, and the difference between the maximum and minimum CR was from 1.2% for Xianggeng 33 to 33.5% for Xiangbao 2 (Table 1).

For CA, the maximum ranged from 7.5% for Xinchanggeng to 63% for Aiganxiangdaowan; the minimum CA ranged from 4.1% for Nangeng 44 to 39.8% of Aiganxiangdaowan. The average CA ranged from 6.3% for Xinchanggeng to 50.3% for Aiganxiangdaowan, the variation between the maximum and minimum CA across the four sowing dates was ranged from 2.5% for Nongxianggeng to 23.2% for Aiganxiangdaowan.

**Table 1.** Chalkiness characters of 21 varieties across four sowing dates in 2014.

Group	Variety	Maximum (%)		Minimum (%)		Mean (%)		Range (%)		r	
		CR	CA	CR	CA	CR	CA	CR	CA	CR	CA
I	Yilatai 104	19.1	15.1	3.7	9.4	13.3	12.4	15.4	5.7	0.969*	0.469
	Nangeng 44	24.8	12.3	7.3	4.1	15.7	8.5	17.5	8.2	0.546	0.479
	Zhengdao 18	19.7	19.2	3.3	12.9	9.7	16.3	16.4	6.3	0.973*	0.466
	9 you 418	19.3	11.7	3.3	5.1	10.6	8.2	16.0	6.6	0.954*	0.499
II	Xiangbao 1	41.6	50.3	23.1	38.7	33.2	44.4	18.5	11.6	0.976*	0.313
	Xiangbao 2	98.4	51.8	64.9	31.7	84.0	41.8	33.5	20.1	0.947	0.437
	Wuxianggeng 23	57.1	30.1	40.7	19.9	46.6	25.7	16.4	10.2	0.943	0.491
	Wuyungeng 23	47.8	25.3	15.2	12.1	33.0	19.3	32.6	13.2	0.965*	0.505
III	Heixiangdao 193	100.0	50.0	95.1	37.1	97.1	43.8	4.9	12.9	0.976*	0.454
	Nongxianggeng 4	6.1	12.5	4.0	7.3	5.1	10.2	2.1	5.2	0.762	0.541
	Baixianggeng	14.7	8.9	10.7	5.1	12.7	7.4	4.0	3.8	0.998**	0.407
	Xiangnuo 1862	59.5	36.7	53.1	26.1	56.7	31.5	6.4	10.6	0.982*	0.35
	Xianggeng 33	4.1	16.9	2.9	9.8	3.5	13.6	1.2	7.1	0.999**	0.693
	Xinchanggeng	20.5	7.5	13.1	4.6	16.1	6.3	7.4	2.9	0.956*	0.601
	Aiganxiangdaowan	95.2	63.0	93.1	39.8	94.4	50.3	2.1	23.2	-0.696	0.442
	Nongxianggeng	16.9	7.8	10.9	5.3	13.0	6.7	6.0	2.5	0.899	0.635
	Huangjinjing	10.5	12.5	5.7	7.6	7.9	10.3	4.8	4.9	0.969*	0.473
	Mors	4.1	15.1	2.8	9.7	3.3	12.4	1.3	5.4	0.463	0.645
	Zhegeng 88	47.1	32.6	40.1	15.3	44.0	23.0	7.6	17.3	0.805	-0.083
	Longyou 1875	22.7	15.0	20.6	4.3	21.5	10.1	2.1	10.7	0.861	0.457
	Xu 68you 201	10.6	13.8	6.7	5.4	8.7	9.3	3.9	8.4	0.936	0.582
All										0.516**	0.161

\* = Significant at 0.05 probability level; \*\* = Significant at 0.01 probability level; CR: Chalkiness rate; CA: Chalkiness area; r: Correlation coefficients between CR and CA with average daily mean temperature respectively from heading to maturity.

Based on the response of grain chalkiness to sowing dates, maximum CR and whether the rice varieties were sensitive or insensitive to heading dates, the twenty-one rice varieties were classified into 3 groups, i.e. four varieties in group I, four varieties in group II and thirteen varieties in group III, respectively (Table 1). In group I and group II, the difference between the maximum and minimum CR of each variety was greater than 15% across the four sowing dates, while that was less than 15% for each variety in group III. The minimum CR of each variety was less than 15% in group I and more than 15% for the varieties in group II; the maximum CR were less than 25% and more than 40% for each variety in group I and group II, respectively; and, the grain chalkiness of all varieties were sensitive to heading dates in group I and group II, while the rice varieties in group III were insensitive to heading dates.

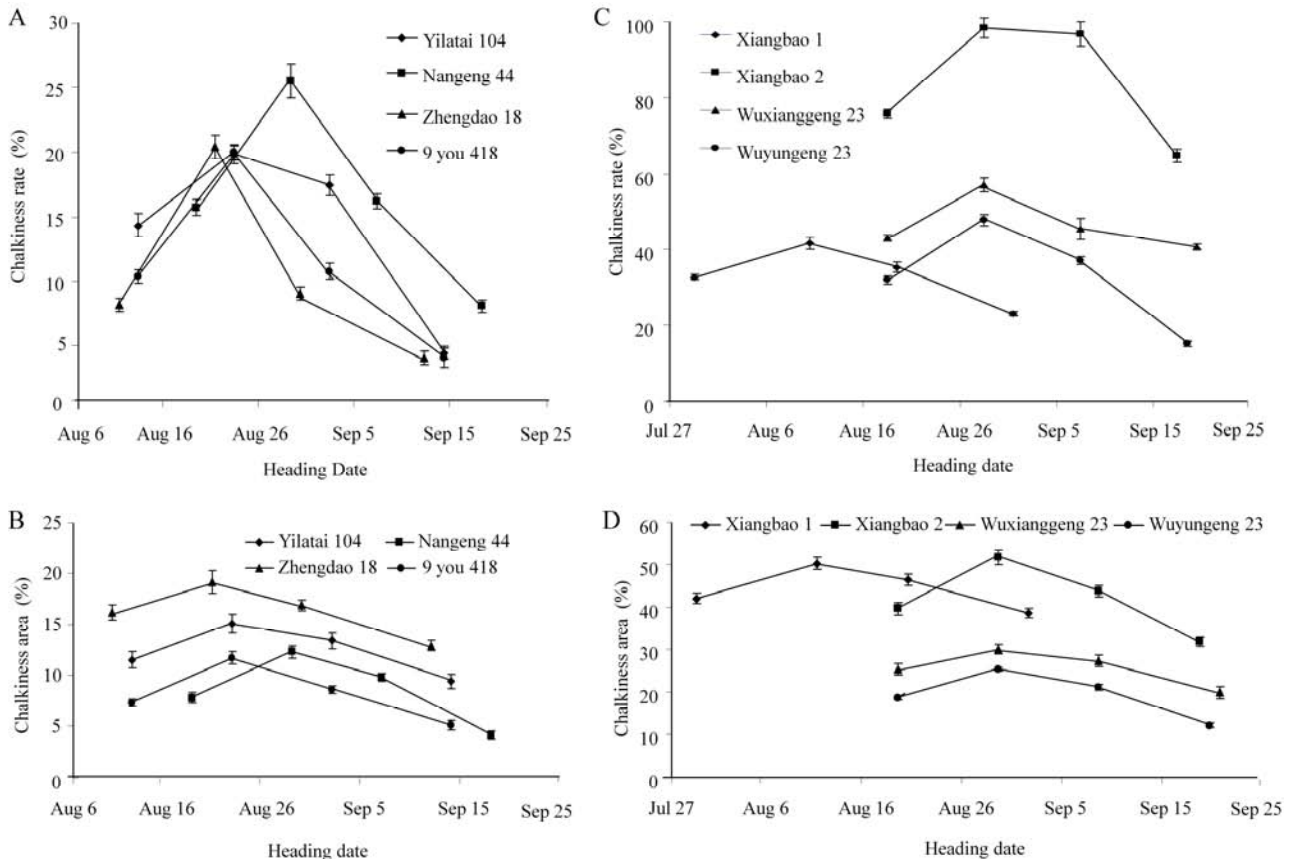
In order to further study the effects of different sowing dates on grain chalkiness, the chalkiness from the seven varieties across six sowing dates were also observed in 2015. Based on the same classification standards in 2014, the seven rice varieties were also divided into 3 groups in 2015 (Table 2). There were three rice varieties in group I, i.e. Nangeng 44, Zhengdao 18 and 9 you 418. Wuyungeng 23 was in group II, and the group III including three rice varieties, Heixiangdao 193, Longyou 1875 and Xu 68 you 201. The minimum CR was less than 15% among the varieties in group I, while in group II; it was more than 15% for Wuyungeng 23. In group I and group II, the difference between the maximum and

minimum CR for each variety was all greater than 15% across the six sowing dates. Compared to group I, the minimum CR was 14.8% for Wuyungeng 23 in Group II, while the maximum CR reached 54.9%. However, the variation between the maximum and minimum CR were all less than 15% for each variety in group III. Noticeably, seven rice varieties were classified into the same groups in 2014 and 2015. The CR of these varieties were sensitive to heading dates in group I and group II, but the varieties in Group III are insensitive to heading dates. In 2015, the maximum CR was 100% for Heixiangdao 193, while the minimum CR was 16.8% for Xu 68 you 201; besides, the minimum of mean CR was 10.4% for Xu 68 you 201 and the maximum was 97.2% for Heixiangdao 193. The minimum of discrepancy between the maximum CR and the minimum CR was 2.8% for Longyou 1875, and the maximum of variation was 40.1% for Wuyungeng 23. The maximum CA ranged from 16.7% for Xu 68 you 201 to 54.8% for Heixiangdao 193; the minimum CA ranged from 6.8% for Nangeng 44 to 50.2% for Heixiangdao 193. The variation range of the maximum CA and the minimum CA ranged from 4.6% for Heixiangdao 193 to 11.1% for Zhengdao, and the mean CA were from 11.1% for Nangeng 44 to 52.5% for Heixiangdao 193. Therefore, our results indicated that there is a wide variation range of grain chalkiness across sowing dates among different rice varieties, and their sensitivity to the temperature during grain filling phase was also different.

**Table 2.** Chalkiness characters of 7 varieties across six sowing dates in 2015.

Group	Variety	Maximum (%)		Minimum (%)		Mean (%)		Range (%)	
		CR	CA	CR	CA	CR	CA	CR	CA
I	Nangeng 44	36.2	15.6	6.4	6.8	20.8	11.1	29.8	8.8
	Zhengdao 18	29.3	23.8	4.1	12.7	17.5	18.8	25.2	11.1
	9 you 418	26.5	15.3	5.8	7.8	14.9	11.8	20.7	7.5
II	Wuyungeng 23	54.9	28.1	14.8	19.0	38.6	23.7	40.1	9.1
III	Heixiangdao 193	100.0	54.8	94.1	50.2	97.2	52.5	5.9	4.6
	Longyou 1875	23.1	17.6	20.3	7.6	21.5	13.3	2.8	10.0
	Xu 68you 201	16.8	16.7	7.1	10.8	10.4	13.3	9.7	5.9

CR = Chalkiness rate; CA = Chalkiness area.



**Figure 2.** Response of grain chalkiness to heading dates in eight varieties in 2014. The chalkiness of these varieties was sensitive to heading dates and remained at low level (A, B) and high level (C, D), respectively.

### 3.3. The Effect of the Temperature on Grain Chalkiness Characters

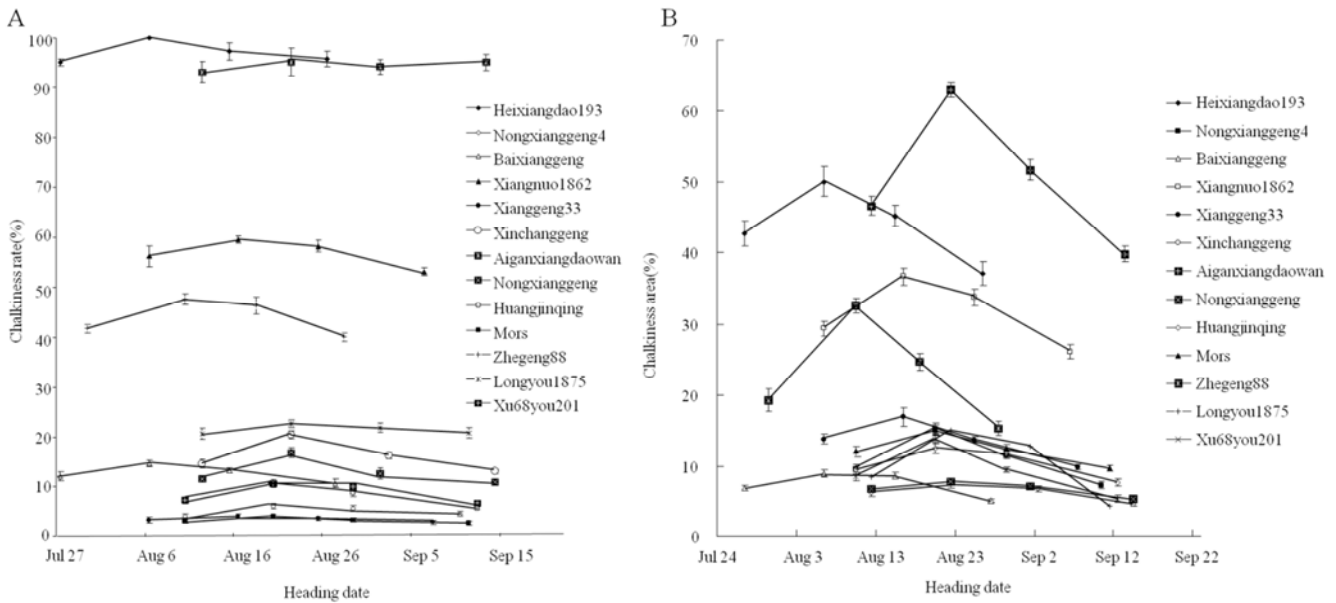
According to the analysis of CR and CA of the twenty-one rice varieties across four sowing dates in 2014, our results showed that the four varieties were extremely sensitive to heading dates in group I. The CR of these four varieties reached the maximum in the second sowing date (i.e. heading date between 20 and 28 August), then, the CR decreased accompanied by the temperatures decreased during grain filling phase, and the change trend of CA was quite similar to that of CR (Figure 2A-B). The four varieties in group II were also sensitive to heading dates, and the change trend of CR and CA was similar to that of varieties in group I, yet the whole change trend was relatively flat compared with group I (Figure 2C-D). When the heading dates were from August

10<sup>th</sup> to August 28<sup>th</sup>, the CR reached maximum, and the CA was also up to the biggest simultaneously. All of the varieties' CR was insensitive to heading dates in group III. Four varieties' CR were more than 40% in group III, and quite different in endosperm CA were observed across four sowing dates; the CR ranged from 40% to 60% in two varieties, and the remaining seven varieties' CR were lower than 25% in group III. However, the variation of CA among these nine varieties (CR was less than 40%) was not big across four sowing dates. Interestingly, Mors, an American variety from the twenty-one rice varieties remained the lowest and most stable CR across sowing dates. CA was small in Xingchanggeng and Nongxianggeng, while high CR and CA were always occurred in Heixiangdao 193 and Aiganxiangdaowan (Figure 3). Thus, for these varieties with sensitivity to heading dates, the chalkiness was affected greatly by the temperature, while

chalkiness characters were less affected on these varieties with insensitivity to heading dates.

The grain CR and CA of the seven rice varieties across six sowing dates was further analyzed in 2015, and the results showed that the heading dates of the varieties with largest CR were between July 29<sup>th</sup> and August 8<sup>th</sup> in group I (Nangeng 44, Zhengdao 18 and 9 you 418) and group II (Wuyugeng 23), and when the heading dates of the varieties were between August 27<sup>th</sup> and September 2<sup>nd</sup>, the CR was relatively minimal among all of the above varieties (Figure 4). The CA reached maximum between August 8<sup>th</sup> and August 18<sup>th</sup> for the three varieties in group I, and with the decrease of the temperature during grain filling period, the CA gradually decreased

(Figure 4B). For the Wuyugeng 23 in group II, the variation range of the CR was large across the six different sowing dates (Figure 4C), and the CR and CA reached the maximum at the third sowing date simultaneously (i.e. the heading period was August 17<sup>th</sup>) (Figure 4C-D). While the overall change trend of the CA was relatively gentle compared with that of CR, and the variation range of the CA was also smaller across the six sowing dates (Figure 4C-D). Moreover, chalkiness of the rice varieties was insensitive to heading dates in group III and the change trend of CR and CA was also very gentle, even though the variations of CR and CA were not obvious across sowing dates (Figure 5).



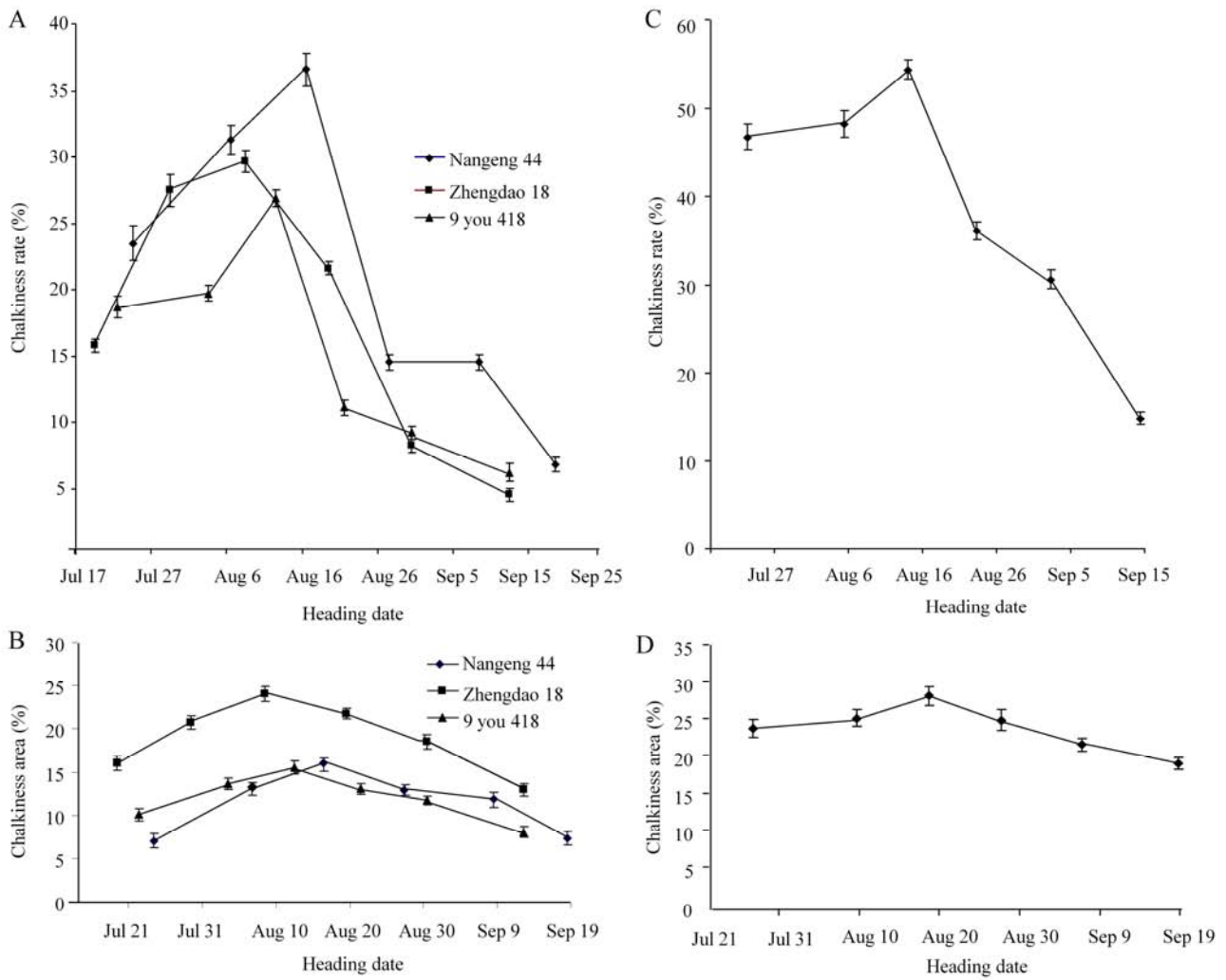
**Figure 3.** Response of grain chalkiness rate (A) and chalkiness area (B) to heading dates in thirteen varieties in 2014. The chalkiness rate of these thirteen varieties was insensitive to heading dates.

**3.4. Correlation Analysis Between Chalkiness and Temperature**

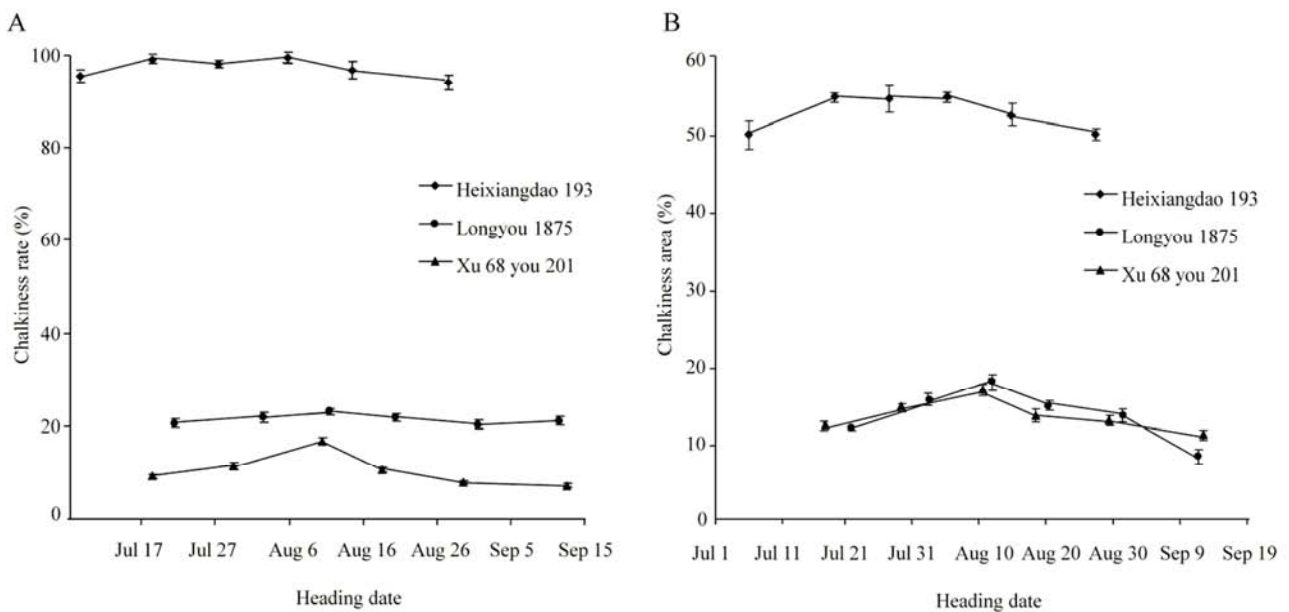
To investigate the correlation between chalkiness characters and the temperatures, chalkiness and average daily mean temperature during grain filling phase from heading date to maturity was also analyzed using the twenty-one rice varieties in 2014. Our results showed that there was a significant correlation between CR and the average temperature at different grain filling stages among the eleven rice varieties (Table 1). Although the correlation coefficient between chalkiness and the daily mean temperature during grain filling phase was larger in seven varieties, from 0.947 to 0.762, there was no significant correlation. When pooled all varieties to calculate the correlation coefficient, it reached to 0.516 ( $P < 0.01$ ), the chalkiness was significantly associated with the

daily mean temperature during grain filling phase in 2014 (Table 1).

We had further analyzed the correlation between chalkiness characters of the seven varieties and the temperatures at different grain filling stages across the six sowing dates in 2015, similar results were obtained (Table 2), and there was a significant correlation between endosperm CR and the daily mean temperature from heading to maturity, and the total correlation coefficient was 0.483 ( $P < 0.01$ ). Based on in-depth analysis of the correlation, we found that the correlation coefficient between CR and the daily mean temperature from heading to maturity was the maximum for all of the seven varieties in 2015, followed by the temperature of two weeks, three weeks and one week.



**Figure 4.** Response of grain chalkiness to heading dates in four varieties in 2015. The chalkiness of these varieties was sensitive to heading dates and was at low level (A, B) and high level (C, D) when heading occurred after August 16.



**Figure 5.** Response of grain chalkiness to heading dates in three varieties in 2015. The chalkiness rate (A) chalkiness area (B) and of these three varieties was insensitive to heading dates.

**Table 3.** Correlation coefficients between chalkiness character and average daily mean temperature during different periods of ripening phase in 2015.

Group	Variety	1WAH		2WAH		3WAH		4WAH		Ripening	
		CR	CA	CR	CA	CR	CA	CR	CA	CR	CA
I	Nangeng 44	0.830*	0.454	0.935**	0.503	0.779	0.108	0.668	0.121	0.711	0.024
	Zhengdao 18	0.945**	0.839*	0.963**	0.749	0.942**	0.480	0.744	0.449	0.945**	0.742
	9 you 418	0.764	0.829*	0.951**	0.753	0.822*	0.471	0.813*	0.551	0.884*	0.917**
II	Wuyungeng 23	0.948**	0.889*	0.942**	0.912*	0.862*	0.649	0.826*	0.605	0.847*	0.673
III	Heixiangdao 193	0.490	0.803	0.964**	0.862*	0.778	0.778	0.636	0.611	0.569	0.555
	Longyou 1875	0.620	0.850*	0.741	0.653	0.370	0.521	0.278	0.497	0.219	0.428
	Xu 68you 201	0.616	0.704	0.570	0.506	0.570	0.491	0.513	0.465	0.393	0.311
All		0.345*	0.380*	0.479**	0.321*	0.351*	0.276	0.299	0.291	0.483**	0.317*

\*= Significant at 0.05 probability level; \*\* = Significant at 0.01 probability level; WAH: Weeks after heading.

Concerning CA, no significant correlation was found between CA and the mean temperature during grain filling phase in 2014 (Table 1), while CA was significantly related to the daily mean temperature at different grain filling stages in general in 2015. Moreover, the correlation coefficient between CA and the one week mean temperature during grain filling phase was biggest, followed by two weeks and maturity stages' daily mean temperature in 2015 (Table 3).

## 4. Discussion

### 4.1. Variation of Chalkiness Characters in Different Rice Varieties

Grain chalkiness is recognized as one of the most important traits in rice. However, the endosperm chalkiness is a complex quantitative trait controlled by polygenes and/or QTLs [9, 21, 22, 23] and influenced by environmental factors [3, 46]. The quality of rice grains is usually poor when chalkiness rate and degree of endosperm chalkiness are high; it could lead to decrease in grain yield and wide variation range of chalkiness among different rice varieties [20, 29]. In the tested twenty-one varieties in 2014, we also found that there was a wide variation range of grain endosperm CR and CA. Such that the maximum CR was 100% for Heixiangdao 193, while the maximum CR was only 4.1% for Xianggeng 33 and Mors. Moreover, the maximum CA was up to 63% for Aiganxiangdaowan, while the maximum CA was only 7.5% for Xinchanggeng (Table 1). In 2015, we also found similar phenomenon: i.e. seven different rice varieties had a larger variation range of chalkiness than that of varieties in 2014. Therefore, our materials have a wide variation range of grain chalkiness among the twenty-one rice varieties.

Although there was a wide variation range of CR and CA, the variations were relatively stable for the same rice variety (Table 1 and Table 2). Based on field survey in two years, we found that the average CR of the seven varieties in 2015 was quite similar to those varieties in 2014. Especially the varieties that the chalkiness was insensitive to heading dates in group III, the variations were not statistically significant, i.e. the average CR was all 21.5% for Longyou 1875 in 2014 and 2015, and minimum CR of Longyou 1875 was 20.6% in 2014 and 20.3% in 2015, respectively. The average CR was 97.0% for Heixiangdao 193 in 2014 and 97.1% in 2015, and the maximum CA was all 100% in the two years. In addition, the

variations were also small for Xu 68 you 201, the minimum CR was only 6.7% in 2014 and 7.1% in 2015.

At the same time, the average CA variations were also smaller for Longyou 1875 and Xu 68 you 201 in two years. Combined with our previous research results [9, 29, 46], all of our results showed that the grain chalkiness was influenced by heredity and environment together. Thus, the rice varieties with low and stable CR and CA across different sowing dates could be considered as core parents in rice breeding, especially for breeding of new rice varieties in South China.

### 4.2. The Effects of the Temperature at Different Grain Filling Stages on Grain Chalkiness

Global warming not only affects the grain yield but also the grain quality, especially during the grain filling phase, the high temperature environment will promote the occurrence of grain chalkiness [16, 24]. In this study, the effect of the grain filling stages' temperatures on the formation of grain chalkiness was very different among the 21 rice varieties in 2014. As previously mentioned, all rice varieties were classified into three groups in the two years, and the varieties in group I and group II were sensitive to heading dates, while the rice cultivars in group III were insensitive to heading dates. The maximum CR of varieties in 2015 (the heading periods were from August 8<sup>th</sup> to August 16<sup>th</sup>) was generally greater than that of varieties in 2014 (the heading periods were from August 20<sup>th</sup> to August 28<sup>th</sup>). This may be due to the mean temperature in August 2015 which was higher than that in 2014, and was consistent with previous research, i.e. the higher the temperature during different grain filling periods, the easier the occurrence of the chalkiness in rice grains [10, 16, 47, 48]. In addition, the maximum CA in 2015 (the heading dates were from July 27<sup>th</sup> to August 17<sup>th</sup>) was also higher than that in 2014 (the heading dates were from August 6<sup>th</sup> to August 28<sup>th</sup>). In the heading date period, the mean temperature in 2015 was higher than that in 2014, and additionally the CA of the seven rice varieties had a significantly positive correlation with the daily mean temperature at grain filling periods in 2015 (Table 3). Therefore, the daily mean temperatures at grain filling stages not only affected CR, but also had an important influence on CA of the grain endosperm in rice.

At present, the problems of high CR and degree of endosperm chalkiness are very common in rice production in many rice-producing areas of the world, needing to improve rice quality urgently [49, 26, 50]. Our results showed that the



CR and CA were both lower for Xianggeng 33 and Mors among the rice varieties across different sowing dates, and these varieties could be considered in the future as parental materials to cultivate new varieties with lower chalkiness characters, especially under the background of global warming; this breeding strategy is even more important. However, some varieties were sensitive to heading dates, such as Nangeng 44, Zhegeng 18, 9 you 418, Wuyungeng 23, the CR and CA in 2015 (the heading dates were from August 8<sup>th</sup> to August 17<sup>th</sup>) were much higher than those in 2014 (the heading dates from August 20<sup>th</sup> to August 28<sup>th</sup>), the reason may be due to the fact that the mean temperature in August 2015 was higher than that in 2014. Meanwhile, the effects of different sowing dates on the grain chalkiness characters were very serious, and even the variation range of the CR could reach more than 15% across the sowing dates (Table 1). Thus, for these varieties, we could reduce grain chalkiness and improve rice grain appearance quality by adjusting sowing dates and lowering the temperatures during grain filling stages in the process of rice production in the future.

#### 4.3. Correlation Analysis Between Chalkiness Characters and the Temperature of Grain Filling Stages

Previous studies have shown that high temperature at grain filling stages will increase the occurrence of grain chalkiness [10]. Some varieties (e.g., Nanjing 11, Aimi, Chengnongshuijing) had the greatest correlation between the CR and the temperature of the two weeks after heading dates, if the hot weather begins to appear four days after flowering, the rice grains will appear with chalkiness characters obviously [16]. In our studies, although some varieties had no significant correlation with the daily mean temperature during grain filling periods in 2014, the correlation coefficient was still very large (Table 1). However, when analysis of the correlation between CR and the daily mean temperature at grain filling stages using all of the rice varieties, the total correlation coefficient was 0.483 ( $P < 0.01$ ), besides, the correlation between CR and the average temperature reached an extremely significant level. There was a similar correlation between CR and the daily mean temperature at grain filling stages among those varieties with insensitive, lower and stable CR in group III in the two years. We also found that the correlation coefficient was biggest between CR and the daily mean temperature from heading to maturity stages, followed by the temperature of two weeks, three weeks and one week after heading dates in 2014, which was basically consistent with previous studies. There was no significant correlation between CA and the daily mean temperature during grain filling periods in 2014, but it was significantly correlated with the daily mean temperature for all of the rice varieties in 2015 (Table 3). The CA was significantly correlated with the daily mean temperature of the one and two weeks after rice heading date, and the correlation coefficients were 0.380 ( $P < 0.05$ ) and 0.321 ( $P < 0.05$ ), respectively. Therefore, our results suggested that there is a certain correlation between CA and the daily mean temperature at grain filling stages.

Meanwhile, grain chalkiness characters were more sensitive to the temperatures at the specific developmental stages in rice grain, which is consistent with previous studies [16, 47, 48]. Therefore, the varieties with insensitivity to heading dates could be selected in the process of rice production [51], and we could select sowing date reasonably to reduce the grain chalkiness and improve the rice quality.

## 5. Conclusions

Our results showed that there is overall positive correlation between the CR and the daily mean temperature during grain filling periods: the higher the temperature during the grain filling stage, the higher the CR in rice grains. The correlation between CR and the daily mean temperature from heading to maturity was biggest, followed by two weeks' daily mean temperature after heading dates. In addition, there was a great variation in the sensitivity of grain chalkiness to the heading date, and changing the sowing dates could be considered in rice production to reduce the grain chalkiness and simultaneously enhance grain quality in rice and, potentially, other staple crops.

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