Amylose Content and Starch Granule Size in Rice Grains are Affected By Growing Season

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Abstract: Amylose content and starch granule size in grains influence rice quality, which differs between the early (ES) and late season (LS). The objective of this study was to determine the variation of amylose content and starch granule size between seasons and find the main reasons (e.g., temperature and solar radiation) for the observed variation. Field experiments with six rice varieties (three high and three low amylose content rice) planted in the ES and LS were conducted in 2016 and 2017, respectively. The mean temperatures during the filling stage were higher in ES, however, the daily temperatures at 7-10 days after flowering (DAF) in 2016, and at 5-10, 13-14 DAF in 2017 were higher in LS. The results showed that amylose content in LS was lower than in ES with high amylose content rice varieties (HACV); the opposite trend occurred with low amylose content rice varieties (LACV). The mean starch granule diameter was higher in LS than ES in 2016, but the opposite result occurred in 2017 with all rice. Our results suggest that higher temperatures increased and decreased the amylose content in HACV and LACV, respectively. Temperatures at 5-15 DAF were important for the formation of starch granules: lower temperatures during 10-14 DAF increased the proportion of larger starch granules ($d > 6.21$ μm, some with $d > 13.3$ μm), and higher temperatures at 5-6 DAF increased the proportion of starch granules with diameter 4.24-6.21 μm.

Keywords: Amylose content; growing season; rice variety; starch granule

1 Introduction

Amylose content is an important factor closely related to rice quality. The variation of amylose content in *indica* rice is mainly governed by genetic effects and environmental conditions [1]. The stability of amylose content at an intermediate level is similar regardless of variety, but hybrid rice with high amylose content is more stable than inbred rice [2]. Temperature is a key factor influencing changes in amylose content [3-5]. Lower temperatures in the heading period increase amylose content [3,6], An experiment by Yao et al. [4] with different sowing dates found that high temperatures at 6-15 days after flowering (DAF) increased amylose content. However, Zhang et al. [7] showed that high temperature stress after flowering decreased the amylose content.

Starch granules are important to amylose, because amylose synthesis occurs within the granule, and it can be influenced by temperature [8,9]. High temperatures increase the average diameter of starch granules and increase the proportion of large starch granules [10]. Starch granules have more variation in shape in low quality rice than in high quality rice [11]. Starch granules are heterogeneous in morphological structure, which influences the amylose content: Elongated starch granules have high amylose content, hollow starch granules have higher amylose content, and polygonal starch granules have

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low amylose content [12] and small particle sizes, mainly falling in the range of 1-10 μm. Amylose content and the size distribution of starch granule particles are correlated in rice varieties [9,13-14]: as amylose content decreases, granule size decreases correspondingly [15]. Seguchi et al. [9] concluded that maintaining starch granule structure requires at least 5% amylose. Cai et al. [16] determined that granule size did not significantly change in rice starch at different levels of amylose content.

Double cropping rice is the most important intensive cropping system for food security in China [17], and it occupies a large portion of rice cropland in southern provinces of the country [18]. Meteorological factors are different in late and early seasons, and temperature is a major component that has a marked effect on grain filling [19]. Temperature and solar radiation are different in the grain-filling stage of early and late seasons, resulting in differences in yield and quality [20,21]. In addition, amylose content and starch granules are influenced by temperature and solar radiation [22]. There has, however, been limited research on changes in amylose content and starch granule size in different rice varieties with changes in temperature and solar radiation in early and late planting seasons.

In this study, we planted three high-amylose and three low-amylose content rice varieties in the early and late seasons in 2016 and 2017. Our aims were to (1) identify changes in amylose content and starch granule particle size with different rice varieties in different seasons, and (2) find the reason of change from temperature factor.

2 Materials and Methods

2.1 Site and Soil

Field experiments were performed in the early and late seasons (ES and LS) from 2016 to 2017 in Yongan Town (28°09′N, 113°37′E, 43 m altitude), Hunan province, China. Soil samples were taken from the 0-20 cm layer before rice transplanting in the early season in 2016. Soil characteristics were: pH 6.07; 36.18g kg^{-1} organic C; 203.17 mg kg^{-1} available N; 16.02 mg kg^{-1} available P; and 190.19 mg kg^{-1} available K.

2.2 Plants and Treatments

The experiments were conducted using a random block design with three replicates and a plot size of 40 m^2. Rice was transplanted using a machine (PZ80-25, Dongfeng Iseki Agriculture Machinery Co., Ltd., Xiangyang, China) with a hill spacing of 25 cm × 11 cm in both planting seasons. The six indica rice varieties were: Luliangyou 996 (a hybrid rice variety), Zhongzao 39 and Zhongjiazao 17 (two inbred varieties) with high amylose content (amylose content: 22%-26%); and Lingliangyou 268 (a hybrid rice variety), Xiangzaoxian 42 and Xiangzaoxian 45 (two inbred varieties) with low amylose content (amylose content: 10%-15%).

In the ES experiments, rice transplanting was done on 20 April with 23-day-old seedlings; in the LS experiment, rice transplanting was done on 23 July with 15-day-old seedlings. All the rice varieties had the same amount of fertilizer in each season; 135 kg N ha^{-1} and 150 kg N ha^{-1} were applied in ES and LS experiments, respectively, as three splits: 50% as basal fertilizer (1 day before transplanting), 20% as tillering fertilizer (7 days after transplanting), and 30% fertilization for head dressing. The rate of N: P_2O5: K_2O was 1:0.5:1 for each season; All P_2O_5 was applied as basal fertilizer and K_2O was applied as basal fertilizer (50%) and head dressing (50%).

2.3 Sampling and Measurements

An automatic weather station (Vantage Pro2, Davis Instruments Crop., Hayward, CA, USA) near the experiment field was used to obtain temperature and incident solar radiation data.

In each season, 10 hills were sampled diagonally from each plot at maturity. Panicles were hand-threshed and the filled spikelets were separated by submerging them in tap water, then air dried for three months. A hulling machine (JLGJ45-B, Taizhou Woliang Foodstuffs Equipment CO., Ltd., Zhejiang China) was used to obtain brown rice. The extraction of starch was based on the alkali digestion method

[23], with some improvements: 30 g of brown rice from every variety was soaked in ultrapure water overnight at 4ºC; rice was then fully ground by hand and the mixture was pressed through four layers of gauze to obtain a solution; the filtered residue was ground repeatedly and pressed in order to fully extract the starch. The collected solutions were filtered successively over 200 mesh and 400 mesh screens then centrifuged at 3000 ×g for 20 mins. The supernatant was discarded and a four-fold volume of 0.4% NaOH solution was added to the sediment, after fully suspending them, the samples were fully shaken with a Platform Constant Temperature Shaking Incubator (THZ-320, Shanghai Jinghong Experimental Equipment Co. LTD, Shanghai, China) at room temperature for 4 hours. The supernatant was poured out after centrifuging at 3000 × g for 20 mins and the 0.4% NaOH solution was replaced with the same volume of ultrapure water; this step was repeated twice. After fully suspending the filter residue in ultrapure water, the pH was adjusted to 7 with HCl solution at 1 mol/L. The supernatant was poured out after centrifuging at 3000 × g for 20 mins, the solutions were filtered through 400 mesh screens. The process of fully suspending, centrifuging, and pouring out the supernatant was repeated until the supernatant was clear, and then the ultrapure water was replaced with absolute ethyl alcohol twice. The sediment was then dried for 48 hours at 40ºC, and the starch was obtained through a 100 mesh screen after full grinding.

Amylose content was determined using iodine-blue colorimetry. The size of starch granules was determined using a laser particle size analyzer (LS-POP6, OMEC Instruments Co. Ltd., Guangzhou, China).

2.4 Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) in Statistix 8.0 software (Tallahassee, FL, USA). The least significant difference test (LSD) was used at the 0.05 probability level among all means of treatments for each year. Correlation analysis was conducted with SPSS 18.0 software (IBM, USA).

3 Results

3.1 Temperature and Solar Radiation

The mean daily temperatures after flowering in ES were higher than in LS (Figs. 1(c) and 1(f)) by 5.98ºC and 4.74ºC in 2016 and 2017, respectively. The flowering day, when all 6 rice varieties flowering in the middle of the panicles and were listed and labeled (19 June and 17 September in ES and LS in 2016, 21 June and 18 September in ES and LS in 2017, respectively.). The maximum, minimum and mean daily temperatures after flowering were higher in ES than in LS in both years (Figs. 1(a)-1(f)). However, in 2016, LS had higher mean and maximum daily temperatures (2.3-5.1ºC and 3.-8.80ºC, respectively) than ES (Figs. 1(a) and 1(c)) from the 7th to 10th DAF, and the minimum temperatures from 8 to 10 DAF were higher in LS than in ES by 0.1-3.9ºC (Fig. 1(b)). In 2017, the maximum and mean daily temperatures in LS were higher (1.3-10.1ºC and 0.2-6.9ºC) than in ES at 5-10 and 13-14 DAF, respectively (Fig. 1(d) and (f)), and there was a higher minimum temperature (1.6-4.6ºC) from 7 to 10 DAF in LS than ES (Fig. 1(e)). The incident radiation from flowering to maturity was higher in ES than in LS in 2016 and 2017 by 29% and 24%, respectively (Fig. 2).

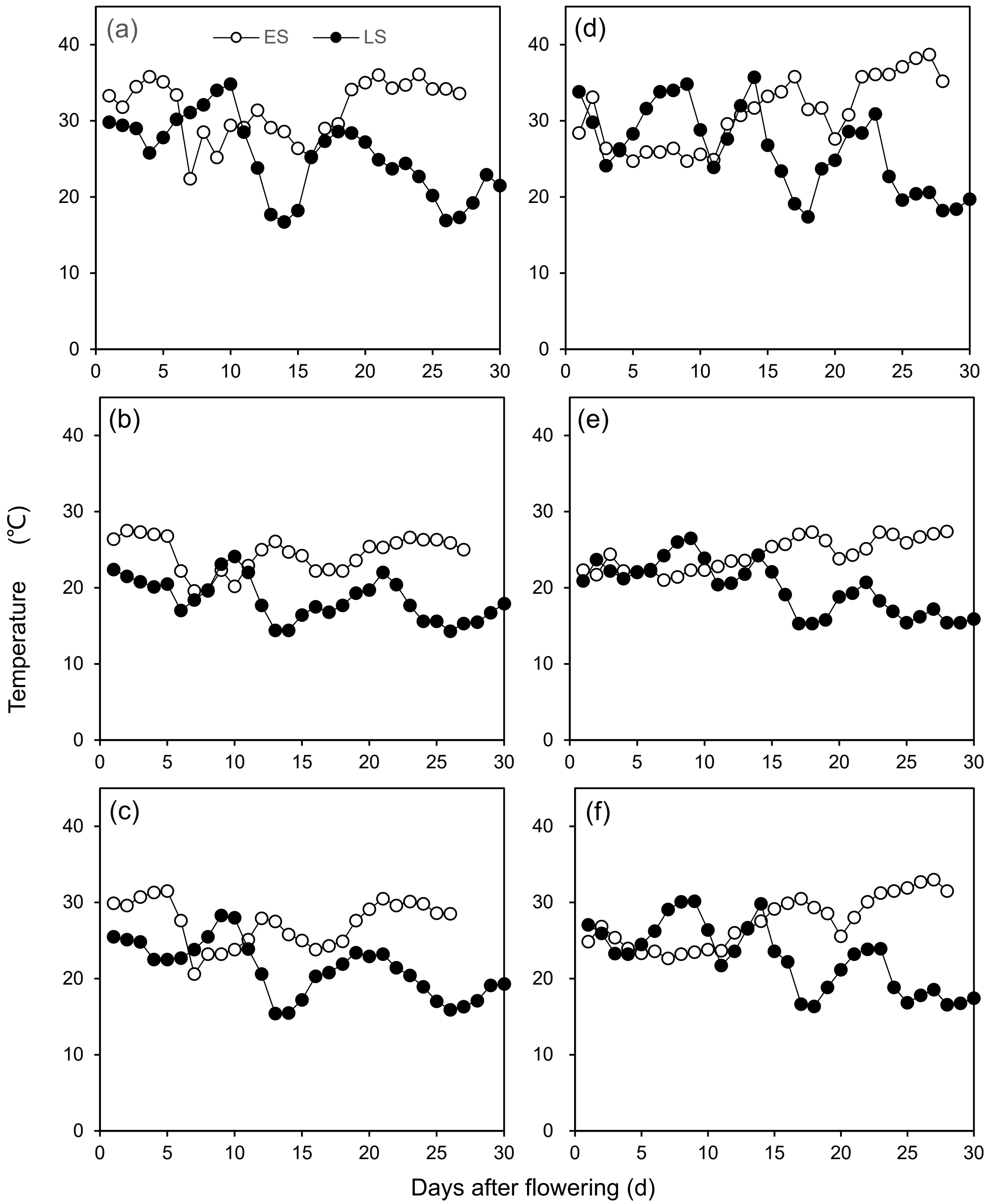

Figure 1: Daily maximum (a and d), minimum (b and e) and mean (c and f) temperatures in filling stage in early season (ES) and late season (LS) in 2016 (a-c) and 2017 (d-f)

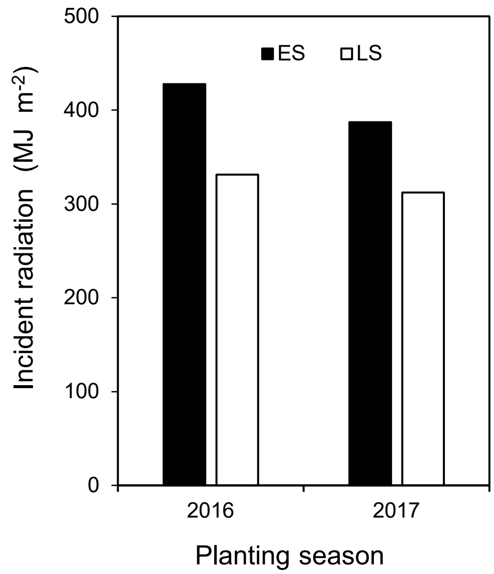

Figure 2: Incident radiation in each planting season after flowering day to maturity

Note: ES: early season, LS: late season

3.2 Amylose Content

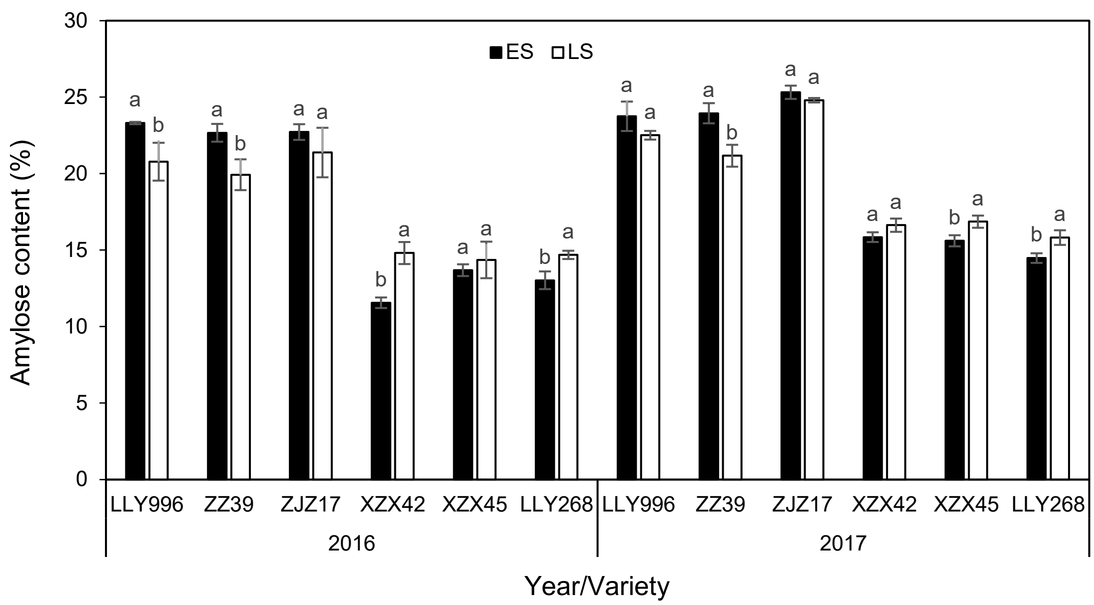

Figure 3: Amylose content of six rice varieties in ES and LS in 2016 and 2017

Note: LLY996, Luliangyou 996; ZZ39, Zhongzao 39; ZJZ17, Zhongjiazao 17; XZX42, Xiangzaoxian 42; XZX45, Xiangzaoxian 45; LLY268, Lingliangyou 268. ES: early season, LS: late season

All of the high amylose content rice varieties (HACV) tended to have higher amylose content in ES than in LS, and the low amylose content rice varieties (LACV) tended to have lower amylose content in ES than in LS both in 2016 and 2017 (Fig. 3). The amylose content of Zhongjiazao 17 (ZJZ17) decreased by 5% in LS compared to ES in 2016, but the difference was not significant. The other two HACV, Luliangyou 996 (LLY996) and Zhongzao 39 (ZZ39), had lower amylose content in LS than in ES, by 10% and 12%, respectively; these differences were significant. Amylose content of Xiangzaoxian 45 (XZX45) in LS had a non-significant increase of 5% compared to that in ES in 2016. In the other two LACV, Xiangzaoxian 42 (XZX42) and Lingliangyou 268 (LLY268), the amylose contents in LS were significantly lower than in ES by 28% and 12%, respectively. In 2017, the amylose content in the three HACV decreased by 2-13% in LS compared to ES. The amylose content of XZX45 and LLY268 increased significantly by 8% and 9% in LS compared to ES. The same trend with XZX42, but the difference was not significant.

3.3 Starch Granules

The particle size of starch granules ranged from 1.11 μm to 19.5 μm, showing a unimodal curve (Fig. 4). In HACV, the peak of the starch granule size distribution was higher in LS than that in ES in both years (Figs. 4(a), 4(c)). The peak of starch granule size distribution occurred at a larger starch granule size in LS (ZJZ17 and ZZ39 at 7.51 μm, LLY996 at 9.09 μm) compared to that in ES (all three varieties had a peak at 6.21 μm). The percentage of large starch granules (d > 6.21 μm in ZJZ17, d > 7.51 μm in ZZ39 and LLY996) increased by 33%, 34%, and 40% in LS compared to ES for ZJZ17, ZZ39, and LLY996, respectively, in 2016 (Fig. 4(a)). The same starch granule size (5.13 μm) had the highest proportion for all varieties in ES and LS. The percentage of starch granules with diameter 4.24-6.21 μm was higher by 4%, 6%, and 3% in LS compared to ES for ZJZ17, ZZ39, and LLY996, respectively, in 2017 (Fig. 4(c)). The mean starch granule particle size was significantly higher by 30-39% in LS compared to that in ES in 2016, however, the mean starch granule particle size decreased by 1-4% in 2017 (Tab. 1). In LACV, starch granules accounting for the largest proportion had a larger or the same starch granule size in ES than in LS in both years. The percentage of large starch granules (d > 6.21 μm in LLY268, d > 13.31 μm in XZX42 and XZX45) increased in LS compared to ES in 2016; the starch granules with diameter 4.24-6.21 μm had a higher percentage in LS than in ES in 2017 (Figs. 4(b), 4(d)). The mean starch granule size was higher in LS compared to that in ES in 2016, with the opposite trend in 2017; these differences were significant (Tab. 1).

Table 1: Mean starch granule particle size (μm) in six rice varieties grown in the early season (ES) and late season (LS) in 2016 and 2017

Year	Season	High amylose content varieties			Low amylose content varieties		
		ZJZ17	ZZ39	LLY996	XZX42	XZX45	LLY268
2016	ES	6.43 ± 0.11b	6.50 ± 0.10b	7.10 ± 0.09b	8.79 ± 0.20a	8.81 ± 0.27b	6.99 ± 0.16b
	LS	8.37 ± 0.08a	8.82 ± 0.03a	9.85 ± 0.06a	9.15 ± 0.32a	9.39 ± 0.07a	7.95 ± 0.26a
2017	ES	5.56 ± 0.05a	5.63 ± 0.04a	5.71 ± 0.02a	5.90 ± 0.02a	6.09 ± 0.02a	6.13 ± 0.05a
	LS	5.50 ± 0.02a	5.40 ± 0.02b	5.63 ± 0.03b	5.67 ± 0.02b	5.81 ± 0.03b	5.90 ± 0.04b

Within a column for each year, data followed by the same letters are not significantly different at the 0.05 probability level. LLY996, Luliangyou 996; ZZ39, Zhongzao 39; ZJZ17, Zhongjiazao 17; XZX42, Xiangzaoxian 42; XZX45, Xiangzaoxian 45; LLY268, Lingliangyou 268. ES: early season, LS: late season.

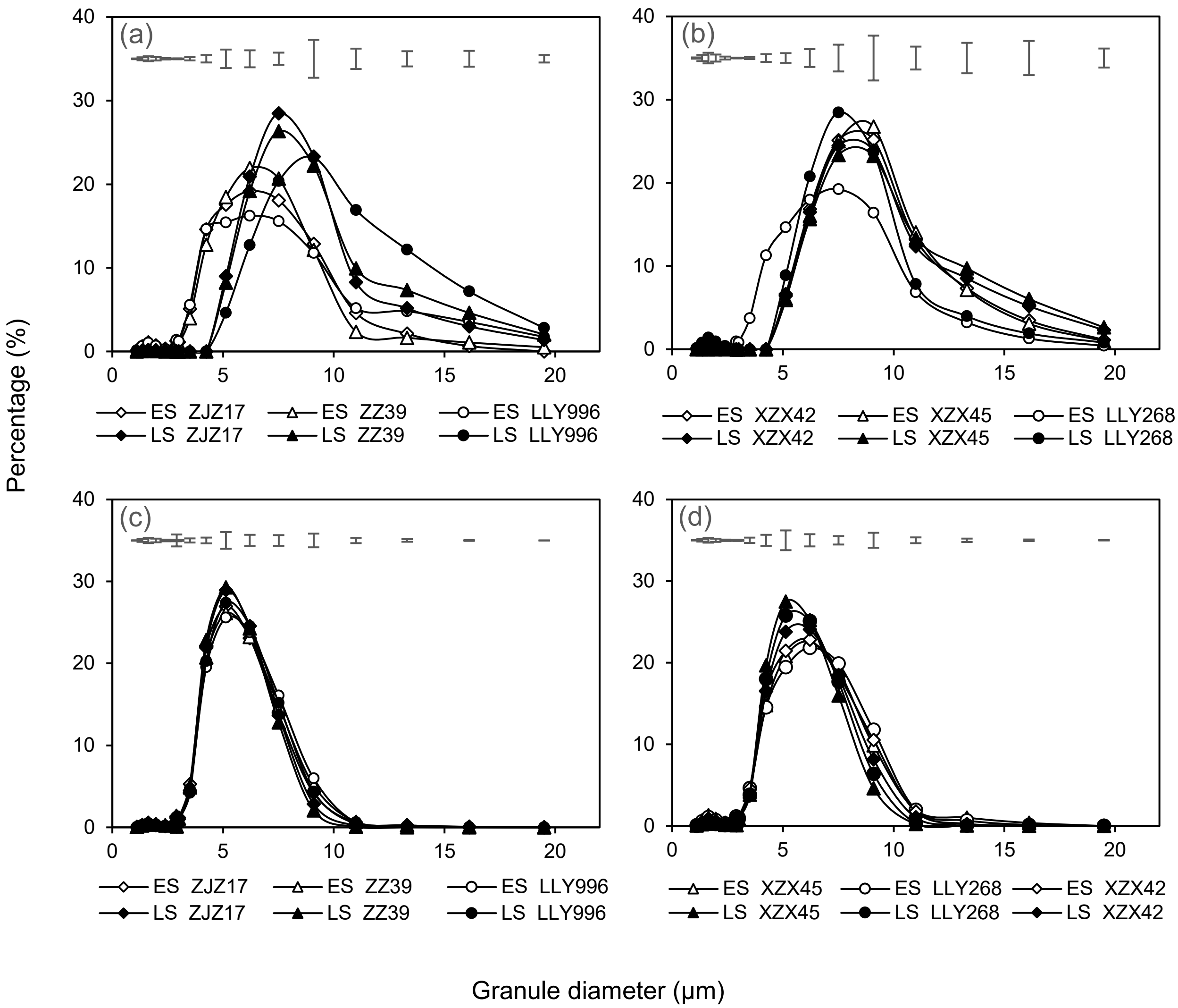

Figure 4: The size distributions of starch granules of high amylose content varieties (a and c) and low amylose content varieties (b and d) in 2016 (a and b) and 2017 (c and d) at maturity. Bars show LSD (0.05) values

LLY996, Luliangyou 996; ZZ39, Zhongzao 39; ZJZ17, Zhongjiazao 17; XZX42, Xiangzaoxian 42; XZX45, Xiangzaoxian 45; LLY268, Lingliangyou 268. ES: early season, LS: late season.

4 Discussion

Amylose content varied with different rice varieties in this study. The environment can influence crop grain quality [24], and particularly, the amylose content and the temperature and incident radiation were different in ES and LS [25]. For nonwaxy rice amylose content is higher in LS than ES [26]. Liu et al [19] showed that incident radiation had a significant positive correlation with amylose content and Kumar et al. [27] observed that the apparent amylose content decreased significantly with increased radiation. Our study showed that the mean temperatures in ES and LS were, respectively, 27.3ºC and 21.3ºC in 2016 and 27.4ºC and 22.7ºC in 2017 (Figs. 1(c),1(f)), the solar incident radiation higher 29% and 24% in ES than in LS in 2016 and 2017, respectively (Fig. 2). The amylose content of HACV was lower in LS compared to ES, while the opposite relationship was observed in LACV (Fig. 3). The temperature is one of major reasons for this result: high temperature at the grain-filling stage increased the amylose content in HACV, while the middle and low amylose content varieties have higher amylose content in low temperature conditions [5,28].

Abeysekera et al. [2] showed that rice with an intermediate level of amylose and hybrid rice with high amylose content had relatively stable amylose content over different seasons. For HACV in our study, the two inbred rice varieties had the same variation in ES and LS in both years, the hybrid rice LLY 996 had significantly lower amylose content in ES than in LS in 2016, and there was a non-significant decrease in LS compared to ES in 2017. In LACV, amylose content in the hybrid rice LLY 268 was significantly higher in LS than in ES in both years, and the differences in amylose content of two inbred rice varieties in ES and LS were slightly different in 2016 and 2017. Our results suggested that amylose contents in inbred rice varieties in HACV and hybrid rice varieties in LACV between ES and LS had relatively stable variation. However, because meteorology, rainfall, sunshine duration, and other factors function directly in determining amylose content and starch granule parameters, physiological changes during the filling stage still need to be studied.

The distribution of starch granule particle size of rice had a single peak, similar to Xu et al. [13], and particle size ranged from 1.11 μm to 19.5 μm (Fig. 4). This result contradicts some other reports where starch granule size ranged from 1-10 μm [29-31]. In our study, the mean starch granule particle size was higher in LS compared to that in ES in 2016, however, the mean starch granule particle size decreased in 2017 with all rice varieties (Tab. 1). The percentage of large starch granules (d > 6.21 μm in ZJZ17, d > 7.51 μm in ZZ39 and LLY996) increased 33%, 34%, and 40% in LS compared to ES for ZJZ17, ZZ39, and LLY996, respectively (Fig. 4(a)), and the percentage of starch granules (d > 6.21 μm in LLY268, d > 13.31 μm in XZX42 and XZX45) increased in LS compared to ES in 2016 (Fig. 4(b)). All rice varieties with diameter 4.24-6.21 μm had a higher percentage in LS than in ES in 2017 (Figs. 4(c), 4(d)).

The daily mean and maximum temperatures after flowering were higher in ES than LS, except for 7-10 DAF in 2016 and 5-10 and 13-14 DAF in 2017. The temperature differences between 2016 and 2017 occurred at 5-6 and 13-14 DAF, with ES compared to LS. The daily maximum and mean temperatures were higher by 4.9-12.1ºC and 3.2-11.9ºC, respectively, in 2016, while they were lower by 0.2-2.6ºC and 1.3-5.7ºC, respectively, in 2017. Starch granules have different sizes and types in different periods after flowering in wheat [32], which may be similar to rice. Our results suggested that lower temperatures during 10-14 DAF increased the proportion of larger starch granules (d > 6.21 μm), and higher temperature at 5-6 DAF increased the proportion of starch granules with diameter 4.24-6.21 μm. Which are differently with that high temperatures during the heading period have been shown to increase the average diameter of starch granules and the proportion of large starch granules (d >2.6 μm) [10]. Zhang et al. [7] showed that high temperature will change the structure of starch granules, but the specific changes are not well understood and must be determined in a future study.

5 Conclusion

Our study showed that amylose content decreased in LS compared to ES for HACV, and the opposite trend occurred in LACV in both years. The mean starch granule particle size was higher in LS compared to that in ES in 2016, however, the mean starch granule particle size decreased in 2017 with all rice varieties. Our data suggested that higher temperatures in ES increased and decreased the amylose content with HACV and LACV, respectively. amylose contents in inbred rice varieties in HACV and hybrid rice varieties in LACV between ES and LS had relatively stable variation. Temperatures at 5-15 DAF are important for the formation of starch granules: lower temperatures during 10-14 DAF increased the proportion of larger starch granules (d > 6.21 μm, partly with d > 13.3 μm), and higher temperatures at 5-6 DAF increased the proportion of starch granules with diameter 4.24-6.21 μm.

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