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Effects of irrigation schemes on the components and physicochemical properties of starch in waxy wheat lines

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Abstract: The waxy wheat shows special starch quality due to low amylose content. However, less information is available concerning the physicochemical properties of starch in different waxy wheat under different irrigation. In this study, two wheat near-isogenic lines (NILs) and a normal wheat cultivar were used to investigate the contents, size distribution and crystallinity of starch by biochemical methods, laser-diffraction and X-ray diffraction analysis. The amylose content in wheat grains was the lowest in waxy wheat lines, SJZ8-N, followed by the partly waxy wheat lines, SJZ8-P, and the highest in the normal wheat, SJZ8, with significant differences among wheat lines. Waxy wheat starch had more B-type granules and a higher degree of crystallinity than normal wheat starch, with the order as SJZ8-N > SJZ8-P > SJZ8. When compared with the conventional and water-saving irrigation, the rainfed treatment showed the lowest starch content, amylose content (except SJZ8-N), amylopectin content and relative crystallinity in the three wheat lines indicating that water deficiency was not benefited starch accumulation and crystal formation in wheat grains. It was concluded that (1) wheat lines not only differed in amylose content but also in size distribution and crystallinity of starch; (2) irrigation markedly influenced the physicochemical characteristics of wheat starch; therefore, the irrigation schemes could be adjusted to achieve high-quality wheat production.

Keywords: *Triticum aestivum* L.; endosperm; water deficit; distribution of granule size; polysaccharide

Starch is the major storage component of wheat endosperm, accounts for 65–75% of dry weight in grains. Wheat starch is composed of amylose and amylopectin, and the content and amylose/amylopectin (Am/Ap) ratios are common indexes to evaluate the starch quality (Ai and Jane 2016). The wheat grain generally possesses two distinct starch granule types, named A-type and B-type starch granules (Peng et al. 1999). The number and volume of starch granules vary among wheat cultivars differing in compositions of amylose and amylopectin. Hence, the starch particle distribution has a significant effect on the rheological and baking properties of wheat flour (Zhong et al. 2016). Moreover, starch granules in the wheat endosperm are semi-crystalline structures with the degree of crystallinity varying from 9% to 40% for different wheat starches. The crystalline property was attributed to the hierarchical organisation of amylopectin, while amylose formed the amorphous regions (Kozlov et al. 2007).

Amylose content is genetically controlled by *Wx* genes which encode waxy proteins (i.e., granule-bounded starch synthase). Common wheat usually has three waxy proteins encoded by the *Wx-A1*, *Wx-B1* and *Wx-D1* genes, respectively, and waxy wheat lack all three Wx proteins. When one or two

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Table 1. The relationship between irrigation date, development period and BBCH code

Irrigation treatment	Irrigation date	Development period	The code of the BBCH development phase
	November 25, 2019	before-wintering	25
Conventional irrigation (W2)	March 25, 2020	jointing	32
	May 3, 2020	anthesis	60
Water-saving irrigation (W1)	March 25, 2020	jointing	32
No irrigation (W0)	–	–	–

Wx genes are non-functional, the wheat cultivars are called partially waxy wheat (Jaksics et al. 2020). Starch from two waxy wheat exhibited different physicochemical properties, such as particle size, pasting viscosity, swelling power, gelatinisation enthalpy and retrogradation enthalpy (Purna et al. 2015). The proportion of long amylopectin chains was the main factor influencing the physicochemical properties of waxy starch (Li et al. 2020). The total starch content, grain yield and average kernel weight of the waxy wheat were lower than those of the normal wheat at maturity, which was mainly due to the reduced conversion of sucrose to starch in the late grain filling stage (Zi et al. 2018).

Studies have shown that the physicochemical properties of starch are both genetically and environmentally controlled. Nitrogen rate and water deficit caused significant changes in starch content and particle size distribution of wheat and then altering the physicochemical properties of starch (Xia et al. 2020). Post-anthesis waterlogging modified the expressions of starch synthase encoding genes, which finally affected the pasting properties of wheat starch (Zhou et al. 2018). Post flowering shading stress affected the particle size distribution and starch components, resulting in the changes of gelatinisation, relative crystallinity and thermodynamic properties of wheat starch (Liu et al. 2017). Despite a lot of research performed on wheat starch, less information is available on starch in waxy wheat under different irrigation. The objectives of the present work were to study the physicochemical properties of starch in wheat near-isogenic lines (NILs) and to test the hypothesis that the impacts of irrigation schemes on the starch quality of wheat flour were related to the modified size distribution and crystallinity of starch granules in wheat endosperm.

MATERIAL AND METHODS

Experiment description. Two wheat (*Triticum aestivum* L.) NILs, SJZ8-N (a fully waxy wheat line, *Wx-A1*/*Wx-B1*/*Wx-D1* null) and SJZ8-P (a partly waxy wheat line, *Wx-D1* null), and the recurrent parent SJZ8 (a widely-grown wheat cultivar) were used in this experiment. This experiment was conducted at the experiment station (37°N, 116°E) of the Institute of Agricultural Science, Dezhou, China, during the wheat growing season of 2019–2020. Three irrigation schemes were used: conventional irrigation (W2); water-saving irrigation (W1), and rainfed irrigation (W0). The irrigation time is shown in Table 1. The amount of irrigation water applied to every stage was 75 L/m^2 (equivalent to 75 mm rainfall). The experiment was a 3 × 3 (three lines and three levels of irrigation) factorial randomised block design with three replicates, and the plot dimension was 4 × 3 m^2. Diseases were controlled chemically at the flag-leaf and filling stage (BBCH 40 and 70, Zadoks et al. 1974) to avoid yielding loss. The irrigation schemes and monthly rainfall during the wheat growing season are presented in Figure 1. The mean temperatures for the growing season are

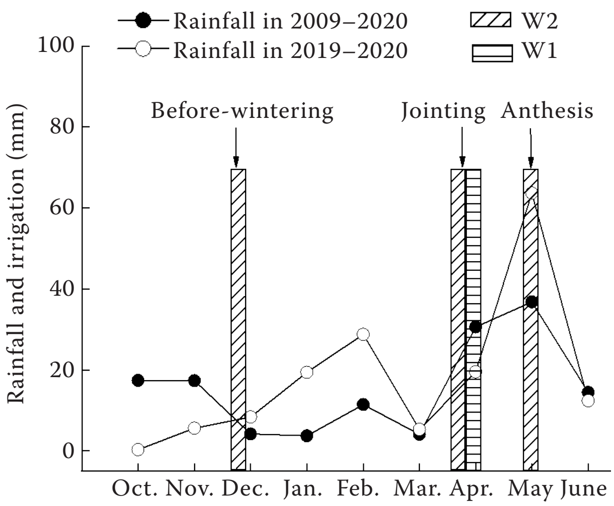

Figure 1. The rainfall and irrigation during the wheat-growing season. W2 – conventional irrigation; W1 – water-saving irrigation;

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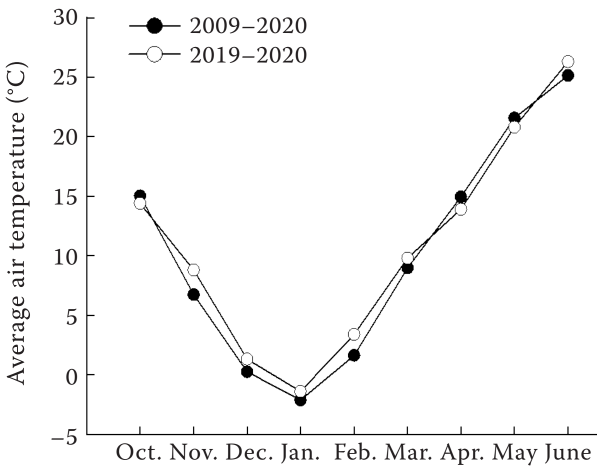

Figure 2. The average temperature during the wheat-growing season

presented in Figure 2. The soil moisture content is shown in Figure 3.

To ensure the uniform grain-filling duration, wheat spikes (about 100 ears in each replication) flowering on the same day were labeled for sampling.

Starch isolation and purification. The isolation and purification of wheat starch were performed according to Peng et al. (1999).

Determination of total starch and amylose content. The total starch content of wheat grains was determined by the Solarbio total starch assay kit (BC0700). Amylose content was determined by the iodine binding colorimetric method (Wang et al. 2015). Amylopectin content was calculated by subtracting amylose content from total starch content.

Granule size analysis. Granule size characteristics of starch were determined by a Microtrac S3500 laser-diffraction particle-size analyser (Microtrac Inc., Florida, USA) according to the method of Zhou et al. (2018).

X-ray diffraction analysis. X-ray diffraction (XRD) was performed using an X-ray diffractometer (Bruker AXS D8 Advance, Frankfurt, Germany) at 40 kV and 40 mA. The degree of crystallinity was calculated using XRD analysis software (Hayakawa et al. 1997).

Scanning electron microscopy. The surface topography of wheat starch granules was observed by a Merlin Compact scanning electron microscopy (Carl Zeiss AG, Jena, Germany) according to the method of Zhang et al. (2013).

Statistical analysis. Analysis of variance was performed with the SPSS statistical analysis package (version 21, IBM, Armonk, USA).

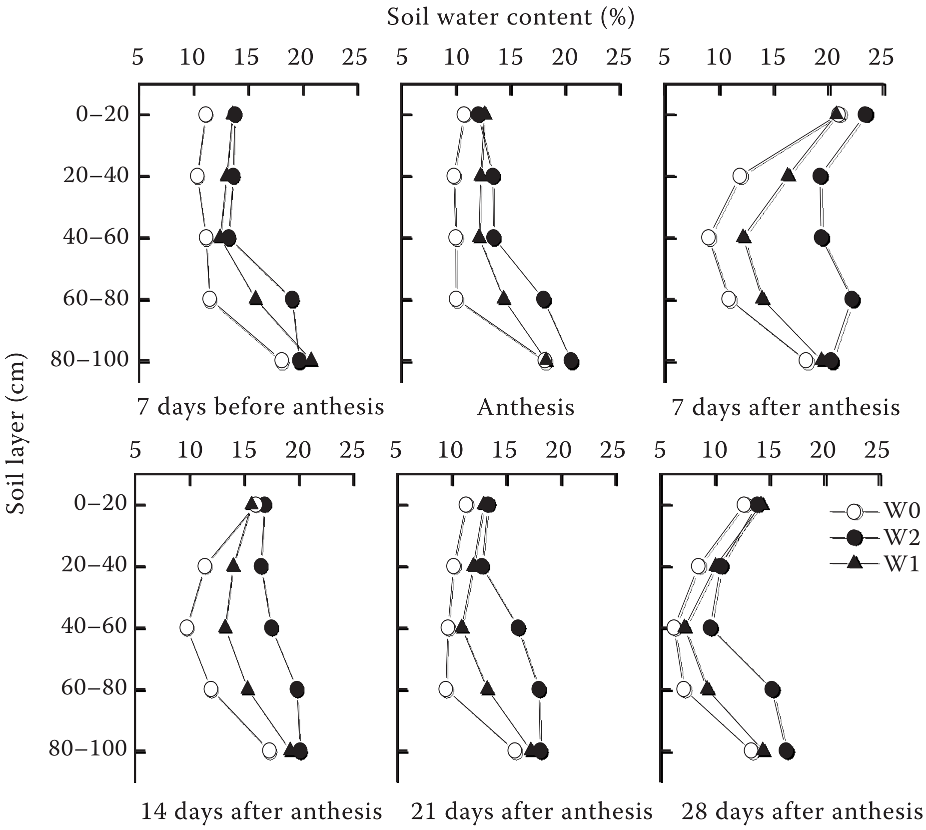

Figure 3. The soil moisture during the wheat growth period. W0 – rainfed irrigation; W2 – conventional irrigation; W1 – water-saving irrigation

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Table 2. Effects of irrigation pattern on starch and its component contents in wheat grains

Cultivar	Treatment	Starch content	Amylose content	Amylopectin content	Am/Ap ration
			(%)		
SJZ8-N	W2	67.89 ± 1.75[ab]	0.67 ± 0.18[f]	67.22 ± 1.91[a]	0.010 ± 0.003[c]
	W1	65.64 ± 1.17[bc]	1.11 ± 0.12[ef]	64.54 ± 1.14[b]	0.017 ± 0.002[c]
	W0	64.79 ± 0.77[c]	1.52 ± 0.19[e]	63.27 ± 0.77[b]	0.024 ± 0.003[c]
SJZ8-P	W2	67.75 ± 1.63[ab]	15.54 ± 0.21[c]	52.21 ± 1.66[c]	0.298 ± 0.011[b]
	W1	66.11 ± 1.46[bc]	14.79 ± 0.26[cd]	51.32 ± 1.34[cd]	0.288 ± 0.007[b]
	W0	65.03 ± 1.02[c]	14.23 ± 0.63[d]	50.80 ± 1.64[cd]	0.280 ± 0.021[b]
SJZ8	W2	69.22 ± 0.31[a]	19.40 ± 0.54[a]	49.83 ± 0.84[cd]	0.389 ± 0.017[a]
	W1	68.95 ± 1.36[a]	19.21 ± 0.67[a]	49.73 ± 1.99[cd]	0.386 ± 0.028[a]
	W0	67.34 ± 1.07[ab]	18.14 ± 0.53[b]	49.21 ± 1.21[d]	0.369 ± 0.017[a]

Data are expressed as mean ± standard deviation from three replicates. Different letters in each column indicate significant differences (P < 0.05). SJZ8-N – fully waxy wheat line; SJZ8-P – partly waxy wheat line; SJZ8 – widely-grown wheat cultivar; W2 – conventional irrigation; W1 – water-saving irrigation; W0 – rainfed irrigation

RESULTS

Changes in starch content and composition. As shown in Table 2, the waxy wheat lines SJZ8-N had significantly lowest amylose content and Am/Ap ration and significantly highest amylopectin content, respectively, in comparison to the other two (P < 0.05). The difference of total starch content in the three wheat lines varied with different water treatments. SJZ8 had the significantly highest starch content in comparison to the other two under W1 (68.95% *vs*. 65.64% and 66.11%, P < 0.05) and W0 treatments (67.34% *vs*. 64.79% and 65.03%, P < 0.05), while there was no significant difference among the three wheat lines under W2 (P > 0.05).

The starch content and composition were also affected by irrigation conditions. The content of total starch, amylose (except SJZ8-N) and amylopectin in wheat grains showed an increasing trend with the increase of irrigation times. As compared to W0, the amylose content in SJZ8 and SJZ8-P and the total starch content in SJZ8-P and SJZ8-N were significantly increased, whereas the amylose content in SJZ8-N was markedly decreased under W2 treatment.

Starch particle size distribution. The percent volume distributions of starch granules in three wheat lines showed a two-peak curve with the mean particle diameter of 3.9–4.6 μm and 18.5–22.0 μm at each peak, whereas there were differences in the peak size (Figure 4). The volume percentages of

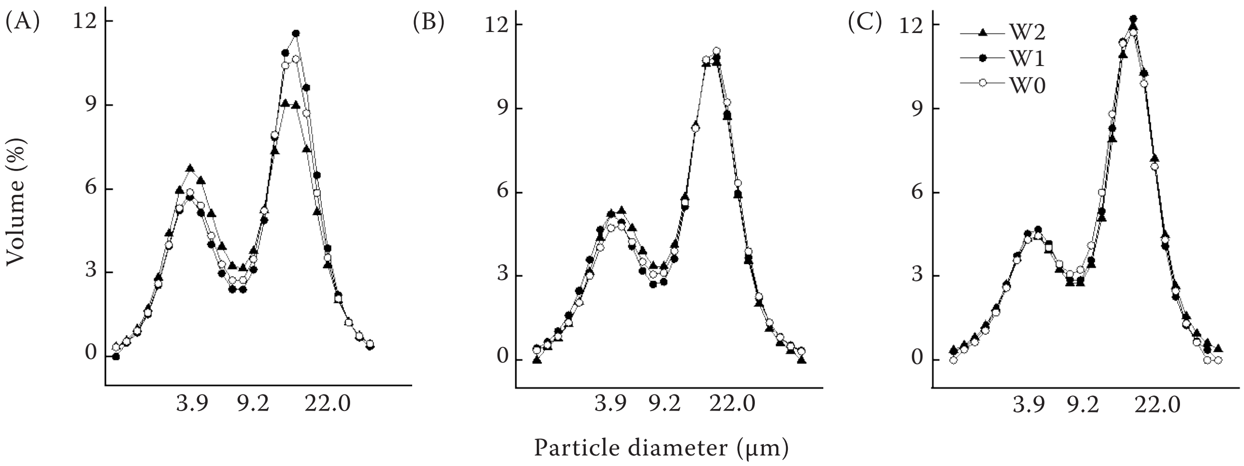

Figure 4. The effects of irrigation pattern on the distribution of starch granule size in wheat grains. (A) fully waxy wheat line (SJZ8-N); (B) partly waxy wheat line (SJZ8-P) and (C) widely-grown wheat cultivar (SJZ8). W2 – conventional irrigation; W1 – water-saving irrigation; W0 – rainfed irrigation

Table 3. Volume distribution of wheat starch granules (%) under different irrigation patterns

Cultivar	Treatment	Starch particle diameter (μm)		Average particle size (μm)
		< 9.25	> 9.25	
SJZ8-N	W2	45.00 ± 0.30^{a}	55.00 ± 0.30^{f}	14.10 ± 0.48^{c}
	W1	37.16 ± 0.15^{c}	62.84 ± 0.15^{d}	16.07 ± 0.70^{ab}
	W0	39.61 ± 0.36^{b}	60.39 ± 0.36^{e}	15.16 ± 0.34^{bc}
SJZ8-P	W2	37.96 ± 0.36^{c}	62.04 ± 0.36^{d}	15.95 ± 1.36^{ab}
	W1	37.41 ± 0.10^{c}	62.59 ± 0.10^{d}	15.84 ± 0.56^{ab}
	W0	36.20 ± 1.09^{d}	63.80 ± 1.09^{c}	15.92 ± 0.46^{ab}
SJZ8	W2	33.31 ± 0.77^{e}	66.69 ± 0.77^{b}	16.90 ± 0.73^{a}
	W1	33.25 ± 0.23^{e}	66.75 ± 0.23^{b}	16.70 ± 0.79^{ab}
	W0	32.26 ± 0.26^{f}	67.74 ± 0.26^{a}	17.49 ± 1.35^{a}

Data are expressed as mean ± standard deviation from three replicates. Different letters in each column indicate significant differences ($P < 0.05$). SJZ8-N – fully waxy wheat line; SJZ8-P – partly waxy wheat line; SJZ8 – widely-grown wheat cultivar; W2 – conventional irrigation; W1 – water-saving irrigation; W0 – rainfed irrigation

B-type (< 9.25 μm) starch granules in SJZ8-N were 37.16–45.0%, i.e., much higher than those in SJZ8-P (36.2–37.96%) and SJZ8 (32.26–33.31%); whereas those of A-type (> 9.25 μm) were quite the opposite (Figure 4, Table 3). This indicated that waxy wheat had a relatively large proportion of B-type starch granules, and normal wheat was mainly A-type. A similar trend was also observed in the average particle size of starch. The average starch size of SJZ8 was significantly higher than that of SJZ8-N under W2 (16.90 μm and 14.10 μm, respectively; $P < 0.05$) and W0 (17.49 μm and 15.16 μm, respectively; $P < 0.05$), except for the non-significant difference among three lines under W1.

As compared to W0, the volume percentages of B-type starch granules were significantly increased (33.31–45.00% *vs.* 32.26–39.61%, $P < 0.05$), and that of A-type was markedly decreased under W2. This result indicates that the soil water deficit is favourable for the increasement of large starch granules in three wheat lines. However, the volume percentages of B-type starch granules in waxy wheat were the lowest under W1, and those in partly waxy and normal wheat were the lowest under W0, indicating the different responses of wheat lines to water stress. The effect of irrigation on starch particle size also varied depending on wheat genotype. The average particle size in SJZ8-N under W1 was significantly higher than under W2; nevertheless, there was no significant difference among irrigation treatments in SJZ8-P and SJZ8.

The crystallinity of wheat starches. Starches from three wheat lines presented the similar characteristic

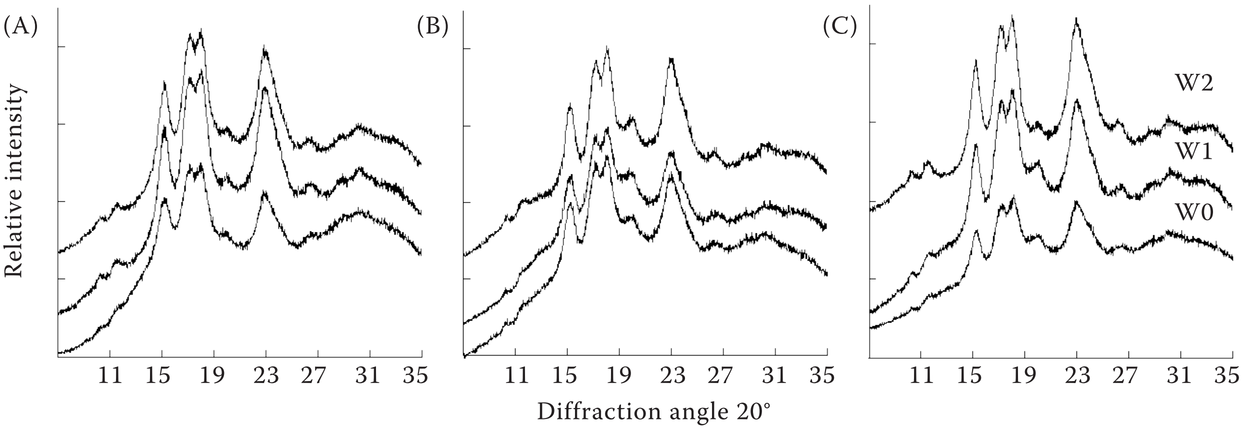

Figure 5. X-ray diffraction patterns of wheat starch. (A) fully waxy wheat line (SJZ8-N); (B) partly waxy wheat line (SJZ8-P), and (C) widely-grown wheat cultivar (SJZ8); W2 – conventional irrigation; W1 – water-saving irrigation; W0 – rainfed irrigation

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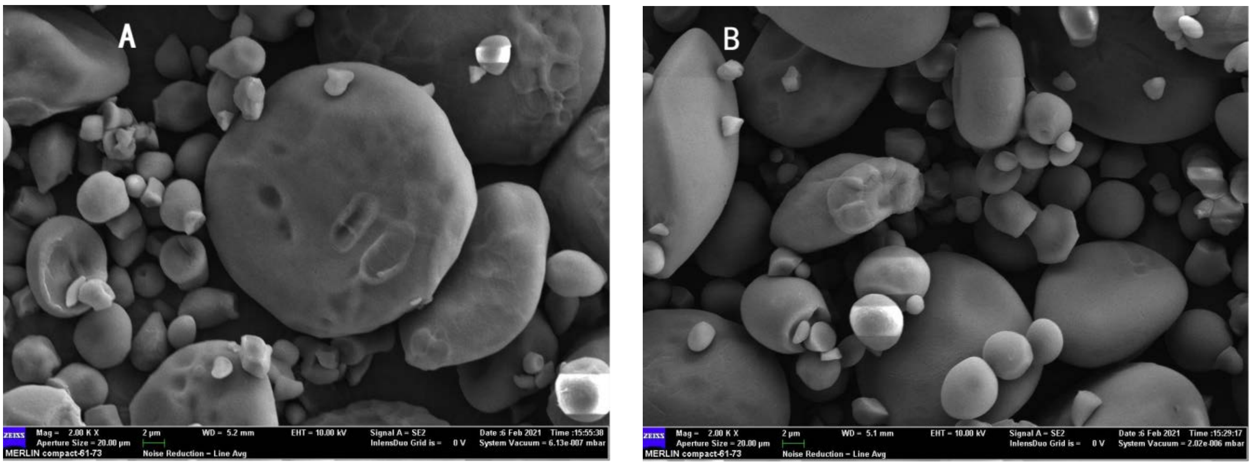
Table 4. Effects of different irrigation patterns on relative crystallinity of wheat starch (%)

Treatment	Cultivar		
	SJZ8-N	SJZ8-P	SJZ8
W2	40.10 ± 0.46[a]	37.73 ± 0.55[a]	35.23 ± 0.25[a]
W1	39.20 ± 0.95[ab]	37.93 ± 0.42[a]	35.17 ± 0.40[a]
W0	38.17 ± 0.65[b]	35.03 ± 0.45[b]	31.47 ± 0.60[b]

Data are expressed as mean ± standard deviation from three replicates. Different letters in each column indicate significant differences ($P < 0.05$). SJZ8-N – fully waxy wheat line; SJZ8-P – partly waxy wheat line; SJZ8 – widely-grown wheat cultivar; W2 – conventional irrigation; W1 – water-saving irrigation; W0 – rainfed irrigation

diffraction peaks at 2θ = 15° (singlet), 17–18° (doublet) and 23° (singlet) under different irrigation treatment, showing the typical characteristics of an A-type diffraction pattern (Figure 5). Compared with SJZ8-P and SJZ8, the peak at 2θ = 20° was not prominent in waxy wheat SJZ8-N. The relative crystallinity of waxy wheat starch was generally higher than that of normal wheat starch, which was ordered as follows: SJZ8-N > SJZ8-P > SJZ8 (Table 4). Irrigation treatments also had a significant effect on the relative crystallinity, but the influence varied among lines. The relative crystallinity under W1 and W2 were increased by 5.06–11.95% and 2.70–11.76% when compared with W0, respectively.

Morphology of the starches. Scanning electron micrographs of wheat starch are shown in Figure 6. All of the different waxy wheat lines contained two different populations of granules, that is, large disk-like granules (type-A) and small spherical granules (type-B). The starch granule surfaces of normal and waxy wheat starches exhibited similar features, but there were still some differences. The surface of some large starch granules in waxy wheat was not smooth with a certain degree of wrinkles or grooves. In addition, some pores were also visible on some large granules. The surface of starch granules in normal and partly waxy wheat was generally smooth, with only a few indentations being visible on starch granules.



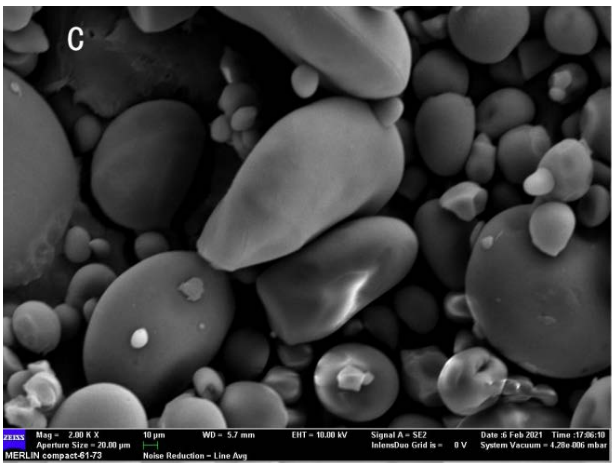


Figure 6. Scanning electron micrographs of wheat starch granules. (A) fully waxy wheat line (SJZ8-N); (B) partly waxy wheat line (SJZ8-P), and (C) widely-grown wheat cultivar (SJZ8)

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Table 5. Pearson correlation analysis derived from the values of 27 repetitions (n = 27) on starch properties of wheat grain

Starch content	Amylose content	Amylopectin content	Am/Ap ration	Starch particle		Average particle size	Crystallinity
				B–type	A–type		
0.527							
–0.357	–0.982**						
0.535	0.999**	–0.980**					
–0.274	–0.791*	0.808**	–0.800**				
0.274	0.791*	–0.808**	0.800**	–1.000**			
0.323	0.772*	–0.777*	0.782*	–0.979**	0.979**		
–0.247	–0.761*	0.782*	–0.776*	0.838**	–0.838**	–0.840**	

*P < 0.05; **P < 0.01

No significant differences in granule morphology were observed between wheat starches under different irrigation schemes (data not shown).

Correlation of starch components and physicochemical properties. There existed a close correlation of starch components to starch particle size and relative crystallinity in wheat grains (Table 5). Correlation analysis showed that amylose content and Am/Ap ration were significantly and negatively correlated with B-type starch granules and crystallinity, while significantly and positively correlated with A-type and average particle size, while oppositely for amylopectin content. Significant negative and positive correlation of B-type starch granules was recorded with average particle size and crystallinity, respectively, while the average granule diameter showed a significant and negative correlation with the crystallinity.

DISCUSSION

Morphology and physicochemical properties of starch in different waxy wheat. The deficiency of waxy protein in wheat endosperm may lead to the decrease of amylose content in grains, thus affecting the physicochemical properties of starch. In the present study, the amylose content in wheat grains was the lowest in the waxy wheat line, followed by the partially waxy genotype, and the highest in the normal variety, with significant differences among wheat lines. Waxy wheat starch contained a larger proportion of B-type granules than normal wheat starch, with the order as SJZ8-N > SJZ8-P > SJZ8. Similar results are obtained by Zhong et al. (2016), who concluded that the amylose content in flour gradually decreased with the loss of waxy protein in grain, and the volume percentage of the B-type granules in the waxy line was the highest, followed by that of the partially waxy lines and the wild line. Liu et al. (2017) also reported that the volume, surface area and number distribution of B starch granules in waxy wheat are higher than those in normal wheat. Nevertheless, Zhang et al. (2013) reported that waxy wheat starch had a smaller proportion of B-type granules and a larger average granule diameter than normal wheat starch. Jung et al. (2015) also reported no significant differences in A-type and B-type starch granule size between waxy and wild-type wheat cultivars. This difference could be due to the different wheat cultivars or the method of starch extraction.

The relative crystallinity was much higher in the waxy lines than in the normal wheat cultivar in this study, which was consistent with previous reports (Jung et al. 2015). It is due to the fact that waxy starch has more amylopectin molecules and can form more double helices, which leads to higher relative crystallinity (Wang et al. 2015).

In this study, the starch granule surfaces of waxy and normal wheat starches exhibited similar features, but there were still some differences. Similar results are obtained by Zhang et al. (2013), who concluded that the morphologies and internal structure of starch granules were found to be similar in waxy and normal wheat starch. On the other hand, some studies proposed that the surface of waxy wheat starch granules was not smooth; more holes were observed on the surface (Chen et al. 2015).

Effects of irrigation pattern on starch characteristics of wheat grains. Soil moisture is one of the important environmental factors affecting the starch composition and characteristics of wheat (Singh et al. 2008, Dai et al. 2016). It has been reported that there exists a close correlation between irrigation schemes and properties of starch content, particle size distribution and relative crystallinity of wheat grains. The suitable soil water is found to be conducive to the maintenance of related indexes, which can improve processing quality. In this study, the content of total starch, amylose (except SJZ8-N) and amylopectin in wheat grains showed an increasing trend with the increase of irrigation times, indicating that water deficiency was not favourable for the accumulation of starch of wheat grain. This was in agreement with previous conclusions that drought stress significantly reduced the starch accumulation and shortened growth duration in wheat but did not affect significantly on the Am/Ap ratio (Song et al. 2017).

In wheat endosperm, starch is present as granules, and the granule size distribution is also closely related to the environment (Shevkani et al. 2016). Under the W2 condition, the volume percentages of B-type starch granules in wheat were significantly increased, and that of A-type was markedly decreased when compared with those under the W0 condition. Similar results were also reported by Tong et al. (2021), who concluded that water-saving irrigation was beneficial to the increase of B-type starch content when compared with non-water control.

In this study, irrigation treatments did not change the crystal type of wheat starch but had a significant effect on the relative crystallinity, which was significantly higher under W2 and W1 treatment when compared with those under W0. This was in agreement with previous conclusions that post-anthesis drought or non-irrigation could decrease the relative crystallinity of starch (Tong et al. 2021). This might be due to the increased proportion of B-type granules, leading to increased amylopectin with its branch chains contributing to the crystal structure (Kim et al. 2010). The opposite result showed that drought stress significantly increased the crystallinity of starch as compared to irrigation (Song et al. 2017). The above inconsistent results might be due to the difference in irrigation pattern and genotype and remains to be investigated in the future.

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