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Effects of shading after pollination on kernel filling and physicochemical quality traits of waxy maize

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ABSTRACT

Understanding the effects of shading after pollination on kernel filling and physicochemical properties of waxy maize could improve kernel quality. Experiments were conducted to investigate the effects of shading (30% and 50% light deprivation, taken plants without shading as control) on kernel weight, size, and physicochemical properties during kernel development in 2013 and 2014 using two waxy maize varieties (Suyunuo 5 and Yunuo 7). Results indicated that shading reduced kernel filling rate and decreased kernel size and weight, and the influence was large under severe light deprivation conditions. The large reduction in kernel weight and volume of Suyunuo 5 in response to shading indicated that it was more sensitive to shading than Yunuo 7. Starch content was reduced and protein content was increased by shading, especially under severe shading after 22 days after pollination (DAP). The iodine binding capacity of Yunuo 7 was not affected by shading at fresh and maturity stages but was gradually decreased by shading at the newly formed stage, while the values for Suyunuo 5 were decreased at 7 and 40 DAP only by moderate shading and were similar among three treatments at 22 DAP. Severe shading decreased crystallinity at all kernel development stages, whereas moderate shading decreased crystallinity at fresh stage and increased it at mature stage for Suyunuo 5. Crystallinity in Yunuo 7 was increased by shading at 7 DAP and decreased by shading at 40 DAP, whereas the value at 22 DAP was increased by moderate shading and reduced by severe shading, respectively. The average gelatinization temperatures at different stages were decreased by shading and showed no difference between two shading levels. The retrogradation percentage at 7 DAP for both varieties was increased by shading. The value at 22 DAP was increased by moderate shading for Suyunuo 5 and decreased by severe shading for Yunuo 7, respectively. The retrogradation percentage at 40 DAP was decreased by shading treatments for Suyunuo 5 and reduced only by moderate shading for Yunuo 7. Peak viscosity was decreased by shading at fresh stage for Yunuo 7 and at maturity for Suyunuo 5. In conclusion, shading after pollination inhibited kernel filling of waxy maize and reduced paste viscosity quality, but kernel retrograde quality, crystallinity and starch iodine binding capacity in response to shading were dependent on stage and variety.

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1. Introduction

Light intensity strongly influences crop photosynthesis, growth, development, and grain yield. Maize, a C_4 crop, needs high light intensity during its growth and development. Even in the summer, sunlight intensity cannot reach a saturation point for maize. Light deprivation decreased kernel set by decreasing photosynthate flux and increasing abscisic acid concentration [1]. Shading after pollination decreased the activity of enzymes involved in starch synthesis and reduced the accumulation of starch in the grain, thereby resulting in grain weight loss [2]. Low-sunlight frequently occurs during maize growth and delayed leaf growth, decreased photosynthetic capacity, degenerated anther tassels, prolonged the tasseling–silking interval, decreased ear setting percentage, reduced kernel dry weight, and accelerated leaf senescence, resulting in kernel yield loss [3,4].

Shading after pollination strongly influences grain quality. In maize, kernel peak, trough, and breakdown viscosities were decreased by reduction of the activities of enzymes involved in starch biosynthesis [2,5]. Shading after pollination also decreased kernel weight, starch content, endosperm cell number, and volume but increased embryo/endosperm ratio, protein content, and fat content [6]. Shading after pollination also increased protein content in barley [7], rice [8], and wheat [9]. However, the increase in protein content is dose-dependent, given that reduction in protein is not as pronounced as reduction in kernel weight [9,10]. In rice, shading reduced kernel filling rate and decreased kernel weight, kernel amylose content, and peak and breakdown viscosities [11]. Li et al. [12] observed that shading decreased the rate of wheat starch accumulation and the activities of enzymes involved in starch synthesis, thereby reduce the starch content in kernels. Shading also reduced the transport of soluble sugars stored in vegetative organs at pre-anthesis stages to kernel and decreased amylopectin content and peak viscosity. However, the effects of shading on trough viscosity and pasting temperature are dependent on the variety of maize [13].

Waxy maize is a special maize type whose starch is composed of nearly 100% amylopectin. Waxy maize can be harvested young [about 22 days after pollination (DAP)] for direct eating after boiling or at maturity (about 40 DAP) for use as viscous food material, thickener, and stabilizer. In a previous study, we found that 30% light deprivation after pollination significantly reduced the starch content and affect the physicochemical properties of fresh waxy maize kernel [14]. However, flour physicochemical properties at other kernel developmental stages (starch early forming stage and maturity) and kernel filling properties in response to weak light after pollination remain unknown. In addition, some researchers have observed that kernel weight and size as well as starch content and size gradually increased, protein content gradually decreased, and starch swelling, chain-length distribution, pasting, and thermal properties changed with maize kernel development [15–20]. Thus, understanding the dynamics of kernel physicochemical characteristics at various developmental stages under shading conditions could help improve kernel quality under low-sunlight conditions. In this study, a field experiment was conducted to investigate kernel filling, pasting, and thermal properties at different stages under moderate (30%) and severe (50%) light deprivation after pollination.

2. Material and methods

2.1. Plant material and growth conditions

Waxy maize varieties (Suyunuo 5 and Yunuo 7) planted over large areas in southern China were grown on the farm of Yangzhou University (Yangzhou, China) in 2013 and 2014. Seeds were sown on July 1 in both years. Plant density was 60,000 plant ha^{-1}, and plot area was 24 m^2. The plants were given a basal dressing of 500 kg ha^{-1} (commercial fertilizer, $N:P_2O_5:K_2O$, 15:15:15) during transplantation and a topdressing of 326 kg ha^{-1} (commercial urea, 46% N) during the jointing stage. Other agronomic operations were performed as for conventional high-yielding cultivation.

The plants were covered with black polyethylene netting that blocked approximately 30% (moderate shading) or 50% (severe shading) of the solar radiation above the canopy during kernel filling (from artificial pollination to maturity). Plants without shading were grown as controls. Nets were placed at least 500 cm higher above the ground to allow good ventilation. The microclimate conditions (CO_2 and temperature) for the control, moderate and severe shading conditions were identical except for light intensity [14], with average values (measured between 11:00 and 12:00 a.m. on 25 August, 3 September, and 16 September, 2013) 1642, 1129, and 832 μmol m^{-2} s^{-1}, respectively. Average growth temperature and total rainfall during kernel filling were 29.0 °C and 90 mm in 2013 and 25.0 °C and 310 mm in 2014.

2.2. Kernel yield determination

Five ears were harvested at each of 7, 15, 22, 30, and 40 DAP to determine kernel fresh weight (mg) and fresh volume (mg μL^{-1}) (determined by drainage). Kernel dry weight (after drying for 1 h at 130 °C and 48 h at 80 °C) was determined and water content (%) was calculated as (1 − dry weight/fresh weight) × 100. All measurements were performed in triplicate.

2.3. Nutrient content analysis

Kernel samples harvested at different stages were dried at 40 °C. After drying, kernel samples (100 g, dry basis) were ground finely and passed through a 100-mesh (0.149 mm) sieve for physicochemical analysis. Nitrogen content in kernel was determined by the Kjeldahl method [21], and protein content was calculated from nitrogen content (protein content = nitrogen content × 6.25). Starch contents in kernel were determined by the anthrone-sulfuric acid method [22]. All measurements were performed in triplicate.

2.4. Starch iodine staining

Starch was isolated by our previously described method [23]. Iodine staining parameters were measured following Fiedorowicz and Rebilas [24]. Spectra in the range of 500 nm to 700 nm were obtained for all samples using a UV–Vis spectrophotometer. Maximum absorption wavelength (λ_{max}) is the peak absorbance value over the range of wavelengths examined. Blue value is the absorbance value at 635 nm. Iodine binding capacity (IBC) is defined as the ratio of absorbance at 635 nm to 520 nm. All measurements were performed in triplicate.

2.5. X-ray diffraction pattern and crystallinity

X-ray diffraction patterns of the flour were obtained with an X-ray diffractometer (D8 Advance; Bruker-AXS, Germany) operated at 200 mA and 40 kV. The scanning region of the diffraction angle (2θ) ranged from 3° to 40° at step size of 0.04° and counting time of 0.6 s. Relative crystallinity (%) was calculated as the percentage of the sum of total crystalline peak areas relative to total diffractogram (sum of total crystalline and amorphous peak areas) using MDI Jade 6 software [25].

2.6. Pasting and thermal properties

The pasting and thermal properties of the flours were evaluated with a rapid visco analyzer (model 3D, Newport Scientific, Australia) and a differential scanning calorimeter (Model 200 F3 Maia, NETZSCH, Germany), respectively, following a previously described method [23], and measurements were performed in duplicate.

2.7. Statistical analysis

Owing to waterlogging and sunlight shortage caused by extended rainfall during maize growth in 2014, only the 2013 data were analyzed. Data were analyzed using DPS 7.05 [26] and Microsoft Excel 2010 [27]. Differences were evaluated by the least significant difference test at the 5% probability level.

3. Results

3.1. Kernel filling

Shading after pollination significantly influenced the kernel filling properties of waxy maize (Fig. 1). With kernel development, the kernel water content gradually decreased, and the dry weight gradually increased for all treatment groups. The kernel fresh weight and volume of Yunuo 7 gradually increased with kernel development. The kernel fresh weight and volume of Suyunuo 5 under the control condition also increased gradually, whereas those two parameters under shading conditions first rose and later fell, peaking at 30 DAP. The kernel water content of Suyunuo 5 was lower under shading than in the control, especially under severe shading. The kernel water content of Yunuo 7 was higher in the control than under shading before 22 DAP, but this difference disappeared at later kernel filling stages (22 DAP to 40 DAP). In comparison with plants under control conditions, those exposed to shading conditions showed lower kernel dry weight, fresh weight, and fresh kernel volume. The differences in these parameters increased with kernel development. The deficiency was more pronounced under severe shading, especially for Suyunuo 5.

3.2. Starch and protein content

The kernel protein content of all samples decreased with kernel development, and the decrease was sharp from 7 DAP to 15 DAP (Fig. 2). Shading increased the kernel protein content throughout the kernel filling stage. Compared with plants exposed to moderate shading, those exposed to severe shading exhibited higher protein content before 30 DAP (Suyunuo 5) and 15–22 DAP (Yunuo 7). However, the protein accumulation amounts (mg g^{-1}) under control and 30% and 50% shading for Suyunuo 5 and Yunuo 7 were 18.5, 18.6, 15.3 and 16.1, 17.9, 17.3, respectively, indicating that protein accumulation amount in response to shading was dependent on variety and light deprivation level. Kernel starch content sharply increased from 7 DAP to 22 DAP and then gradually increased with kernel development for all treatments. Moderate shading had no influence on Suyunuo 5 starch deposition throughout kernel development, whereas severe shading decreased starch deposition after the fresh stage (22 DAP to 40 DAP). Starch content of Yunuo 7 increased under shading conditions at early stage (15 DAP) but decreased after 22 DAP. The decrease became more pronounced under severe shading.

3.3. Starch staining

The blue value for all treatments decreased as kernel filling progressed, and the decrease was sharp from 7 DAP to 22 DAP for both varieties (Table 1). There was no evident effect of shading on the blue value of either variety. The λ_{max} values varied from 532.8 to 537.1 nm for Suyunuo 5 and 533.7 to 539.5 nm for Yunuo 7, indicating a waxy character. The IBC decreased sharply from 7 DAP to 22 DAP for both varieties. For Suyunuo 5, shading exerted no influence on IBC at 22 DAP, and the values at 7 and 40 DAP were decreased only by moderate shading. The IBC of Yunuo 7 was not affected by shading at fresh and maturity stages but was gradually decreased by shading at the newly formed stage (7 DAP).

3.4. Kernel X-ray diffraction pattern and crystallinity

Shading did not alter kernel X-ray diffraction patterns at any point during kernel development. As shown in Fig. 3, all samples presented a typical A-type diffraction pattern, with peaks at 2θ = 17°, 18°, 15°, and 23°. In addition, the peak intensity at 20° was very low, indicating a waxy character. The crystallinity of Suyunuo 5 gradually increased with kernel development. Severe shading decreased crystallinity at all kernel development stages, whereas moderate shading decreased crystallinity at fresh stage and increased it at mature stage for Suyunuo 5. Crystallinity in Yunuo 7 was increased by moderate and severe shading at 7–22 DAP and 7 DAP, respectively. Thereafter, the value was decreased by shading.

3.5. Thermal properties

Gelatinization enthalpy (ΔH_{gel}) changed with kernel development (Table 2). For Suyunuo 5, the ΔH_{gel} under the control condition initially increased and then decreased, whereas those under 30% and 50% shading gradually increased with

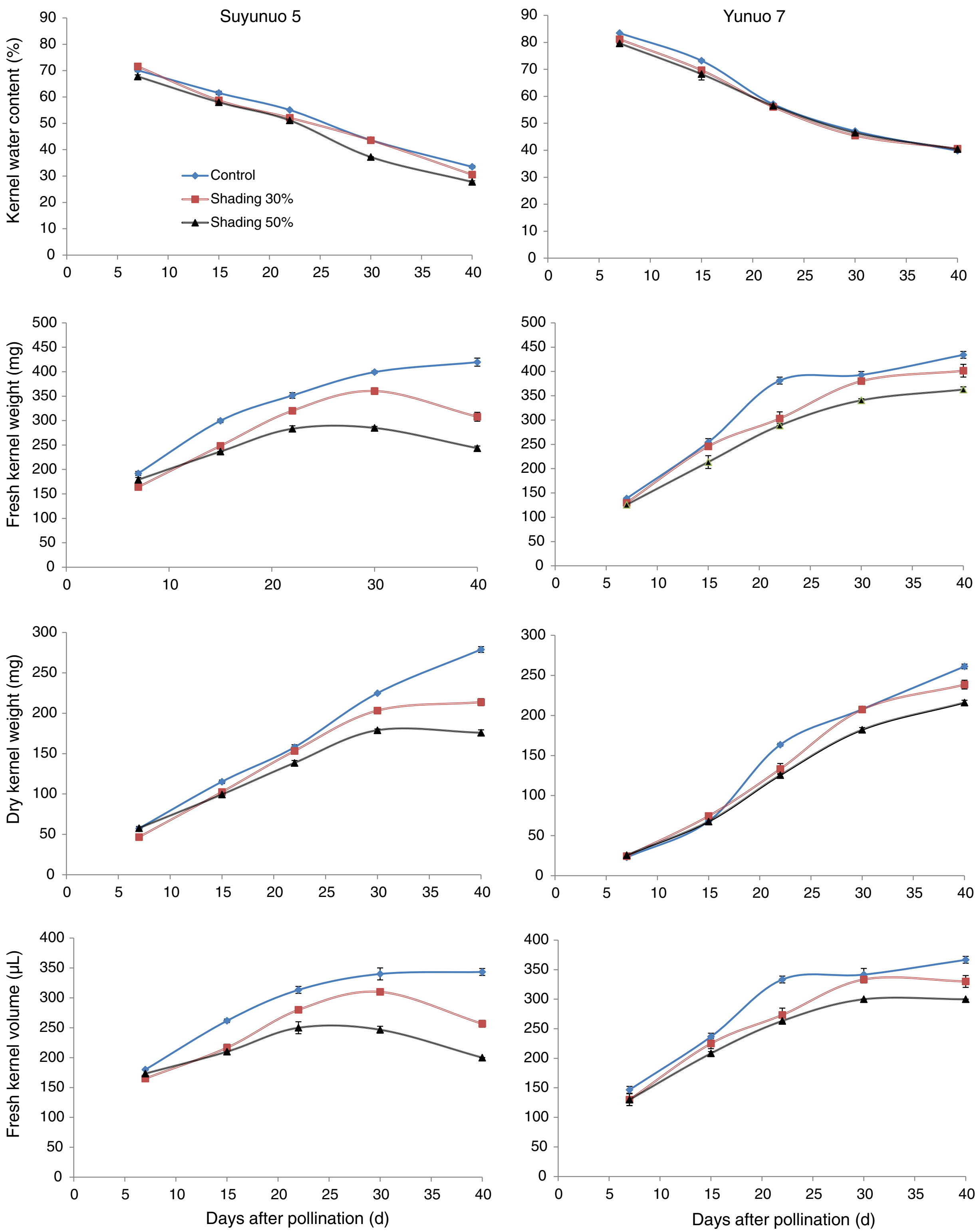

Fig. 1 – Effects of shading after pollination on kernel filling of waxy maize (2013).

kernel development. For Yunuo 7, the ΔH_{gel} under control and 50% shading initially increased and then decreased, whereas that under 30% shading gradually increased with kernel development. Taking the control as reference, moderate shading decreased ΔH_{gel} of Suyunuo 5 at 7 and 22 DAP and then increased it at maturity, whereas moderate shading increased ΔH_{gel} of Yunuo 7 only at maturity. Severe shading increased ΔH_{gel} at 7 and 40 DAP in Suyunuo 5 and at all stages in Yunuo 7. Kernel gelatinization temperatures gradually decreased with kernel filling. The effects of shading on gelatinization temperatures varied with kernel developmental stage and variety. For instance, peak temperature (T_p) of

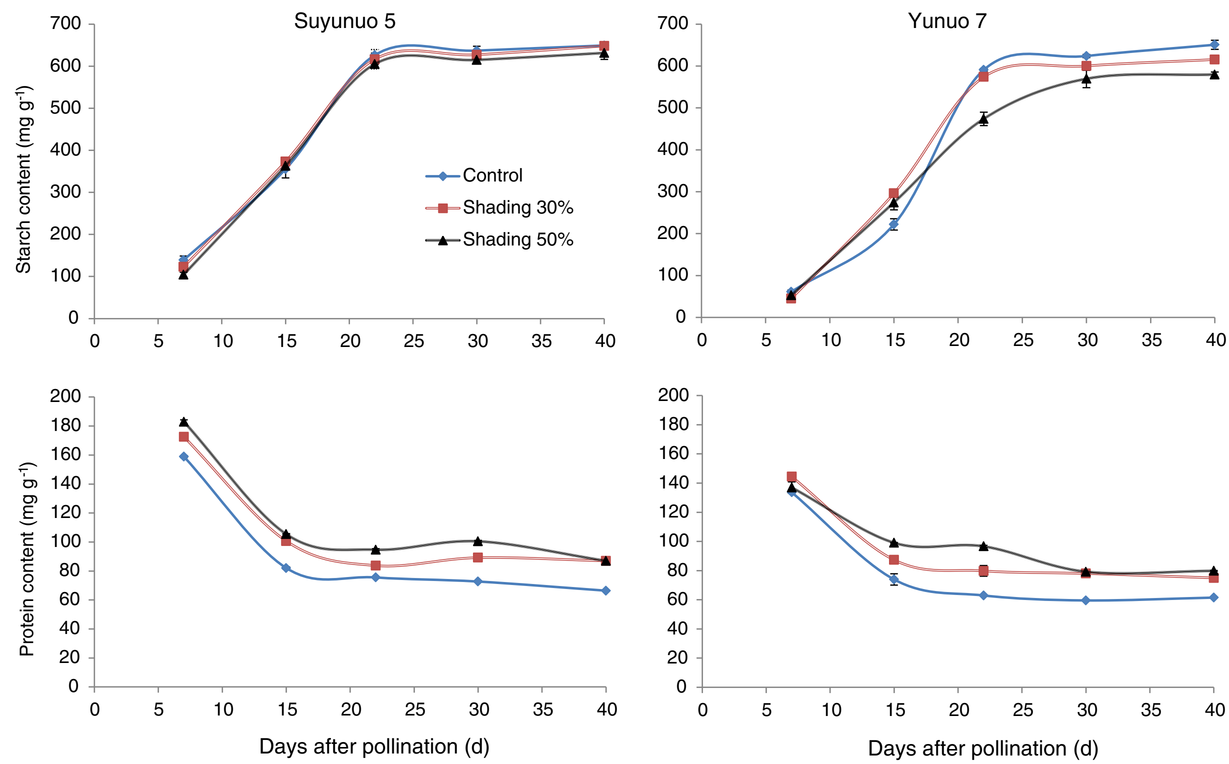

Fig. 2 – Effects of shading on starch and protein content of waxy maize during kernel development (2013).

Suyunuo 5 was not affected by shading at newly formed and fresh stages but was increased at mature stage. T_p of Yunuo 7 was decreased by shading at all stages, but no significant difference was observed between the two shading conditions at fresh and mature stages.

Table 1 – Effects of shading after pollination on starch iodine staining of waxy maize during kernel development[a] (2013).

Variety	DAP	Shading (%)	Blue value	Maximum absorption wavelength (nm)	Iodine binding capacity
Suyunuo 5	7	0	0.1640 a	537.1 bc	0.5554 d
		30	0.1576 a	535.8 cde	0.5329 e
		50	0.1576 a	536.0 cd	0.5538 d
	22	0	0.1392 bc	534.4 efg	0.5054 hi
		30	0.1238 efg	532.8 h	0.5027 i
		50	0.1319 cde	532.8 h	0.5046 hi
	40	0	0.1138 h	534.7 def	0.5297 e
		30	0.1205 gh	534.3 fg	0.5141 ghi
		50	0.1179 gh	535.2 def	0.5333 e
Yunuo 7	7	0	0.1429 b	539.5 a	0.6215 a
		30	0.1353 bcd	538.2 ab	0.5981 b
		50	0.1406 b	537.8 b	0.5708 c
	22	0	0.1296 def	534.1 fgh	0.5143 gh
		30	0.1355 bcd	534.0 fgh	0.5093 ghi
		50	0.1230 fg	533.7 gh	0.5104 ghi
	40	0	0.1224 fg	534.5 efg	0.5175 fg
		30	0.1188 gh	534.7 def	0.5267 ef
		50	0.1163 gh	534.2 fg	0.5272 ef

[a] Mean values in the same column followed by different lowercases are significantly different ($P \leq 0.05$).

Retrogradation occurred after gelatinized samples were stored at 4 °C for 7 days. The retrogradation enthalpy (ΔH_{ret}) and retrogradation percentage (%R) under control and severe shading conditions increased gradually with kernel filling, but the value initially increased and then decreased under moderate shading for both varieties. Compared to the control, moderate shading increased ΔH_{ret} only at 7 DAP, and no significant influence was observed thereafter. Severe shading increased ΔH_{ret} at 7 and 40 DAP but not at 22 DAP in Suyunuo 5. ΔH_{ret} was lower at 7 DAP in Yunuo 7, resulting in lower %R than Suyunuo 5. ΔH_{ret} of Yunuo 7 at 22 DAP was not affected by shading, and it was decreased only at maturity by moderate shading. The %R for both varieties at newly formed starch stage was increased by shading, and the increase became pronounced under moderate shading. %R at the fresh stage was increased only by moderate shading in Suyunuo 5 and decreased by severe shading in Yunuo 7. Thus, %R was decreased by shading at maturity, and the decrease was significant under moderate shading for both varieties.

3.6. Pasting property

With kernel development, the peak, trough, final, and setback viscosities gradually increased for both varieties (Table 3, Fig. 4). The breakdown viscosity for Suyunuo 5 gradually increased under control and moderate shading conditions, but it initially decreased and then increased under severe shading. The breakdown viscosity for Yunuo 7 gradually increased under shading, but it initially increased and then

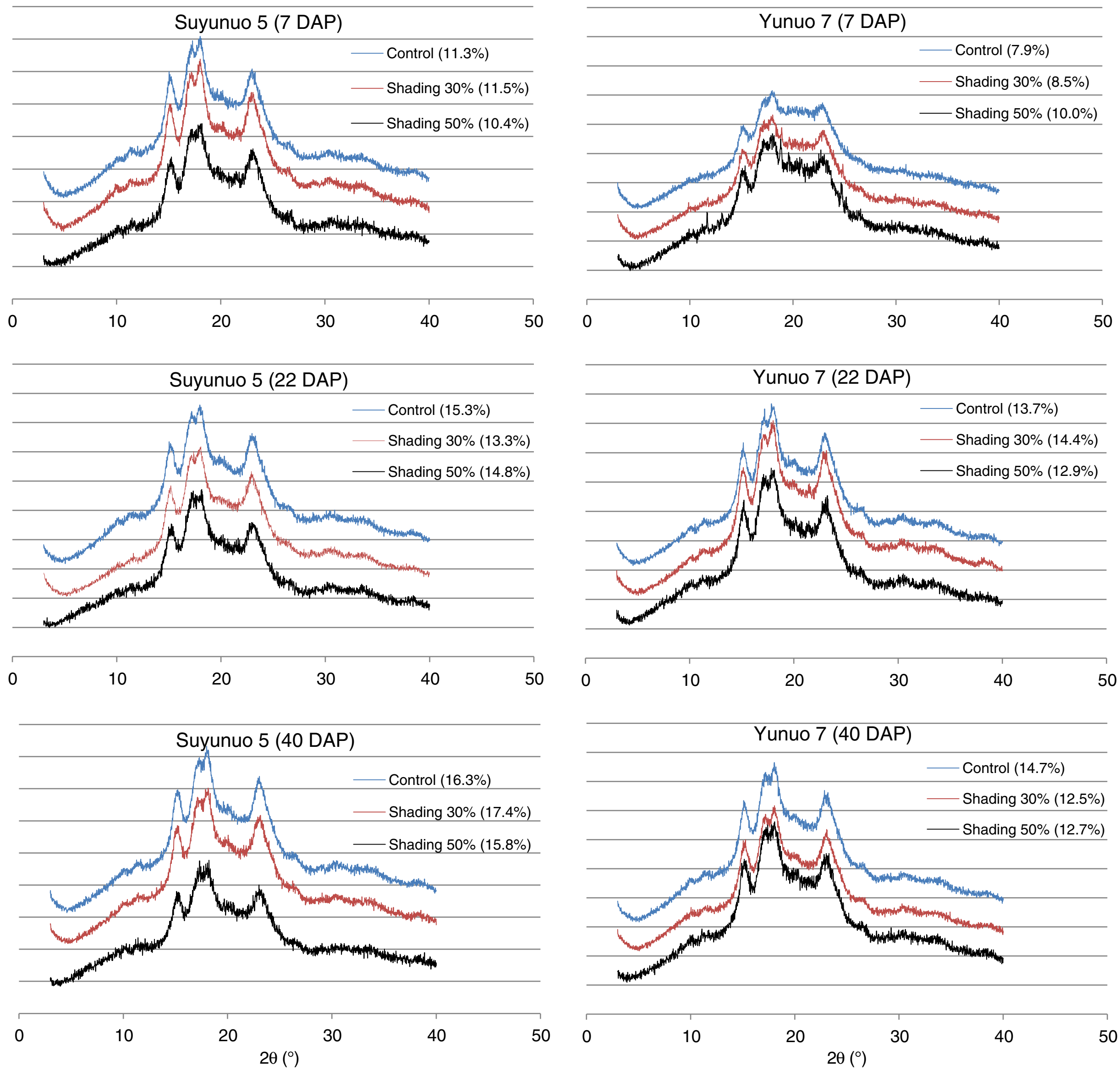

Fig. 3 – Effects of shading after pollination on flour diffraction pattern and crystallinity of waxy maize during kernel development (2013). The values in the plots represent the relative crystallinity of flours.

decreased under the control condition. Waxy maize starch was composed of approximately 100% amylopectin, and the setback viscosity was lower than 309 cP, consistent with its waxy property (Fig. 4). Shading exerted no influence on Suyunuo 5 at early starch formation stage (7 DAP), and Yunuo 7 kernel could not paste at this stage. At fresh stage (22 DAP), the peak viscosity for Suyunuo 5 was not affected by shading, and the trough and final viscosities were decreased only by moderate shading, resulting in higher breakdown rate. Pasting temperature at this stage was not affected by shading for Yunuo 7, but gradually increased with shading for Suyunuo 5. The peak and breakdown viscosities for Yunuo 7 were decreased by shading, and the decreases were more severe under 50% shading conditions. The trough and final viscosities were decreased only by severe shading. At maturity, the trough and final viscosities were not affected, but shading decreased the pasting temperature of both varieties. The peak and breakdown viscosities of Yunuo 7 were also not affected by shading. However, shading decreased both parameters for Suyunuo 5, and the effect was stronger under severe shading conditions.

4. Discussion

In comparison with plants under control conditions, plants under shading after 15 DAP showed lower kernel dry weight, fresh weight, and fresh kernel volume, especially under severe shading, indicating that kernel development is retarded by shading. The retardation may be attributed to reduction by light deprivation of both nonstructural carbohydrate transfer to the kernel [13] and kernel filling rate [11], which

Table 2 – Effects of shading after pollination on flour thermal property of waxy maize during grain development [a] (2013).

Variety	DAP [b]	Shading (%)	ΔH_{gel} (J g^{-1})	T_o (°C)	T_p (°C)	T_c (°C)	ΔH_{ret} (J g^{-1})	%R
Suyunuo 5	7	0	7.7 g	75.3 bc	80.2 bc	85.6 ab	3.0 h	39.0 h
		30	7.4 h	75.4 bc	80.2 cd	85.4 ab	3.8 ef	52.0 c
		50	8.0 ef	75.6 bc	79.9 cd	85.5 ab	3.9 e	49.2 de
	22	0	8.7 b	72.8 e	78.0 ef	83.9 def	4.4 bc	50.9 cd
		30	8.0 ef	72.3 f	77.8 f	83.6 gef	4.5 ab	56.4 a
		50	8.5 cd	72.7 ef	78.1 ef	84.2 de	4.3 cd	50.9 cd
	40	0	7.9 f	70.5 i	75.9 j	82.3 j	4.3 cd	54.2 b
		30	8.4 d	70.8 hi	76.3 i	82.7 hij	4.2 d	50.1 d
		50	8.9 a	71.6 g	76.8 h	83.1 ghi	4.6 a	51.9 c
Yunuo 7	7	0	3.9 j	77.0 a	81.6 a	85.8 a	0.2 l	4.1 k
		30	3.7 j	75.7 b	80.5 b	85.0 bc	1.1 j	29.4 i
		50	4.7 i	75.2 c	79.9 d	84.5 cd	0.7 k	14.8 j
	22	0	8.0 ef	73.4 d	78.3 e	84.4 cd	3.7 fg	46.0 f
		30	8.0 ef	72.8 e	77.3 g	83.5 fg	3.7 fg	46.5 f
		50	8.6 bc	72.6 ef	77.3 g	83.3 fgh	3.6 fg	41.9 g
	40	0	7.7 g	72.3 f	77.0 gh	83.8 ef	3.6 g	47.5 ef
		30	8.6 bc	71.3 g	76.1 ij	82.7 ij	2.7 u	31.1 i
		50	8.1 e	71.1 gh	76.2 ij	82.4 j	3.7 fg	46.1 f

[a] Mean values in the same column followed by different lowercases are significantly different ($P \leq 0.05$).
[b] DAP, days after pollination; ΔH_{gel}, gelatinization enthalpy; T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; ΔH_{ret}, retrogradation enthalpy; and %R, retrogradation percentage.

decrease endosperm cell number and volume [6] as well as kernel set because of accelerated senescence [1]. A similar result has also been observed in rice [28]. Li et al. [29] found that short day length and low temperature during kernel filling after late sowing induced slower filling rate and lighter kernel weight.

Cui et al. [30] observed that protein accumulation in maize kernels increased when the ear was covered with an opaque bag after pollination. In the present study, kernel protein content gradually decreased with kernel development and shading increased kernel protein content throughout the kernel filling stage. Protein accumulation increased for Yunuo 7 under both shading conditions, whereas that for Suyunuo 5 was not affected under moderate shading condition but decreased under severe shading conditions. This difference in response between the two varieties may due to the decrease in kernel weight being more pronounced for Suyunuo 5. The high protein content of kernels under shading conditions may be due to effects of shading on carbon and nitrogen metabolism that favor the transfer of nitrogen from

Table 3 – Effects of shading after pollination on flour pasting property of waxy maize during kernel development [a] (2013).

Variety	DAP [b]	Shading (%)	PV (cP)	TV (cP)	BD (cP)	FV (cP)	SB (cP)	P_{temp} (°C)
Suyunuo 5	7	0	391 g	226 e	165 ghi	310 f	85 j	78.3 a
		30	361 gh	181 ef	180 fgh	256 fg	75 j	77.8 ab
		50	290 gh	148 ef	142 hi	230 fg	82 j	77.8 ab
	22	0	1251 d	1074 b	177 fgh	1325 c	251 d	76.3 ef
		30	1209 de	937 c	272 cd	1102 d	165 hi	76.8 cde
		50	1142 de	1071 b	71 j	1285 c	214 ef	77.2 ab
	40	0	1732 a	1227 a	505 a	1507 ab	280 bc	76.4 ef
		30	1590 b	1195 ab	396 b	1504 ab	309 a	75.6 gh
		50	1515 bc	1248 a	267 cd	1551 a	303 ab	75.3 h
Yunuo 7	7	0	165 ij	136 ef	29 jk	154 gh	19 k	–
		30	76 j	64 f	12 k	90 h	26 k	–
		50	259 hi	231 e	29 jk	257 fg	27 k	–
	22	0	1243 d	939 c	304 c	1135 d	196 fg	76.5 def
		30	1116 e	923 c	193 ef	1074 d	151 i	76.5 def
		50	899 f	767 d	133 i	939 e	173 ghi	76.0 fg
	40	0	1427 c	1212 a	215 ef	1404 bc	192 fgh	77.1 cd
		30	1475 bc	1235 a	240 de	1495 ab	260 cd	76.4 ef
		50	1468 bc	1255 a	213 ef	1490 ab	235 de	76.2 efg

[a] Mean values in the same column followed by different lowercases are significantly different ($P \leq 0.05$).
[b] DAP, days after pollination; PV, peak viscosity; TV, trough viscosity; BD, breakdown viscosity; FV, final viscosity; SB, setback viscosity; P_{temp}, pasting temperature.

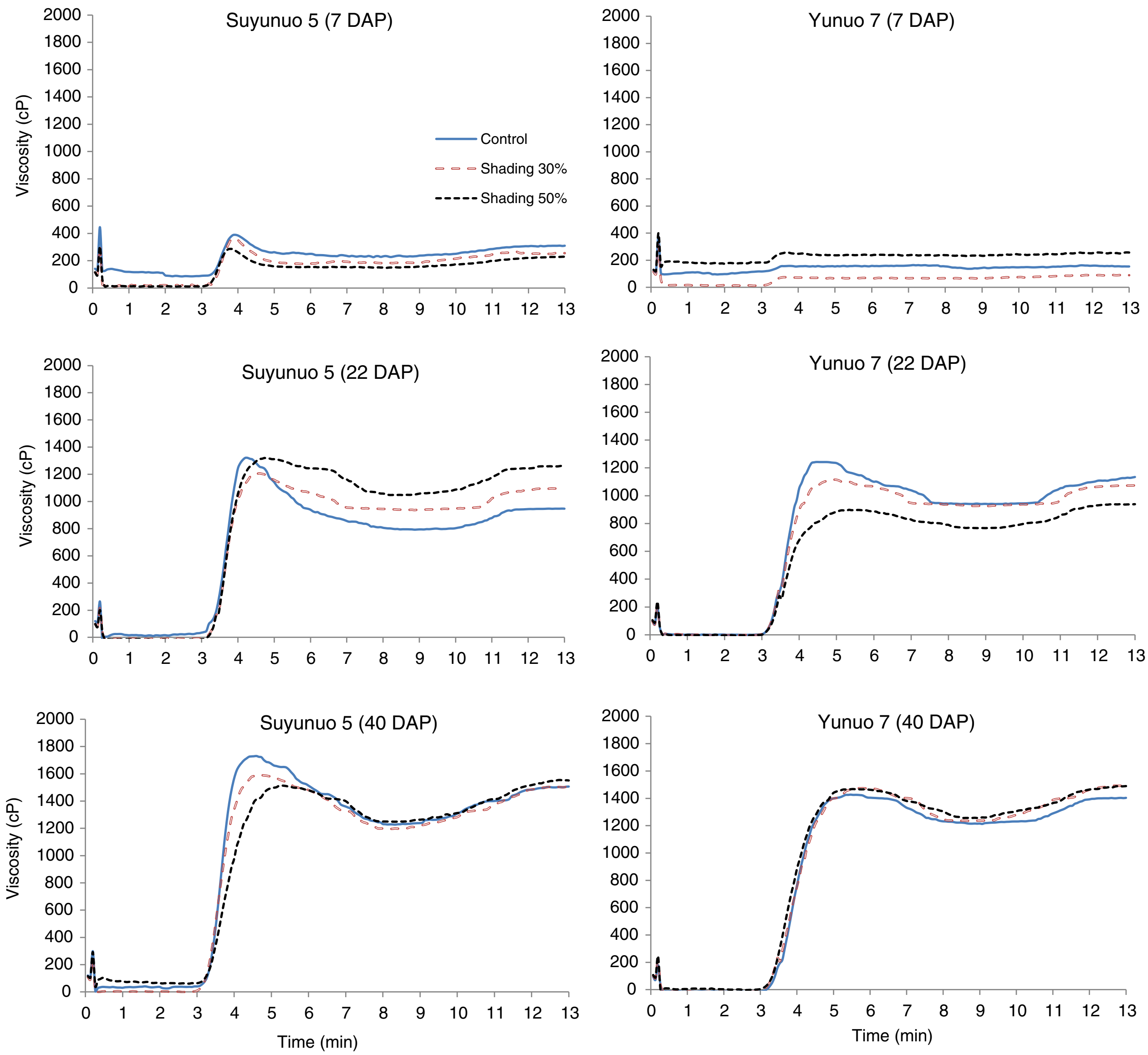

Fig. 4 – Effects of shading after pollination on flour pasting profiles of waxy maize during kernel development (2013).

stem, leaf, and ear to kernel. In addition, increased exposure to blue light under shading treatment has been found to accelerate the accumulation of protein and non-carbohydrate molecules [28]. Kernel starch content sharply increased from 7 to 22 DAP and then gradually increased with kernel development across all treatment conditions. Shading reduced starch deposition, especially under severe shading condition for Yunuo 7. Kernel starch deposition was also reduced by ear shading [30]. Interestingly, the starch content of Yunuo 7 increased under shading at an early stage (15 DAP). A possible reason is a larger decrease in kernel numbers for Yunuo 7 than for Suyunuo 5 (kernel numbers under control and 30% and 50% light deprivation conditions were 436, 368, and 310, respectively, for Yunuo 7 and 395, 367, and 337 for Suyunuo 5). The low sink capacity results in high distribution of accumulated carbohydrate, leading to high starch content at an early stage. With prolongation of shading, reduction of starch accumulation gradually increases, owing to reduction in the activities of starch biosynthesis-associated enzymes such as soluble starch synthase, granule-bound-starch synthase, and sucrose synthase [2,5,12].

IBC reflects the proportions of long chains in amylopectin [24]. IBC sharply decreased from 7 to 22 DAP in both varieties. A similar decreasing trend has been observed previously [20]. The reduction of IBC may be due to increased activities of starch branching enzyme [31], which produces many starch branches, leading to the decrease of proportion of long chains. Ketthaisong et al. [32] found that with kernel development, the change in average chain length was variety-dependent. In the present study, IBC decreased with shading at 22 DAP, as previously observed for fresh waxy maize [14]. IBC was decreased only by moderate shading at maturity (40 DAP) in Suyunuo 5, whereas it was significantly increased by high temperature during kernel filling [20]. The low IBC under weak light conditions may be due to an increase in the activities of starch branching enzymes with shading [5]. Under natural environmental conditions, sunlight intensity and duration and temperature have been found to influence maize growth synergistically, and high temperature is often accompanied by long sunlight duration [29]. Further research on amylopectin chain length distribution during kernel filling under different sunlight intensities and temperature conditions may be helpful for clarifying the difference of starch structure.

Shading did not alter kernel X-ray diffraction patterns at any point during kernel development. Our previous study revealed that shading increased crystallinity at fresh stage [14]. In the present study, crystallinity in response to shading was dependent on kernel development. The different effects of shading degrees on intensities and crystallinity may be attributed to differences in kernel component content, starch granule size, and amylopectin structure.

With kernel development, ΔH_{gel} of Suyunuo 5 under control and 30% shading conditions initially increased and then decreased, whereas that under 50% shading gradually increased. The value for Yunuo 7 under control and 50% shading conditions initially increased and then decreased, whereas that under 30% shading gradually increased. In our previous study, ΔH_{gel} initially increased and then decreased under control and high-temperature conditions [20]. In rice kernels, changes in ΔH_{gel} are variety-dependent [33]. The ΔH_{gel} in response to shading also depended on variety and kernel developmental stage. A variety-dependent response of ΔH_{gel} was also observed in fresh waxy maize [14]. The change in ΔH_{gel} among different kernel developmental stages and different light conditions was caused by the change in starch content ($r = 0.75$, $P < 0.01$), crystallinity ($r = 0.78$, $P < 0.01$) and IBC ($r = -0.88$, $P < 0.01$), indicating that the kernel has higher starch content and crystallinity, and the higher proportion of short chains in amylopectin need more energy to gelatinize the sample. The kernel gelatinization temperatures gradually decreased with kernel filling, and the decrease was sharp from 7 to 22 DAP. Similar results have been found by other groups [20,32]. The higher gelatinization temperature at 7 DAP may have been due to the greater number of long chains in starch than at 22 and 40 DAP, as observed for normal maize [15]. The effects of shading on gelatinization temperatures were dependent on kernel developmental stage and variety. Our previous study also revealed that the response of gelatinization temperatures of fresh waxy maize kernel to 30% light deprivation was variety-dependent [14]. The %*R* under control and severe shading conditions gradually increased with kernel filling, but the value initially increased and then decreased under moderate shading conditions for both varieties. The %*R* for both varieties at newly formed stage was increased by shading, and the increase became pronounced under moderate shading. The %*R* at fresh stage was decreased only by moderate shading in Suyunuo 5 and by severe shading in Yunuo 7. The effects of shading on %*R* have also been investigated in waxy maize at fresh stage [14]. The %*R* was decreased by shading at maturity, and the decrease was greater under moderate shading stress for both varieties. Compared to Suyunuo 5, Yunuo 7 harvested at fresh and mature stages had lower %*R*, indicating its lower retrograde tendency. The ΔH_{ret} and %*R* were correlated with starch content ($r = 0.72$ and 0.62, $P < 0.01$), crystallinity ($r = 0.81$ and 0.74, $P < 0.01$) and IBC ($r = -0.87$ and -0.81, $P < 0.01$), indicating that Yunuo 7 kernel had higher starch content and crystallinity than that of Suyunuo 5 and that a higher proportion of short chains in amylopectin were readily retrogradable.

With kernel development, the peak, trough, final, and setback viscosities gradually increased for both varieties, in agreement with previous study [20]. The increase in flour viscosities may be due to kernel starch accumulation and starch granule size enlargement [20]. Waxy maize starch peak viscosity initially increases and then decreases as kernel filling progresses [32]. However, peak viscosity gradually decreases with rice kernel development [33]. The breakdown viscosity for Suyunuo 5 gradually increased under control and moderate shading conditions, but it initially decreased and then increased under severe shading. The peak viscosity for Yunuo 7 was decreased by shading, and the decrease was more severe under 50% shading. The trough and final viscosities were decreased only by severe shading. A similar result has previously been observed in fresh waxy maize [14]. At maturity, the trough and final viscosities were not affected, but shading decreased the pasting temperature of both varieties. The peak and breakdown viscosities of Yunuo 7 were also unaffected by shading. However, shading decreased those two parameters for Suyunuo 5, and the decrease became pronounced under severe shading. The low viscosities under shading were due to the kernel lower starch content and crystallinity and higher protein content and higher proportion of long chains in amylopectin, as the viscosities were positively correlated with starch content and crystallinity and negatively correlated with protein content and IBC. The small granule size under shading also contributed to lower viscosities [14,34]. Some studies have found that shading reduced the activities of starch biosynthesis-associated enzymes [2,5,12], resulting in lower starch and higher protein contents [5,35]. In rice, the starch pasting characteristics in response to shading are also variety dependent [36]. Moreover, shading during kernel filling decreases peak viscosity in normal maize [5], wheat [13], and rice [11].

5. Conclusion

Shading decreased kernel size and weight (dry and fresh), and the decrease was severe under 50% shading. Shading, especially severe shading, reduced starch accumulation and increased protein content. IBC was decreased by moderate shading and was not affected by severe shading at all stages for Suyunuo 5. In Yunuo 7, IBC was not affected by shading at fresh and mature stages, but gradually decreased at 7 DAP as the amount of light decreased. Crystallinity of Suyunuo 5 was decreased by severe shading during all kernel stages, and was decreased by moderate shading at fresh stage and increased at maturity. In Yunuo 7, crystallinity was increased by moderate and severe shading at 7–22 DAP and 7 DAP, respectively, and decreased by shading thereafter. ΔH_{gel} changed with kernel development, but its response to shading was dependent on variety and growth stage. The influences of shading on %*R* were also dependent on variety and kernel developmental stage, and %*R* was higher for Suyunuo 5 at all stages. Peak viscosity gradually increased with kernel development, but its value was decreased by shading at fresh stage for Yunuo 7 and at maturity for Suyunuo 5.

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Supplementary data

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