

Effects of shading on the photosynthetic characteristics and mesophyll cell ultrastructure of summer maize

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Abstract A field experiment was conducted to study the effects of shading on the photosynthetic characteristics and mesophyll cell ultrastructure of two summer maize hybrids Denghai605 (DH605) and Zhengdan958 (ZD958). The ambient sunlight treatment was used as control (CK) and shading treatments (40 % of ambient sunlight) were applied at different growth stages from silking (R1) to physiological maturity (R6) (S1), from the sixth leaf stage (V6) to R1 (S2), and from seeding to R6 (S3), respectively. The net photosynthetic rate (P_n) was significantly decreased after shading. The greatest reduction of P_n was found at S3 treatment, followed by S1 and S2 treatments. P_n of S3 was decreased by 59 and 48 % for DH605, and 39 and

43 % for ZD958 at tasseling and milk-ripe stages, respectively, compared to that of CK. Additionally, leaf area index (LAI) and chlorophyll content decreased after shading. In terms of mesophyll cell ultrastructure, chloroplast configuration of mesophyll cells dispersed, and part of chloroplast swelled and became circular. Meanwhile, the major characteristics of chloroplasts showed poorly developed thylakoid structure at the early growth stage, blurry lamellar structure, loose grana, and a large gap between slices and warping granum. Then, plasmolysis occurred in mesophyll cells and the endomembrane system was destroyed, which resulted in the dissolution of cell membrane, karyotheca, mitochondria, and some membrane structures. The damaged mesophyll cell ultrastructure led to the decrease of photosynthetic capacity, and thus resulted in significant yield reduction by 45, 11, and 84 % in S1, S2, and S3 treatments, respectively, compared to that of CK.

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Abbreviations

Ch	Chloroplast
C_i	Intracellular CO ₂ concentration
CK	Control
CM	Chloroplast membrane
CW	Cell wall
GL	Grana lamella
G_s	Stomatal conductance
LAI	Leaf area index
Mi	Mitochondria
N	Nucleus
Ne	Nuclear envelope
Nu	Nucleolus

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P	Particles
P_n	Net photosynthetic rate
R1	Silking stage
R3	Milk-ripe stage
R6	Physiological maturity
S1	Shading from R1 to R6
S2	Shading from V6 to R1
S3	Shading from seedling to R6
SL	Stroma lamella
V6	The sixth leaf stage
VT	Tasseling stage

Introduction

For half a century, global solar radiation has decreased by an average of 1.4–2.7 % per decade (Stanhill and Cohen 2001; Ramanathan and Feng 2009). The effective sunlight duration decreased by 1.28 % per decade in China (Chen et al. 2005). According to the light requirement characteristics of summer maize, optimum grain yield is achieved when sunlight duration reaches 600–850 h per growing season. In Tai'an city, Shandong province, China (where we conducted our research), sunlight duration averaged only 489 h per summer maize growing season (from July to September) in 2009 to 2011, which was reduced by 117 h compared to the same time period in 2000 to 2002 due to increased precipitation and cloud cover (Cui et al. 2012). Periods of rain occurred frequently during the maize growing season resulting in insufficient solar radiation and yield reduction of summer maize. Light intensity is only 10–20 % of ambient sunlight with rainy or cloudy conditions (Cui et al. 2012; Ren et al. 2014).

Maize is highly sensitive to low-light intensities because of specific anatomical, biochemical, and energetic complexities associated with C_4 metabolism (Ubierna et al. 2011). Although up to 50 % of C_4 crop canopy photosynthesis may be carried out by shaded leaves (Baker and Long 1988), light limitations play an important role in limiting canopy productivity and severe effects on net canopy photosynthetic uptake have been reported (Kromdijk et al. 2008). The most direct impacts of reduced sunlight on plant growth are about photosynthesis which provides primary energy for assimilation (Burkey and Wells 1991). Thus, it is vital to investigate low-light stress effects on the photosynthetic characteristics of summer maize. Recently, there are some reports focused on the efficiency of C_4 photosynthesis under low-light conditions (Ubierna et al. 2013; Bellasio and Griffiths 2014). Under low light, the activity of the carbon-concentrating mechanism generally decreases, associated with an increase in leakiness (Φ), the ratio of CO_2 retrodiffusing from the bundle sheath relative to C_4 carboxylation. The increase of Φ was theoretically associated with a decrease in biochemical operating efficiency because a proportion of canopy photosynthesis is carried out

by shaded leaves, leading to potential productivity losses at field scale (Bellasio and Griffiths 2014). It was demonstrated that under low light, neglecting respiratory fractionation and assuming that CO_2 concentration in the bundle sheath cells is much larger than that in mesophyll cells lead to overestimation of Φ (Ubierna et al. 2013). Currently, there is no complete understanding of how C_4 photosynthetic efficiency responds to low light, yet it is an important implication to select traits in C_4 plants for enhanced photosynthetic efficiency under various light environments (Sun et al. 2012; Ubierna et al. 2013; Bellasio and Griffiths 2014). The improvement of C_4 photosynthesis efficiency is helpful to explain that shading at any stage reduced biomass of corn. Furthermore, Di Benedetto and Garcia (1992) and Nyitrai et al. (1994) reported that more chlorophyll and light-harvesting complex (LHCII) were synthesized in plants grown under weak illumination. Others reported that chlorophyll synthesis and photosynthetic capacity were reduced in the seedling stage under low light (Zhang et al. 2007; Li et al. 2010; Mu et al. 2010), which was attributed mainly to the damaged chloroplast morphology and destroyed ultrastructure of functional leaves (Li et al. 2010). The morphology and internal structure of mesophyll cells, a fundamental component of photosynthesis, play an important role in photosynthetic capacity. Under different light conditions, leaf cell structure and physiological and biochemical metabolism of arabidopsis and watermelon (*Citrullus lanatus*) have been found to change significantly (Pan 2008; Duan et al. 2014). When subjected to various environmental changes, chloroplasts and mitochondria of all organelles in mesophyll cells were the most sensitive to low light (Weston et al. 2000; Pessarakli 2005; Xu et al. 2008). Chloroplast number in ginseng and ginger was reduced after shading (Xu et al. 1994; Zhang et al. 1999). Thus, the investigation of low-light effect on the cellular level characteristics is important. Previous studies have more comprehensively analyzed the effects of shading on growth and development of summer maize (Zhang et al. 2007; Li et al. 2010; Mu et al. 2010). However, very little attention has been given to study the effect of shading on leaf photosynthesis characteristic at the cellular level. In the work we presented here, changes in mesophyll cell ultrastructure of summer maize were studied under long-term low-light conditions to better understand the effects of shading from silking (R1) to physiological maturity (R6), from the sixth leaf stage (V6) to R1, and from seeding to R6 on the physiological characteristics and yield of summer maize.

Materials and methods

Experimental site

The field experiment was conducted during 2010 and 2011 at the State Key Laboratory of Crop Biology and the

experimental farm of Shandong Agricultural University, China (36° 10' N, 117° 04' E; 151 m.a.s.l.). The region is characterized by alfisols and has a temperate continental monsoon climate.

Experimental design

Summer maize hybrid Zhengdan958 (ZD958) was used for experimental material in 2010 and a second hybrid Denghai605 (DH605) was included in 2011, which were widely planted in Shandong Province, China. Maize was sown on June 16 in both years with a plant density of 67,500 plants per hectare. Every experimental treatment unit was 27 m² (3 m wide × 9 m long) in size and consisted of five rows of maize spaced 0.6 m apart, which was the recommended spacing for summer maize in our region. Four treatments were arranged in a randomized complete block design with three replicates: the ambient sunlight treatment was used as the control (CK) and three shading treatments (40 % of ambient light intensity) were applied during the following growth periods: silking stage (R1) to physiological maturity stage (R6) (S1), from the sixth leaf stage (V6) to R1 (S2), and from seeding to R6 (S3). Shade cloth (Hongda Shade Cloth Company, Shouguang City, China) supported with scaffolding allowed almost 40 % of ambient light intensity to pass through, which were measured by a TES-1332 digital lux meter (TES, Taiwan) at different hours for 5 days under shading and natural ambient conditions. A distance of 2 m between the shade cloth and the top of maize canopy was maintained to keep the microclimate under the cloth consistent with the control. Shade cloth had no effect ($P > 0.05$) on soil temperature, relative humidity, air speed, or CO₂ concentration when measured by a TNHY-11-G handheld weather tester (Top instrument co., LTD, China) in 2010 and 2011. There was also no difference in light quality between shading and naturally cloudy conditions at ten wavelengths between 460 and 870 nm, but slight differences at 950 and 1100 nm for light quality.

Prior to seeding, P and K were applied at 52 kg P per hectare as calcium dihydrogen phosphate (7.4 % P) and 165 kg K per hectare as muriate of potash (50 % K). Nitrogen was applied as urea (46 % N) at V6 at 96 kg N per hectare and at the twelfth leaf stage (V12) at 144 kg N per hectare. Disease, weeds, and pests were well-controlled in each treatment. The 10 % difenoconazole were diluted 1000 times by water and sprayed by 750 L/ha at the third leaf stage (V3) to control *Physoderma maydis*. The herbicide of 90 % atrazine acetochlor was diluted 2000–3000 times and sprayed on the whole field surface by 600 L/ha before germinating to control weeds; the pesticides of 50 % phoxim emulsifiable concentrate were diluted 1000 times by water and sprayed by 750 L/ha at the ninth leaf stage (V9) to control corn borers.

Leaf area index

At the tasseling stage (VT) and milk stage (R3), 15 representative plants were obtained from each plot to measure leaf length (L) and maximum leaf width (W) for the largest leaf on the individual tagged plants, and then leaf area and leaf area index (LAI) were calculated according to the method of Qi et al. (2012).

$$\text{Leafarea} = L \times W \times 0.75$$

$$\text{LAI} = (\text{leafareaperplant} \times \text{plantnumberperplot}) / \text{plotarea}$$

Gas exchange parameters

Net photosynthetic rate (P_n), stomatal conductance (G_s), and intercellular CO₂ concentration (C_i) were measured on the ear leaf of five representative plants on clear days between 10:00 AM and 12:00 PM with a portable infrared gas analyzer (CIRAS II, PP System, Pentney, UK) at VT and R3 stages.

Chlorophyll concentration

Ten 0.7-cm-diameter leaf disks were obtained for chlorophyll extraction from fresh ear leaves of five plants from the center of each plot at VT and R3. Leaf disks were soaked in 15 ml of 95 % ethanol for 48 h. Chlorophyll a and b concentrations in the supernatant were determined by measuring light absorbance at 663 and 645 nm, respectively, with an ultraviolet spectrophotometer (UV-2450, SHIMADZU, Japan). Chlorophyll concentrations were calculated according to the method of Li (2000).

$$\text{Chl a} = 12.72 A_{663} - 2.59 A_{645}$$

$$\text{Chl b} = 22.88 A_{645} - 4.67 A_{663}$$

$$\text{Chl a} + \text{b} = \text{Chl a} + \text{Chl b} = 20.29A_{645} + 8.05A_{663}$$

Where A is the absorption at the referenced wavelength, Chl a is concentration of chlorophyll a, Chl b is concentration of chlorophyll b, and Chl a + b is the total chlorophyll concentration.

Leaf tissue preparation and microscopy

In 2011, the illuminated sides of five ear leaves were obtained from the center of each plot at VT and R3 stages. A square section of a leaf (0.5 × 0.5 cm) near the center vein of each leaf was removed with a blade. After fixation with 2.5 % glutaraldehyde for 4 h, leaf cells were post-fixed with osmic acid at 4 °C for 4 h and then dehydrated with ethanol. When embedded in Spurr resin at 70 °C for 8 h, thin sections were cut from leaf samples with an LKB-V ultramicrotome and placed upon 250 mesh grids. Samples were double stained using stem uranyl acetate and lead citrate and then observed and randomly

photographed using a Hitachi-600 transmission electron microscope (Xu et al. 2008).

Grain yield

At R6, 30 ears harvested from three rows at the center of each plot were used to determine yield and ear traits including length, width, weight, row number, kernels per row, barren tip length, cob weight, and cob width. All the kernels were air-dried and grain yield was calculated at 14 % moisture, the standard for maize storage or sale in China (GB/T 29890–2013).

$$\begin{aligned} \text{Grain yield (kg ha}^{-1}\text{)} &= \text{Harvest ear (ear sha}^{-1}\text{)} \\ &\quad \times \text{kernel number per ear} \\ &\quad \times 1000\text{-grain weight (g 1000 grains}^{-1}\text{)} / 10^6 \\ &\quad \times (1 - \text{sample moisture content \%}) / (1 - 14\%) \end{aligned}$$

Statistical analysis

Data was analyzed with SPSS 17.0 (SPSS Institute Inc.). Data was subjected to two-factor (i.e., shading and hybrid) analyses of variance (ANOVA) in a randomized complete block design. Comparisons among the various treatments were based on Duncan's test at the 0.05 probability level ($P \leq 0.05$).

Results

Chloroplasts configuration

With ambient sunlight, leaf mesophyll cells of summer maize were well-formed and close to the cell wall, appearing long-oval or spindle shaped at VT and R3 for both hybrids (VT-DH605 (Fig. 1a); VT-ZD958 (Fig. 2a); R3-DH605 (Fig. 3j); R3-ZD958 (Fig. 4j)). Normal configuration and well-formed structure of chloroplasts in the mesophyll cells are fundamental for normal photosynthesis of maize leaves. However, the number of chloroplast per mesophyll cell was significantly reduced after shading (Table 1). The greatest reduction (19 %) occurred in the S3 treatment, followed by S2 treatment (7 %) at VT. At R3, chloroplast number of S3 treatment was decreased by 15 % compared to that of CK, while there were no significant difference among S1, S2, and CK. Chloroplast morphology was most affected by shading when shading occurred from seeding to R6 (S3), followed by shading from R1 to R6 (S1) and V6 to R1 (S2). An "edema" was evident in parts of the chloroplasts after shading; they were shorter and wider and changed in external form from long and oval to elliptical or nearly circular (VT-DH605 (Fig. 1d, g); VT-ZD958 (Fig. 2d, g); R3-DH605 (Fig. 3m–s); R3-ZD958 (Fig. 4m–s)). Shading reduced the length of chloroplasts, but increased their width (Table 1). The greatest reduction of

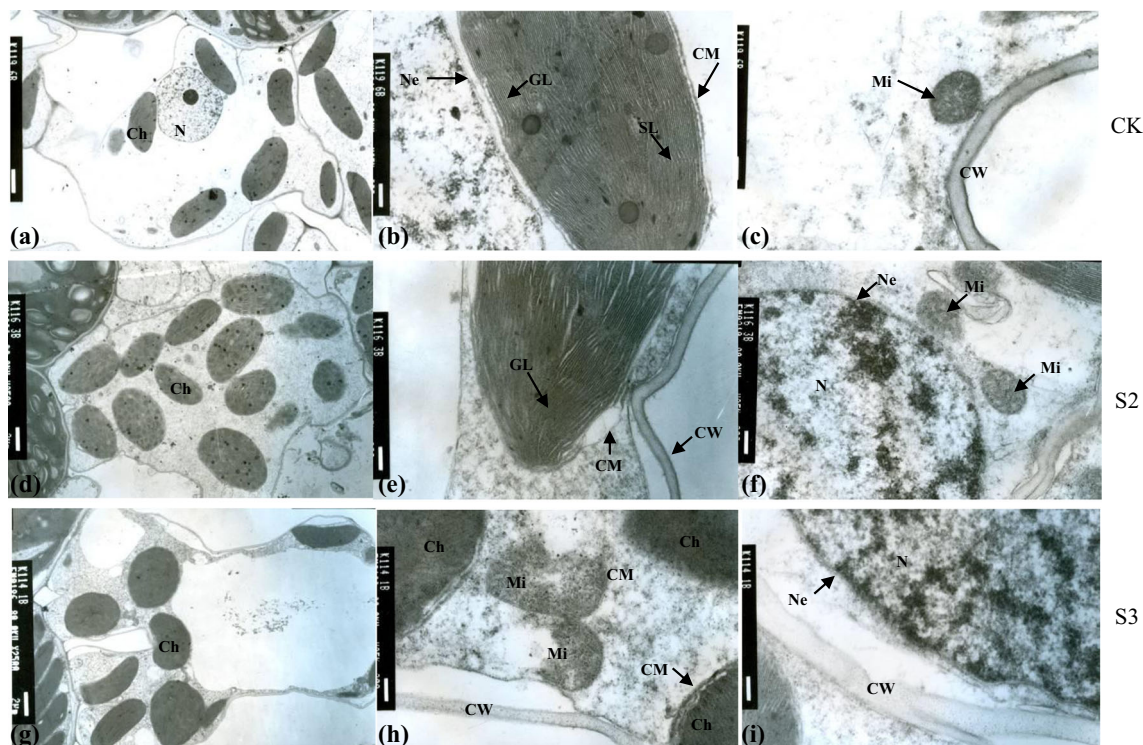


Fig. 1 Mesophyll cell and chloroplast ultrastructure changes in ear leaf of maize hybrid DH605 at tasseling. The leftmost column (a, d, g) shows the chlorophyll mesophyll cells and topography ($\times 2500$). Shown in the middle column (b, e, h) and the 3rd column (c, f, i) is the ultrastructure

($\times 25,000$) of mesophyll cells. Micrographs in rows a–c, d–f, and g–i, respectively, were from plants receiving ambient light or 40 % of ambient sunlight from the sixth leaf stage (V6) to R1 (S2) and from seeding to R6 (S3)

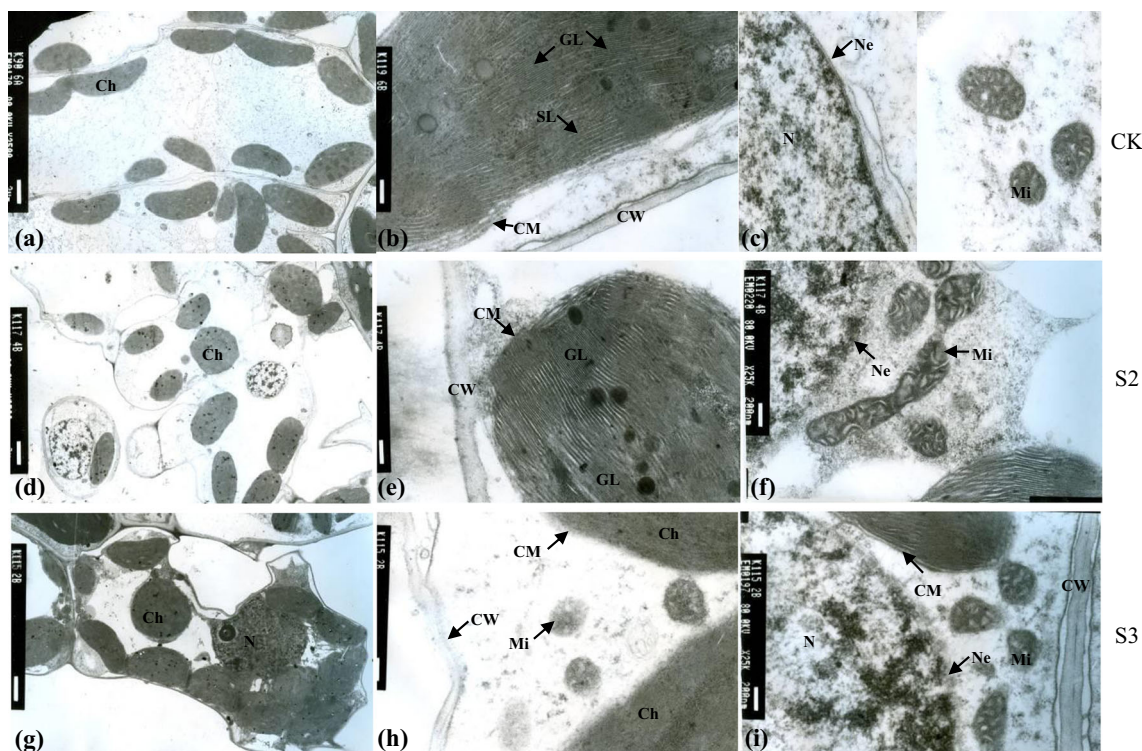


Fig. 2 Mesophyll cell and chloroplast ultrastructure changes in ear leaf of maize hybrid ZD958 at tasseling. The leftmost column (a, d, g) shows the chlorophyll mesophyll cells and topography ($\times 2500$). Shown in the middle column (b, e, h) and the 3rd column (c, f, i) is the ultrastructure

($\times 25,000$) of mesophyll cells. Micrographs in rows a–c, d–f, and g–i, respectively, were from plants receiving ambient light or 40 % of ambient sunlight from the sixth leaf stage (V6) to R1 (S2) and from seeding to R6 (S3)

chloroplast length was found in S3, followed by S1 and S3. The chloroplast length of S3 at VT and R3 were decreased by 16 and 15 % compared to CK, respectively. However, chloroplast width was increased after shading. The greatest increase in chloroplast width, compared to CK, occurred with the S3 treatment by 25 and 26 % for DH605 at VT and R3, respectively. ZD958 decreased by 11 and 33 % compared to that of CK, respectively.

Chloroplasts ultrastructure

As for the CK plants, chloroplasts had a complete external envelope and clear boundaries, and the thylakoid systems were well-developed. Lamella structure pile folds were in order; grana and stroma lamella were compact and clear (VT-DH605 (Fig. 1b); VT-ZD958 (Fig. 2b); R3-DH605 (Fig. 3k); R3-ZD958 (Fig. 4k)). However, the chloroplast internal structure became poor, and the numbers of grana and grana lamella were reduced to varying degrees after shading. The most significant reductions of grana and grana lamella number were found in S3 with decreases of 17 and 31 % at VT and 12 and 19 % at R3, respectively, for DH605, compared with CK while the corresponding values for ZD958 decreased by 10 and 37 % at VT and 25 and 33 % at R3, respectively (Table 1). For S3, most chloroplasts were similarly round and showed external envelope degradation; the differentiated structures of stroma lamella and grana lamella were damaged

and difficult to recognize (VT-DH605 (Fig. 1h); VT-ZD958 (Fig. 2g, h)), and few chloroplasts were clearly differentiated per unit of mesophyll cells at VT; the lamellar structure was clearly visible at R3, showing the adaptability of plants to low-light environments in the long-term. However, swollen thylakoids, disorderly arranged grana lamellae, and large cracks among lamellae led to severely dissipated external envelopes, a fine-grained exosmotic substance, and partially disintegrated chloroplasts (R3-DH605 (Fig. 3t); R3-ZD958 (Fig. 4t)). The chloroplast grana and stroma lamella of S1 and S2 were well-developed and exhibited only partial adventitia fractures. However, lamellar structure was loosely placed and cracks among lamellae were apparent, such that the grana gradually became twisted (R3-DH605 (Fig. 3n, q); R3-ZD958 (Fig. 4q, t)).

Mitochondria

Mitochondria are the “motive power processing factory” of plants. Shading influenced mitochondrial structures to varying degrees. Under natural illumination, mitochondria were scattered in the cytoplasm and most were circular or elliptical (VT-DH605 (Fig. 1c); VT-ZD958 (Fig. 2c)). After exposure to shading, mitochondria were in a clustered arrangement in the chloroplast (VT-ZD958 (Fig. 2f, i); R3-DH605 (Fig. 3t); R3-ZD958 (Fig. 4r)), which would reduce the energy loss due to mitochondrial respiration when energy was in the transport process. After shading,

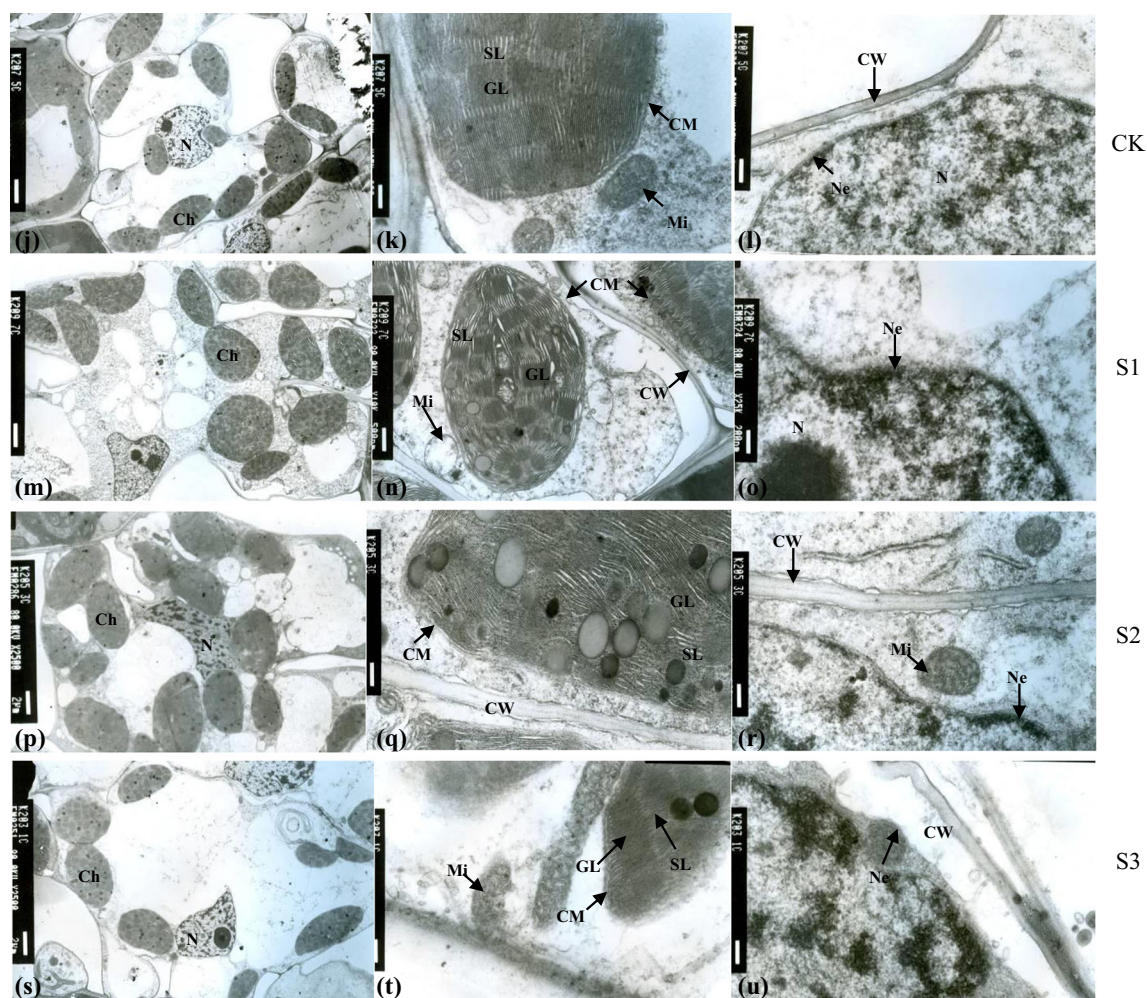


Fig. 3 Mesophyll cell and chloroplast ultrastructure changes in ear leaf of maize hybrid DH605 at milk stage. The leftmost column (**m**, **p**, **s**) shows the chlorophyll mesophyll cells and topography ($\times 2500$). Shown in the middle column (**n**, **q**, **t**) and the 3rd column (**o**, **r**, **u**) is the ultrastructure ($\times 25,000$) of mesophyll cells. Micrographs in rows **j**–**l**,

m–**o**, **p**–**r**, and **s**–**u**, respectively, were from plants receiving ambient light or 40 % of ambient sunlight from silking (R1) to physiological maturity (R6) (S1), from the sixth leaf stage (V6) to R1 (S2), and from seeding to R6 (S3)

mitochondrial outer membrane dimmed, and the fine-grained substance exsposed and diluted (VT-ZD958 (Fig. 2f); R3-DH605 (Fig. 3t)). Moreover, mitochondrial longitudinal diameter became long and thread-like, while the internal ridges were out of shape, dissolved gradually, and would lose their physiological function. Shading had the greatest influence in S3 (VT-ZD958 (Fig. 2h); R3-DH605 (Fig. 3t)), resulting in the mitochondrial disintegration. Mitochondria in S2 were damaged by shading, but function gradually recovered with the removal of shading (VT-ZD958 (Fig. 2f); R3-ZD958 (Fig. 4r)). Therefore, mitochondria were less susceptible to damage from reduced illumination than chloroplast.

Membrane structure

Regular membrane structure plays an important role in normal physiological function of cells. However, cell wall structure was incomplete and exhibited indistinct gradation, lower

density, and a loose edge after shading (VT-DH605 (Fig. 1i); VT-ZD958 (Fig. 2h)). Plasmolysis and degradation in part of the cell membrane were also evident. The damage to mitochondria and chloroplast membranes was more serious than that to the cell membrane. There were not any complete and defined membrane structure in both mitochondria and chloroplasts after shading. However, complete membrane structures of mitochondria and chloroplasts were present after shading was removed for S2, although the structure was seriously damaged. Therefore, the membrane systems of mitochondria and chloroplasts were more susceptible to reduced illumination than other membrane structures, which may be related to their physical function.

Leaf area index and net photosynthetic rate

Leaf area index (LAI) of DH605 and ZD958 decreased significantly with shading (Table 2). LAI was reduced

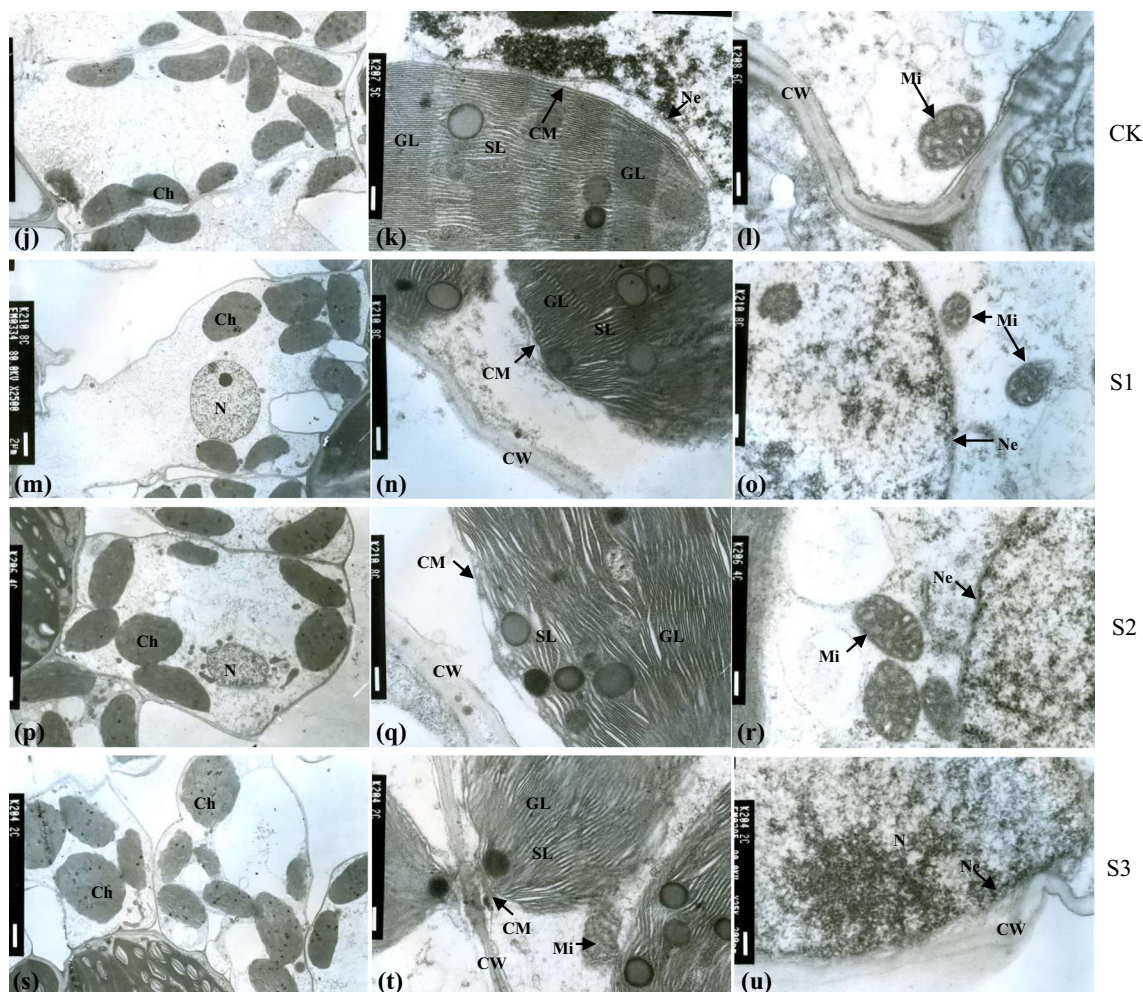


Fig. 4 Mesophyll cell and chloroplast ultrastructure changes in ear leaf of maize hybrid ZD958 at milk stage. The leftmost column (m, p, s) shows the chlorophyll mesophyll cells and topography ($\times 2500$). Shown in the middle column (n, q, t) and the 3rd column (o, r, u) is the ultrastructure ($\times 25,000$) of mesophyll cells. Micrographs in rows j–l,

m–o, p–r, and s–u, respectively, were from plants receiving ambient light or 40 % of ambient sunlight from silking (R1) to physiological maturity (R6) (S1), from the sixth leaf stage (V6) to R1 (S2), and from seeding to R6 (S3)

most when shading occurred from seeding to R6 (S3), followed by shading from R1 to R6 (S1), and least with shading from V6 to R1 (S2). There were no significant hybrid \times shading treatment interaction effects on LAI. At VT in 2011, the greatest reduction (23 %) was found at S3 treatment, followed by S2 (19 %) across hybrids, while the greatest reduction (18 %) was found at S3 treatment, followed by S2 treatment (15 %) and S1 treatment (9 %) across hybrids at R3. In 2010, the greatest reduction was also found at S3 treatment with 33 and 27 % for ZD958, respectively, compared to that of CK. Net photosynthetic rate (P_n) was significantly reduced by shading (Table 2). There were significant hybrid \times shading treatment interaction effects on P_n . The greatest reduction was found at S3 treatment with 59 and 48 % for DH605, and 39 and 43 % for ZD958 at VT and R3, respectively, compared to that in CK (averaged over in 2010 and 2011).

Chlorophyll content

Chlorophyll is the key pigment for luminous energy absorption and photosynthetic transformation. Chlorophyll content of leaves is an important indicator of abnormal chloroplast changes. Chlorophyll a and b contents of ZD958 and DH605 were lower with shading than with the CK treatment (Table 3). Chlorophyll contents were most susceptible to damage when shading occurred from seeding to R6 (S3), followed by shading from R1 to R6 (S1) and V6 to R1 (S2). There were no significant hybrid \times shading treatment interaction effects on chlorophyll contents. At VT in 2011, Chl a + b contents of S2 and S3 were 26 and 39 % across hybrids, respectively, lower than those of CK. At R3, the most reduction of Chl a + b contents (around 27 %) was also found in S3 treatment, followed by S2 treatment (around 15 %) and S1 treatment (around 12 %) across hybrids. Similar results were found in both 2010 and 2011 (Table 3).

Table 1 Effects of shading at 40 % of ambient sunlight from silking (R1) to physiological maturity (R6) (S1), the sixth leaf stage (V6) to R1 (S2), and seeding to R6 (S3) and ambient light conditions (CK) on chloroplast ultrastructure characteristics of mesophyll cells of ear leaves at tasseling (VT) and milk stage (R3) (2011)

Hybrid (H)	Treatment (T)	The number of chloroplast/ mesophyll cell	Chloroplast size		Number of grana	Number of grana lamellae
			Length (μm)	Width (μm)		
2011—VT						
DH605	CK	8.9 a	17.4 a	7.9 b	24.6 a	20.6 a
	S2	8.2 b	17.2 a	9.3 a	23.1 b	18.5 b
	S3	8.1 b	14.0 b	9.9 a	20.4 c	14.3 c
ZD958	CK	10.0 a	17.2 a	7.3 b	24.0 a	21.1 a
	S2	9.4 a	15.8 b	7.8 ab	22.5 b	17.0 b
	S3	7.1 b	15.0 b	8.1 a	21.6 c	13.3 c
ANOVA	H	NS	NS	NS	NS	NS
	T	*	*	*	*	*
	H × T	NS	NS	NS	NS	NS
2011—R3						
DH605	CK	10.4 a	17.3 a	7.2 c	23.5 a	19.4 a
	S1	8.7 b	14.6 c	8.6 b	20.6 c	15.0 c
	S2	9.5 a	15.4 bc	8.1 b	22.4 b	18.2 b
	S3	8.3 b	14.3 c	9.1 a	20.6 c	15.8 c
ZD958	CK	8.5 a	17.4 a	6.9 c	25.9 a	23.0 a
	S1	8.7 a	15.6 b	8.1 b	22.8 c	17.9 c
	S2	8.5 a	17.2 a	9.2 a	24.5 b	19.5 b
	S3	7.7 b	15.3 b	9.2 a	19.4 d	15.5 d
ANOVA	H	NS	NS	NS	NS	NS
	T	*	*	*	*	*
	H × T	NS	NS	NS	NS	NS

S1 treatment was not applied at VT. Means within a column for each hybrid and growth stage followed by the same letter did not differ at 5 % probability level. The values are means of three replicate blocks

NS not significant

*Significant at the 0.05 probability level; **significant at the 0.01 probability level

Yield and yield components

Rows per ear and kernels per row were affected by shading treatments (Table 4). The number of rows per ear was minimally affected by shading—typically a reduction of only 1 to 2 rows per ear with S3 and 0 to 1 row per ear with S1 or S2, in comparison to CK. Kernels per row decreased significantly with respect to the control after shading at different stages. The most yield reduction (around 84 %) was found in S3 treatment, followed by S1 treatment (around 45 %) and S2 treatment (around 11 %) across hybrids and years. Kernel weight was most affected by S1 than by S2 or S3. Ear number per hectare was reduced more by S3 than by S2 or S1 (Table 4). The changes of yield components, inducing by shading, resulted in yield reduction of summer maize. There were no significant hybrid × shading treatment interaction effects on grain yield. Grain yields decreased significantly with respect to the control after shading at different stages.

The most yield reduction (around 92 %) was found in S3 treatment, followed by S1 treatment (around 55 %) and S2 treatment (around 19 %) across hybrids in 2011. In addition, biomass was significantly reduced by shading. The most reduction of biomass was found in S3 treatment by 52 %, followed by S1 treatment (around 35 %) and S2 treatment (around 28 %) in 2011. Similar results were found in both 2010 and 2011 (Table 4).

Discussion

Leaves are the most important organs by which plants utilize light energy and conduct photosynthesis. The size of leaf area generally reflects photosynthesis capacity. LAI plays an important role in crop production. However, our study indicated that the significant decreases in LAI occurred due to shading, which accelerated the senescence process, and negatively

Table 2 Effects of shading at 40 % of ambient sunlight from silking (R1) to physiological maturity (R6) (S1), the sixth leaf stage (V6) to R1 (S2), and seeding to R6 (S3) and ambient light conditions (CK) on leaf area index (LAI) and net photosynthetic rate (P_n) of summer maize at tasseling (VT) and milk stage (R3)

Hybrid (H)	Treatment (T)	VT		R3	
		LAI	P_n ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	LAI	P_n ($\mu\text{mol m}^{-2} \text{s}^{-1}$)
2010					
ZD958	CK	4.48 a	27.5 a	3.09 a	28.4 a
	S1	–	–	2.87 b	22.9 c
	S2	3.28 c	19.6 b	2.42 c	25.3 b
	S3	2.98 d	16.9 c	2.33 c	20.8 d
ANOVA	T	**	*	**	*
2011					
DH605	CK	3.70 a	29.1 a	3.38 a	33.2 a
	S1	–	–	3.15 a	27.0 c
	S2	3.16 b	17.0 b	2.90 b	32.0 b
	S3	2.99 b	12.0 c	2.66 c	22.5 d
ZD958	CK	4.29 a	25.5 a	3.39 a	25.4 a
	S1	–	–	3.02 b	19.9 c
	S2	3.30 b	17.6 b	2.86 c	23.3 b
	S3	3.18 b	15.4 c	2.88 c	17.1 d
ANOVA	H	NS	NS	NS	*
	T	*	*	*	**
	H × T	NS	NS	NS	*

S1 treatment was not applied at VT. Means within a column for each year, hybrid, and growth stage followed by the same letter did not differ at 5 % probability level. The values are means of three replicate blocks

NS not significant

*Significant at the 0.05 probability level; **significant at the 0.01 probability level

affected the photosynthetic properties. Chlorophyll plays an important role in photon absorption, transmission, and transportation and is closely related to P_n in leaves (Baig et al. 2005). Bell and Danneberger (1999) stated that weakened illumination due to short-term shading accelerated chlorophyll synthesis, by avoiding the damage to chloroplasts. However, under long-term shading, the chlorophyll content decreased and the green color of leaves faded. In our study, summer maize chlorophyll content at VT and R3 under shaded conditions was significantly lower than that of the control, which is consistent with the result of Zhang et al. (2007). The significant reduction in chlorophyll content induced by shading indicated that shading affected leaf photosynthesis of summer maize and weakened the photosynthetic assimilation capacity, resulting in a reduction in grain yield. The negative effects of shading on LAI and chlorophyll content resulted in a decline in leaf photosynthetic performance of summer maize, and thus led to the decrease of light-contracting product. Our study also showed leaf photosynthesis of summer maize was most susceptible to damage when shading occurred from seeding to R6 (S3), followed by shading from R1 to R6 (S1) and V6 to R1 (S2). However, at R3, P_n increased to near control levels upon removal of shading from V6 to VT, which indicated that summer maize could display a certain acclimation for long-term low-light conditions.

Chloroplasts are the main organelles for photosynthesis, of which ultrastructure is correlated with photosynthetic performance (Anderson 1982). Zhang and Li (2005) reported photosynthetic capacity was enhanced due to increased granum and grana lamella number and a more dense grana lamella arrangement. The integrity and arrangement of thylakoid structures play an important role in effective light energy conversion for photosynthesis (Bertamini et al. 2006). However, our study showed that chloroplasts were found to be damaged after shading; granum and grana lamella numbers per unit granum were reduced in response to the hypoplasia or disintegration in a portion of the granum. Some chloroplasts swelled to become round, and thylakoid structures swelled and were arranged in a disorderly manner. These damages of chloroplast indicated that the chloroplast has gradually lost the function of photosynthesis after shading, leading to the decrease of photosynthetic assimilation capacity, and thus resulted in a reduction in grain yield. Our results also indicated that most of chloroplast grana were poorly developed at VT, while the grana lamella was well-developed. However, some chloroplast internal structures were disordered and were dissolving gradually at R3. The grana lamellae in S1 and S2 were clear, but inattentive degrees were

Table 3 Effects of shading at 40 % of ambient sunlight from R silking (R1) to physiological maturity (R6) (S1), the sixth leaf stage (V6) to R1 (S2), and seeding to R6 (S3) and ambient light conditions (CK) on chlorophyll concentration of ear leaves ($\mu\text{g Fw cm}^{-2}$) at tasseling (VT) and milk stage (R3)

Hybrid (H)	Treatment (T)	VT			R3		
		Chl a	Chl b	Chl a + b	Chl a	Chl b	Chl a + b
2010							
ZD958	CK	51.0 a	19.3 a	70.3 a	54.9 a	17.5 a	72.4 a
	S1	–	–	–	50.7 b	16.1 a	66.8 b
	S2	46.3 bc	14.3 b	60.6 b	47.0 c	14.2 b	61.2 c
	S3	42.6 c	13.2 b	55.8 c	46.8 c	13.8 c	60.6 c
ANOVA	T	**	*	**	*	*	**
2011							
DH605	CK	53.3 a	14.8 a	68.1 a	55.3 a	16.3 a	71.5 a
	S1	–	–	–	53.1 a	15.9 a	69.1 a
	S2	36.8 b	10.0 b	46.8 b	51.2 b	13.9 b	65.2 b
	S3	28.9 c	8.4 b	37.3 c	46.6 c	13.2 b	59.8 c
ZD958	CK	52.4 a	15.1 a	67.5 a	64.8 a	19.0 a	83.8 a
	S1	–	–	–	51.6 b	15.0 b	66.6 b
	S2	40.9 b	12.0 b	52.9 b	51.7 b	14.5 b	66.2 b
	S3	35.5 c	9.9 c	45.4 c	46.2 c	13.4 c	59.6 c
ANOVA	H	NS	NS	NS	NS	NS	NS
	T	*	*	*	*	*	*
	H × T	NS	NS	NS	NS	NS	NS

S1 treatment was not applied at VT. Means within a column for each year, hybrid, and growth stage followed by the same letter did not differ at 5 % probability level. The values are means of three replicate blocks

NS not significant

*Significant at the 0.05 probability level; **significant at the 0.01 probability level

higher, while the granum became twisted and gaps among lamellas increased. Illumination is necessary for the normal development of grana lamella. The asynchronous development of chloroplasts could make sure it that plants were able to conduct photosynthesis and form assimilation products under shaded conditions (Mitsuya et al. 2000).

Burkey and Wells (1991) and Kusaba et al. (2007) pointed out that a portion of cell membranes would be damaged by shading stress, causing apoptosis and loss of photosynthetic capacity. Our data also showed that plasmolysis in mesophyll cells of summer maize was obvious, and that endomembrane systems were destroyed, and other membrane structures that surrounding cell, mitochondria, and nucleus were dissolved gradually under shading conditions. The cause of membrane damage and destruction was most likely to increased free-radical levels under environmental stress (Yu et al. 2011). Additionally, respiration was influenced by the dissolved mitochondrial membrane structures and mitochondria appeared as “empty bubbles.” These changes would counteract normal photosynthesis, which depends on respiration, and vice versa. Therefore, damage to mitochondria further reduced photosynthesis in plants. Visibly, the damaged mesophyll cell ultrastructure of functional leaf induced by shading would lead to

the decline of photosynthetic ability, and thus resulted in a significant yield reduction of summer maize.

The intensity and duration of sunlight is important to obtain high and stable yields of maize. Increased shading during the reproductive period of corn development decreased more grain yield than during the vegetative growth stages (Earley et al. 1967; Zhang et al. 2007). In our study, yield reduction decreased in the order: season-long shading > shading from R1 to R6 > shading from V6 to R1. Thus the effects of shading after silking on yield were more significant than those before silking. Dry matter accumulation is the basis of grain yield formation. However, summer maize biomass was significantly reduced by shading, resulting in a significant yield reduction of summer maize. Assimilate supply to the developing ear of maize is an important determinant of grain yield (Reed et al. 1988; Zhang et al. 2007). Under limiting light, the activity of biochemical carbon-concentrating mechanism generally decreased, causing an increase in leakiness (Φ), the ratio of CO_2 retrodiffusing from the bundle sheath relative to C_4 carboxylation processes (Bellasio and Griffiths 2014). Our study indicated that effects of shading on photosynthetic characteristics and mesophyll cell ultrastructure of summer maize significantly affected dry matter accumulation and distribution, and then influenced the formation of grain yield. On the other

Table 4 Effects of shading at 40 % of ambient sunlight from silking (R1) to physiological maturity (R6) (S1), the sixth leaf stage (V6) to R1 (S2) and seeding to R6 (S3) and ambient light conditions (CK) on biomass, grain yield and yield components of summer maize

Hybrid (H)	Treatment (T)	No. of rows per ear	No. of kernels per row	Kernel weight (1000 g per kernel)	Ears per hectare	Grain yield (kg ha ⁻¹)	Biomass (kg ha ⁻¹)
2010							
ZD958	CK	15.0 a	37.1 a	332 a	58,065 a	10,728 a	22,250 a
	S1	13.9 c	21.3 c	252 c	39,392 c	2939 c	14,777 c
	S2	14.3 b	30.5 b	295 b	50,504 b	6498 b	17,462 b
	S3	13.7 c	4.2 d	303 b	19,272 d	336 d	10,579 d
ANOVA	T	*	**	*	**	**	**
2011							
DH605	CK	16.1 a	32.2 a	321 b	60,735 a	10,107 a	19,087 a
	S1	15.5 b	14.6 c	307 c	59,072 b	4104 c	13,105 b
	S2	15.4 b	28.3 b	329 b	59,992 c	8602 b	13,302 b
	S3	14.4 c	4.8 d	336 a	27,600 d	641 d	8162 c
ZD958	CK	14.9 a	32.8 a	335 a	60,951 a	9979 a	21,372 a
	S1	14.7 a	20.5 b	289 b	55,471 b	4831 c	13,268 b
	S2	14.5 a	32.0 a	324 a	51,638 c	7763 b	15,725 b
	S3	13.2 b	7.3 c	343 a	30,861 d	1020 d	7409 c
ANOVA	H	*	NS	NS	NS	NS	NS
	T	*	**	*	**	**	**
	H × T	*	NS	NS	NS	NS	NS

Means within a column for each year, hybrid, and growth stage followed by the same letter did not differ at 5 % probability level. The values are means of three replicate blocks

NS not significant

*Significant at the 0.05 probability level; **significant at the 0.01 probability level

hand, shading from V6 to VT and from VT to R6 decreased significantly ear number and kernel weight, the effect of shading from VT to R6 on kernel weight was much higher than that of shading from V6 to VT. Ear number reduction should be owing to the effects of shading on tassel and ear differentiation, fertilization, and kernel set rate. Decreased kernel weight may be caused by the effects of shading on grain yield formation and accumulation and distribution of dry matter. In further, the effects of shading on spike differentiation and grain yield formation of summer maize would be examined.

Conclusions

After shading, chloroplast arrangement dispersed in mesophyll cells and thylakoid structure was destroyed, causing reduced chlorophyll synthesis per unit leaf area. The damaged membrane systems and mitochondria were primarily responsible for the decreased photosynthetic capacity. The decline of photosynthetic capacity induced by shading resulted in a significant reduction of grain yield of summer maize. Summer maize was most susceptible to damage when shading occurred from seeding to physiological maturity stage, followed by

shading from silking stage to physiological maturity stage, and shading from the sixth leaf stage to silking stage.

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