Pak. J. Bot., 49(5): 1681-1688, 2017.

PHOTOSYNTHESIS AND CHLOROPHYLL FLUORESCENCE REACTION TO DIFFERENT SHADE STRESSES OF WEAK LIGHT SENSITIVE MAIZE

JIAN WANG[1Ɵ], HAIJIAO HUANG[2Ɵ], SEN JIA[3], XUEMEI ZHONG[1*], FENGHAI LI[1*], KUANGYE ZHANG[1] AND ZHENSHENG SHI[1]

[1]*Special Corn Institute, Shenyang Agricultural University, Shenyang 110866, P.R. China*
[2]*Tibet Academy of Agricultural and Animal Hubbandry Sciences, Lhasa 850000, P.R. China*
[3]*Department of Agronomy, Suihua University, Suihua 152061, P.R. China*
[Ɵ]*Indicates the authors who contributed equally to this study*
[*]*Corresponding author's email: xuemei.zhong@163.com; lifenghai@126.com*

Abstract

A split-plot experimental study was conducted to evaluate the effect of different shade stresses on photosynthesis and chlorophyll fluorescence of maize leaves.The experiment was designed on the south farm of Special Corn Institute, Shenyang Agricultural University, China.Data was collected from the day maize tasseled (Jul. 21) to the beginning of grouting (Aug.12) under 18%, 28%, 38%, 60%, and 75% shade stress to determine indexes such as photosynthesis and chlorophyll fluorescence after 15 days of shade treatment. Pairs of near-isogenic lines (NILs) of Shennong 98A (a barren stalk inbred line) and Shennong 98B (an un-barren stalk inbred line) were used as experimental materials to further reveal photosynthetic mechanisms of weak light sensitive maize when exposed to weak light conditions. Thus, a foundation was establishedfor high density-resistant (shade resistant) corn breeding,while identifying weak light sensitive varieties. After shading treatment, chlorophyll a and total chlorophyll content of both varieties increased, chlorophyll b content first increased, followed by a decrease, while the net photosynthetic rate and stomatal conductance showed a gradually decreasing trend. The changing trends of photochemical quenching coefficient(q_P) and effective quantum yield of PSII photochemistry (Φ_{PSII})were similar, Φ_{PSII} and q_P increased significantly as shading stress increased from 18% to 38%;however, Φ_{PSII} and q_P declined significantly under 60% and 75% shading stresses. The changing trend of NPQ was opposite to Φ_{PSII} and q_P. A comparison of both inbred lines showed that photosynthesis and chlorophyll fluorescence characteristics of Shennong 98B were superior to Shennong 98A. This study revealed the relationships between weak light sensitive lines and shade intensities by comparing differences in photosynthesis and chlorophyll fluorescence parameters.

Key words: Barren stalk, Lines, Shade, Photosynthesis, Chlorophyll fluorescence.

Introduction

Maize (*Zea mays* L.) is one of the most important food crops in the world, and is occupying an important position in agriculture. As a C4 plant, it has higher photosynthetic rates than C3 plants (Ehleringer & Pearcy, 1983; Pearcy & Ehleringer, 2010). Furthermore, the metabolism of maize is highly sensitive to limited light intensitiesdue to specific anatomical, biochemical, and energetic complexities (Ubierna *et al.*, 2013). Climatic factors, especially light conditions play an increasingly important role for maize production (Brown, 2013; Lobell *et al.*, 2011; Xiao & Tao, 2016). Light intensityin particular plays a leading role for high-yield maize with no apparent light saturation point (Early *et al.*, 1967). During the growing and developing stage, maize often encounters cloudy days and days of scant-sunlight, resulting in decreased photosynthetic capacity, reduced spikelet fertility, and even barren stalks (Kiniry & Ritchie, 1985; Zhong *et al.*, 2011; Zhong *et al.*, 2014). Therefore, maize must have the strong ability to adapt to different light conditions in field production. One of these abilities is a weak light adaptability, which means that maize still retains high photosynthetic efficiency under scant light conditions. Up to now, many domestic and international studies have focused on how adverse weather affects maize production (Tollenaar & Daynard, 1978;Ubierna *et al.*, 2013; Cui *et al.*, 2015). In most experiments, an artificial shade canopy has been applied to control illumination intensity. Many studies showed that, under shady condition, leaf number changed slightly, leaves were thinner (Struik, 1983), and the specific area was increased significantly (Li *et al.*, 2015). Maize grain yield decreased due to shading, while the maize production capacity has been shown to reduce even due to short-term shading. This was particularly true for grain yield with the reducing degree being closely related to the shading period (Wang *et al.*, 2008;Cui *et al.*, 2015; Zhang *et al.*, 2006). Light intensity had significant influence on leaf photosynthetic rate, transpiration rate, stomatal conductance, light compensation point and light saturation point (Bellasio& Griffiths, 2014;Li *et al.*, 2007; Ubierna *et al.*, 2013). Therefore, the study of light stress, especially weak light stress, has important practical significance for the maize production. Due to the light requirement characteristics of maize, the level of shading, the shading period, and the time length of weak light stress exposure changed the influence of week light stress on maize. Different light sensitive maize responded differently to weak light stress, and to some degree, the difference in the weak light sensitivity of maize is related to the shade-tolerance of maize via an inverse proportion: more sensitivity of maize to weak light will result in decreased shade-tolerance, and vice versa. Pairs of near–isogenic lines (NILs) of Shennong 98A (a barren stalk inbred line) and Shennong 98B (an un-barren stalk inbred line) were used to reveal different shade stress reactions in photosynthesis and fluorescence characteristics of weakly light sensitive maize. Detecting changes of physiological indexes (changes of photosynthetic physiological indexes in particular) further explained photosynthetic mechanisms of weak light sensitive maize in weak light weather. Screening comprehensive indexes that could analyze weak light sensitive maize, formed a foundation for high density-resistant (shade-resistant) corn breeding, while identifying weak light sensitive varieties.

Materials and Methods

Experimental design: The experiment was conducted at the south farm of the Shenyang Agricultural University (41°49' N, 123°4' E), an area that belongs to the northern temperate zone, with monsoon-affected semi-humid continental climate. The average annual temperature was 8°C, the annual average rainfall was 628 mm, and the frost-free period was 150-170 days (Zhong *et al.*, 2014). Asplit-plot experimental design was utilized with three replications, using inbred lines and shade intensities separately as main plots and subplots. The fundamental nutrient content of the tested soil was 26.59 g kg^{-1} of organic matter, 2.35 g kg^{-1} of total nitrogen, 108.75 mg kg^{-1} of alkaline hydrolysed nitrogen, 11.19 mg kg^{-1} of available phosphorous, and 102.83 mg kg^{-1} of available potassium. Shennong 98A is a barren-stalk defective inbred in weak light, and Shennong 98B is a spikes well inbred through previous field investigation. Both were pairs of near-isogenic lines of maize and were adopted for test, bred by the Institute of Specialty Corn of the Shenyang Agricultural University. Planting density was 60,000 plants hm^{-2}.

Shading intensity:18%, 28%, 38%, 60%, and 75% shading intensities were used, and natural light was used as control. The plot area was 3.6 m x 4.0 m. The shade treatment was started from the day maize tasseled (Jul. 21) and lasted to the beginning of grouting (Aug. 12). The experiments were conducted in the field, adopting a method of installing a shade shelter. The average height from the shade shelter ceiling to the canopy was 1 m, which ensured that the temperature was identical both under and outside the shade shelter (Table 1).

Field microclimate: The photometric quantity, CO_2 density, andrelative humidity wereobserved via TES-1335 illuminometer and Li-6400 portable photosynthesis apparatus (LI-COR Inc., Lincoln, NE, USA) at 10:00 am. A normal thermometer and geothermometer (HG04-2, Tuopu Inc., Zhejiang, China) were used to detect the temperatures of canopy, surface, and 20 cm below the surface.

Chlorophyll contents: Chlorophyll contents were determined 15 days after shading treatments. 80% acetone solution was used to extract the ear leaf and to determine the absorbance at 663 nm and 646 nm. Chlorophyll a, Chlorophyll b, total chlorophyll content, and chlorophyll a/b values were calculated according to formula $C_a = 12.21\times D_{663} - 2.81\times D_{646}$, $C_b = 20.13\times D_{646} - 5.03\times D_{663}$, $C_t = C_a + C_b$, and$C_v = C_a/C_b$.

Photosynthetic parameters: The photosynthetic parameters of ear leaves were measured 15 days after shading treatments, including the net photosynthetic rate (P_N), intercellular CO_2 concentration(C_i), stomatal conductance(G_s), and transpiration rate(E), using the Li-6400 portable photosynthesis system (LI-COR *Inc.*, Lincoln, NE, USA) on a sunny day from 9:00 am to 11:30 am. Three plants were measured, and every measurement was repeated thrice. A light response curve was determined under the condition of a CO_2 flow rate of 500 μmolm^{-2} s^{-1}, leaf chamber temperature 25°C, and PFD range of 0-1800 μmol m^{-2} s^{-1}. Regression method was used to calculate the light saturation point and light compensation point.

Chlorophyll Fluorescence Parameters: The basis of fluorescence under dark adaptation (F_o), maximum fluorescence (F_m), and the optical system II (Φ_{PSII}) photochemical efficiency (F_v/F_m) of ear leaves were measured with the Li-6400 portable photosynthesis system. Actual photochemical efficiency (Φ_{PSII}), electron transport rate (ETR), photochemical quenching (q_P), and nonphotochemical quenching (NPQ) were calculated according to the following formulas $(F_m' - F_s)/F_m'$, $\Phi_{PSII} \times PAR \times 0.5 \times 0.84$, $(F_m' - F_s)/(F_m' - F_0')$ and $F_m/F_m' - 1$, respectively. Three plants were measured, and every measurement was repeated thrice. The leaves were placed under a dark-adaptation state (DAS) for 30 min using light exclusion clips prior to measurement. Clips were randomly sampled at the centre of the leaves, but not at the nervure.

Data analysis: Microsoft Excel and Origin8.0 were used for data processing and mapping, and SPSS12.0 software was used to analyze significance. $p<0.05$ was considered a significant difference (Steel *et al.*, 1997).

Table 1. Effect of different shading treatments on microclimate of maize population.

Shading intensity [%]	Light intensity [lux]	CO_2 concentration [μmolmol^{-1}]	Relative humidity [%]	Temperature at different positions [°C]		
				Top canopy	Surface	Ground
0(CK)	75871.25 ± 2.00a	368.28 ± 1.03a	69.18 ± 0.56a	29.05 ± 0.45ab	26.40 ± 0.07a	22.67 ± 0.47a
18	62214.22 ± 1.03b	368.35 ± 0.53a	68.72 ± 0.34a	29.18 ± 0.42ab	26.30 ± 0.19a	22.18 ± 0.35a
28	54627.12 ± 1.17c	368.37 ± 0.43a	68.95 ± 0.56a	29.09 ± 0.38ab	25.00 ± 0.34ab	22.08 ± 0.02a
38	47291.67 ± 1.23d	368.52 ± 1.05a	67.84 ± 0.53a	29.32 ± 0.37ab	25.13 ± 0.13ab	22.10 ± 0.43a
60	30716.67 ± 1.00e	368.59 ± 0.65a	68.40 ± 0.49a	29.45 ± 0.37a	25.27 ± 0.25ab	21.60 ± 0.52a
75	17346.60 ± 0.79f	368.63 ± 0.57a	73.00 ± 0.49a	28.85 ± 0.42b	23.70 ± 0.17b	21.57 ± 0.39a

0, no shading; 18%, 28%, 38%, 60% and 75%, shaded respectively by sunshade nets with 18%, 28%, 38%, 60% and 75% shading rates. Values followed by different letters within the same column mean significant difference at 5% level.

Table 2. The effects of shading on maize leaf chlorophyll content.

Shading treatment	C_a [mg g^{-1}]		C_b [mg g^{-1}]		C_{a+b} [mg g^{-1}]		C_a/C_b [mg g^{-1}]	
	Shennong 98A	Shennong 98B	Shennong 98A	Shennong 98B	Shennong 98A	Shennong 98B	Shennong 98A	Shennong 98B
Control	2.348±0.04a	2.362±0.03a	0.795±0.03a	0.743±0.02a	3.143±0.06abc	3.105± 0.5a	2.960± 0.05a	3.179±0.03a
18%	2.318±0.13a	2.425±0.03ab	0.818±0.10a	0.869±0.02bc	3.136±0.23abc	3.294±0.04b	2.893±0.18b	2.790±0.04bc
28%	2.397±0.05a	2.444±0.04ab	0.841±0.05a	0.897±0.03b	3.239±0.09ab	3.342±0.07b	2.867±0.10b	2.731± 0.06c
38%	2.384±0.08a	2.394±0.04b	0.880±0.03a	0.841±0.04bc	3.264±0.06a	3.235±0.09ab	2.714±0.05ab	2.860±0.10b
60%	2.529±0.03a	2.564±0.08b	0.808±0.01a	0.802±0.03ab	3.337±0.03bc	3.366±0.11b	3.131±0.03b	3.196±0.09a
75%	2.597±0.08a	2.772±0.01c	0.806±0.02a	0.841±0.01bc	3.404±0.09c	3.613±0.02c	3.221±0.01c	3.298±0.03a

Vertical bars denote SE (n = 5). Small letters indicatedifferences under different shade stresses at p<0.05, according to theleast significant difference (LSD) test

Table 3. The changes of photosynthetic indexes of maize leaves under different shading treatments.

Shade intensity (%)	P_N [μmol m^{-2} s^{-1}]		G_s [mol (H_2O) m^{-2} s^{-1}]		C_i [μmol(CO_2) mol^{-1}]		L_s	
	Shennong 98A	Shennong 98B	Shennong 98A	Shennong 98B	Shennong 98A	Shennong 98B	Shennong 98A	Shennong 98B
CK	22.50±1.21a	24.60±1.01a	0.21±0.05a	0.22±0.07a	87.61±5.29a	67.77±3.98a	0.78±0.04a	0.80±0.01a
18	21.07±4.73a	25.03±1.27a	0.17±0.08a	0.22±0.06a	62.32±0.54a	68.77±4.68a	0.80±0.03a	0.82±0.03a
28	20.32±3.63a	20.05±1.10b	0.19±0.04ab	0.21±0.12a	97.12±4.62b	94.73±6.65b	0.73±0.03a	0.68±0.07a
38	15.40±0.12b	20.05±1.45b	0.13±0.01bc	0.17±0.18a	97.55±0.14b	85.13±3.60ab	0.72±0.02a	0.76±0.03a
60	9.23±3.41c	10.83±1.80c	0.07±0.03cd	0.08±0.01b	90.32±2.62b	89.42±0.82ab	0.74±0.01ab	0.75±0.02a
75	5.29±1.82d	7.41±1.39c	0.04±0.02d	0.06±0.01b	117.17±1.41b	106.82±5.53b	0.66±0.01b	0.69±0.02a

Vertical bars denote SE (n = 5). Small letters indicate differences under different shade stresses at p<0.05, according to the least significant difference (LSD) test

Results

The effects of shading on maize leaf chlorophyll content: Chlorophyll content was closely related to shade-tolerance in plants. High chlorophyll content of plants was associated with high shade-tolerance, and vice versa. Therefore, the chlorophyll content is an important index to judge how much of the weak light is utilized in maize. Chlorophyll a is given priority to absorb long wave blue-purple light, while diffuse light, and scattered light, which are two types of short wave light, can be effectively absorbed by chlorophyll b. A reduction of chlorophyll a/b is beneficial for the absorption of red light, so that the plants can thrive in weak light conditions (Zhang *et al.*, 2012; Milne *et al.*, 2015). The illumination intensity directly influences the formation and distribution of pigments (Hamamoto *et al.*, 2000). Our experiment showed that the chlorophyll content difference was not apparent in these pairs of near-isogenic lines: Shennong 98A (a barren stalk inbred) and Shennong 98B (an un-barren stalk inbred) under normal light conditions (Table 2; Fig. 1).After shading treatment, chlorophyll a and total chlorophyll content of both lines were increased, and the degree of increase was proportionate to the increase of shading intensity, with a maximum below 75% shading intensity.In response to different shading stresses,chlorophyll b content of Shennong 98A and Shennong 98B showed a trend of rising increasing and then falling and below 38% shading intensity, a maximum of 0.880 mg g^{-1} and 0.841mg g^{-1} appeared, respectively. As we continued to increase shading intensity, chlorophyll b content increased at a lower rate, but still remained higher than the control. When comparing varieties, chlorophyll content of Shennong 98B was higher than that of Shennong 98A, suggesting that Shennong 98B had a strong adaptability to shading stress, thus absorbance in shading environment could be increased by improving the synthesis of chlorophyll, while the shading sensitive variety had a weak adaptability to shading stress.

Chlorophyll content (especially chlorophyll b content) increased significantly in response to 18%, 28%, and 38% shade condition, and the effect of slight shading on chlorophyll b was greater than that on chlorophyll a, which led to a lower chlorophyll a/b than in the control. When under 60% and 75% shading, chlorophyll a content increased rapidly, and chlorophyll b content increased at an accelerated rate, especially for Shennong 98A with a significant increase, leading to an apparently lower chlorophyll a/b value of Shennong 98A than for the control. The result showed that the effect on chlorophyll b was greater than that of chlorophyll a under a condition of slight shade (18%–38% shade condition), while under moderate and severe shading (60% and 75% shading condition), the results were reversed.

The influence of shading on maize leaf photosynthetic rate and related parameters: Differences in photosynthetic rate were found in the different maize varieties (Zhao *et al*., 1999; Li *et al*., 2007). Table 3 shows that under both natural and shade stresses, the weak light sensitive line Shennong 98A, had a slightly lower net photosynthetic rate than Shennong 98B. In addition to the 18% and 28% shade stress, net photosynthetic rate decreased significantly in the remainder of the treatment, and the more severe the shading was, the greater the observed decline was. In response to 28% shading treatment, weak light sensitive varieties Shennong 98A and its near-isogenic line Shennong 98B showed small differences in net photosynthetic rate (P_N). As the shading intensity continuously increased to 38%, the photosynthetic rate of Shennong 98A was lower than that of Shennong 98B. Compared to the 38% shading condition, the net photosynthetic rate (P_N) of both lines decreased significantly while under 60% and 75% shading intensity; however, the difference was not obvious between both lines. The above explained that the main reason for the decreasing net photosynthetic rate (P_N) was the light intensity, and the difference of weak light adaptability was determined by the photosensitive properties.

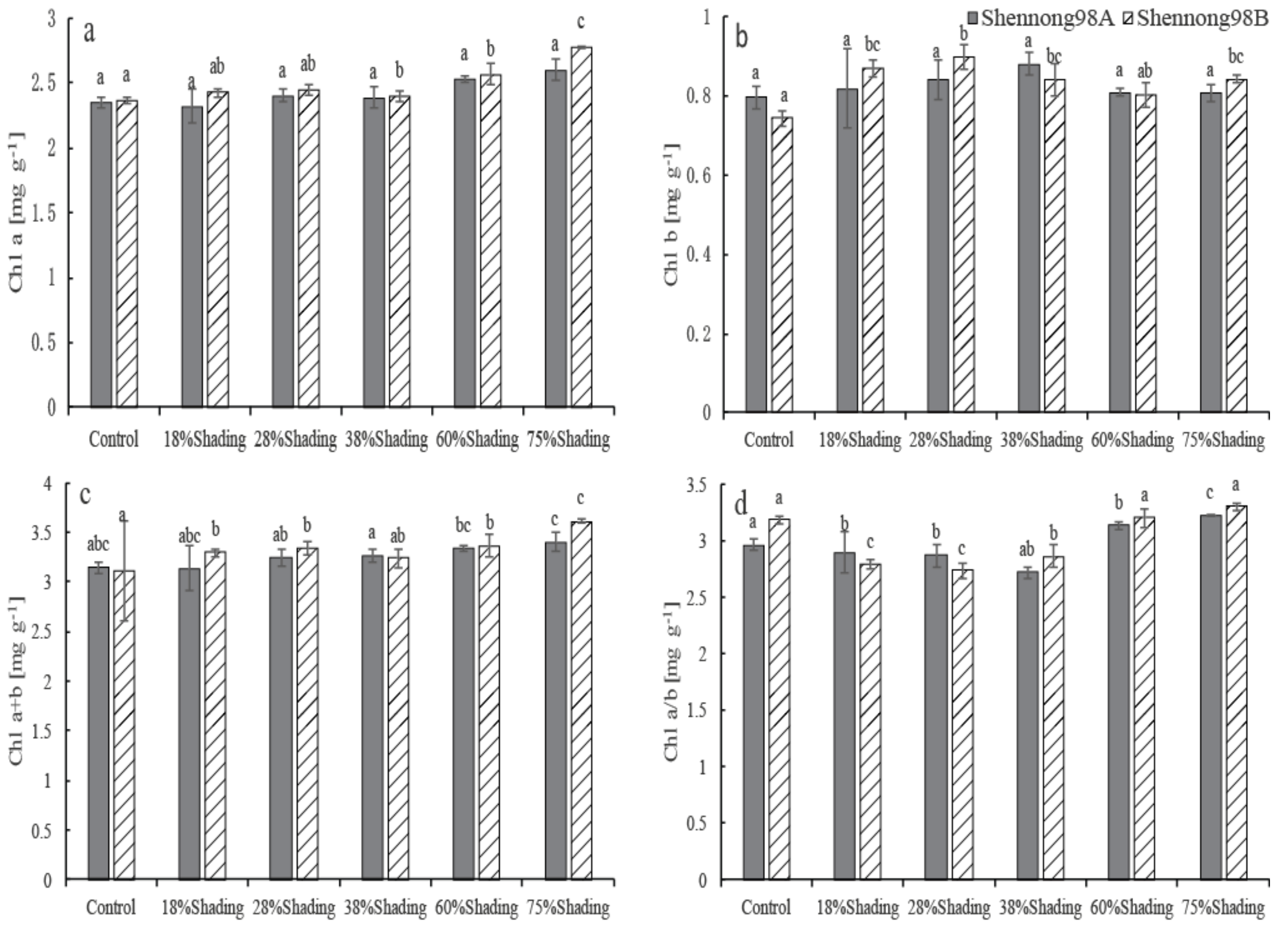

Fig. 1. The effects of shading on maize leaf chlorophyll content. Vertical bars denote SE (*n* = 5).

In comparison to the photosynthetic rate and its related photosynthetic parameters, it could be found that the stomatal conductance (G_s) of the leaves correlated positively with the photosynthetic rate, and the concentration (C_i) correlated negatively with the cell gap CO_2 concentration, except for the 18% shading condition. The main reason for this decrease of photosynthesis rate was stomatal limitation or non-stomatal limitation, and the key index was C_i. The reason for the observed C_i decrease and L_s increase was stomatal limitation; however, if C_i increased and L_s decreased, the reason was non-stomatal limitation (Farquhar& Sharkey, 1982). The C_i of Shennong 98A decreased, and L_s increased under the 18% shading condition, and the L_s of Shennong 98A continued to decrease, while C_i began to increase, which showed that the main reason for the reduction of the photosynthetic rate was stomatal limitation transferred to non-stomatal limitation.

Effects of shading on photosynthetic curves and related parameters on maize leaves: The response of different treatments and varieties to the light intensity and the natural light was basically identical, and the photosynthetic rate increased with increasing light intensity (Fig. 2). When the light intensity moved beyond a certain range, the photosynthetic rate flattened out or had a tendency to decrease. With increasing shade intensity, the light response curves of each treatment were lower than the control, and a higher shading intensity caused a lower light response curve.

The apparent quantum efficiency (AQY) and the light compensation point (LCP) reflect the light energy use efficiency in plants, especially the weak light utilization efficiency, which is one of the important indexes of plant resistance to negative stimuli (Demming *et al*., 1996; Buykey & Wells, 1991). Under natural light conditions (Table 4), the light saturation point (LSP), light compensation point (LCP), maximum net photosynthetic rate (P_{max}), and apparent quantum efficiency (AQY) of Shennong 98A were lower than those of its near-isogenic lines Shennong 98B. The maximum net photosynthetic rates (P_{max}) of both lines were significantly lower than those of the control after shade stress. The P_{max} of Shennong 98A was lower than Shennong 98B in each treatment, and the decreasing range was always higher than that of Shennong 98B. With the increase of shading intensity, the light compensation point (LCP) of maize declined, and apparent quantum efficiency (AQY) first increased and then decreased. Under the 18% and 28% shade stress, the LCP of Shennong 98B changed little, while the LCP of Shennong 98A declined significantly under 28% shading intensity. The LCP of Shennong 98A was lower than that of Shennong 98B in each treatment, which showed that Shennong 98A could photosynthesize during a relatively weaker light intensity. The tendency of the apparent quantum efficiency (AQY) was different: under 28% shading condition, the AQY of Shennong 98A was maximal and then it began to decrease, while the AQY of Shennong 98B reached its maximum under 38% shading stress. Although Shennong 98A can photosynthesize under a weak light environment, its photosynthetic efficiency had always been lower than that of Shennong 98B.

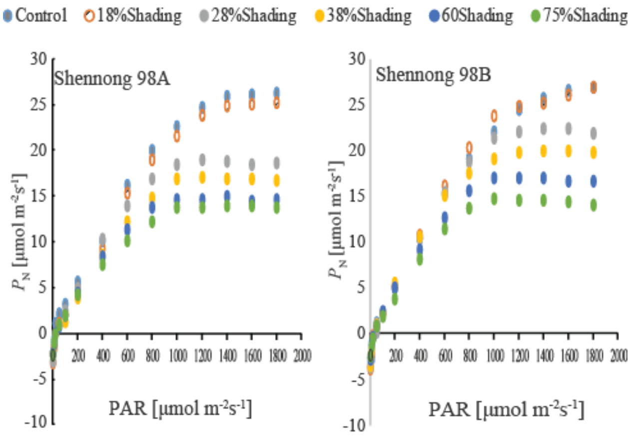

Fig. 2. Photosynthetic response curves of Shennong 98A and Shennong 98B leaves under different shading treatments.

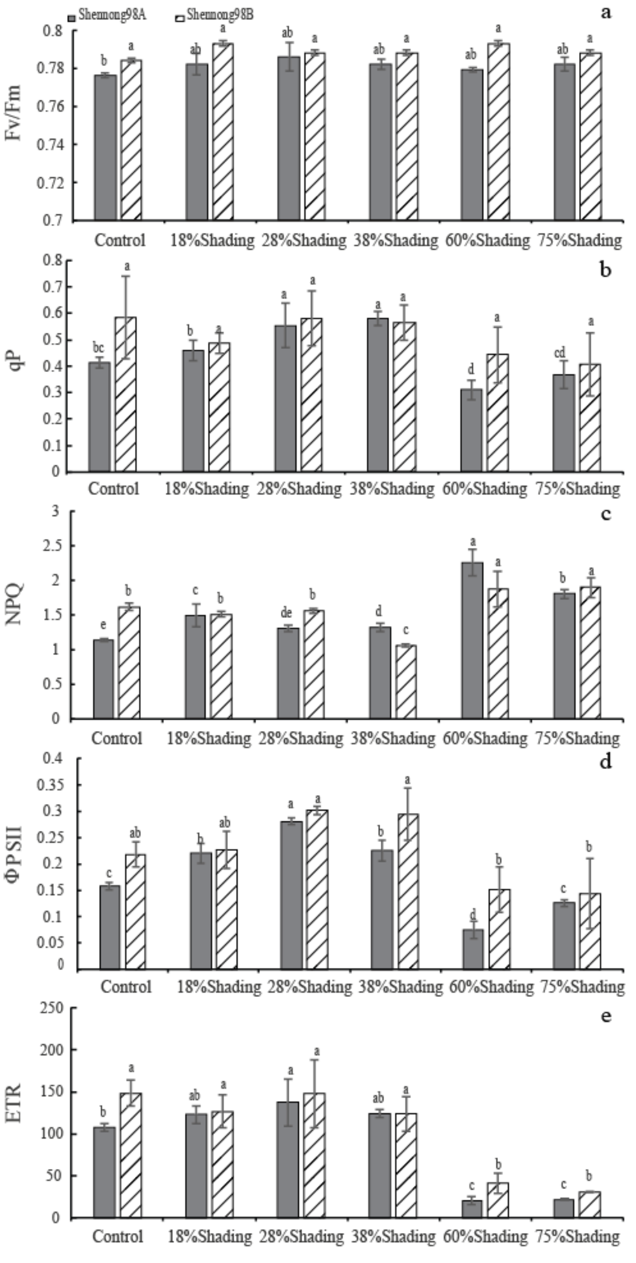

Fig. 3. Variation in characteristics of F_v/F_m, NPQ, q_P, Φ_{PSII} and ETR in Shennong 98A and Shennong 98B under different shade stresses. Vertical bars denote SE (*n* = 5).

Effect of shading on chlorophyll fluorescence parameters of maize leaves: F_v/F_m represents the rate of photochemical conversion efficiency and potential activity in plant leaves (Demmig-Adams *et al.*, 1990; Falbel *et al.*, 1994). Under shading condition, the maximal photochemical efficiency (F_v/F_m) analysis showed that the F_v/F_m of Shennong 98B increased significantly with increased shading stress, and Shennong 98A also showed an increasing trend, but this was not obvious. F_v/F_m did not decrease, but remained higher than the control under short-term shading stress. Under shading stress, the maximum light conversion efficiency of PSII reaction center could be increased to reduce the damage of maize. Comparing the F_v/F_m of both lines (both under natural light or shade stress), the maximum photochemical efficiency (F_v/F_m) of Shennong 98A was always lower than that of Shennong 98B. This showed that the F_v/F_m response to the weak light stress differed in different light sensitive varieties, indicating that the weak light physiological regulation mechanism of shade-tolerant varieties was better than that of weak light sensitive varieties.

Chlorophyll fluorescence quenching is a method of dissipating excess energy, and it can be divided into photochemical quenching and non-photochemical quenching (Schreiber *et al.*, 1986). The fluorescent chemical quenching coefficient (q_P) is commonly used to indicate the openness of the PSII reaction center, as it reflects the efficiency of the conversion of light energy into chemical energy via the PSII antenna pigment. NPQ refers to the non-photochemical quenching coefficient, i.e. the absorption of light energy by the PSII antenna pigment cannot be used in photosynthetic transfer, but dissipates in the form of heat, and this dissipation of excess light energy is reflected by the photosynthetic system (Krause & Weis, 1991). The changes of chlorophyll fluorescence quenching were different in both inbred lines in response to different shading stress (Fig. 3). When shading stress was increased from 18% to 38%, q_P increased while NPQ was decreased, which showed that, under mild to weak light stress, the light energy was captured by the PSII antenna pigment to increase the photochemical electron transport, while reducing the harm of weakened intensity on the photosynthetic system, representing the effects on photosynthetic rate, to ensure normal photosynthetic conduct. When the shade intensity was increased to 60% and 75%, q_P decreased significantly, while NPQ increased significantly, which illustrated that the shading intensity had been beyond the shade-tolerance range of the line. Comparing both lines, regardless of whether the plants were subjected to natural light or shade stress condition, q_P of Shennong 98A was generally lower than that of Shennong 98B.

The actual photochemical efficiency (Φ_{PSII}) reflects the photochemical efficiency of PSII when its reaction center was closed, which reflects the PSII actual photochemical reaction activity (Govindjee, 2002). The changing trend of Φ_{PSII} was similar to the changing trend of qP (Fig. 3). During mild shading (18%–38% shading), Φ_{PSII} increased, especially the Φ_{PSII} of Shennong 98A increased significantly ($p<0.05$); to take full advantage of the weak light intensity, the absorbed light energy was mainly used for the photochemical reaction. Φ_{PSII} declined significantly under 60% and 75% shading stress. ETR reflected the efficiency of the apparent electron transfer under the actual light intensity, which had a strong linear relationship with the photosynthetic rate. When under

60% and 75% shading stress, the ETR of pairs of near-isogenic lines both reduced significantly, the electron transfer rate decreased, and almost in the stop state. This may be one of the important reasons for the rapid decline of P_N under 60% and 75% shading stresses.

Discussion

Effect of shading on photosynthesis of weak light sensitive cultivars: Environmental factors such as temperature, illumination, and precipitation are essential to plant growth. Plants absorb light energy for photosynthesis, which is where the energy conversion and material accumulation happen. For agricultural production, the effective solar radiation impact on crop yield was more significant than that of temperature and precipitation (Xiao & Tao, 2016). During the last 40–50 years, the Chinese climate change has already become an indisputable fact. From 1960 to 2000, the duration of effective solar radiation reduced by 1.28% on average every ten years (Che *et al.*, 2005). For Liaoning, the annual sunshine time of the entire province is 2216.0 to 2942.1 h, decreasing from the west to the east. However, it is detrimental to agriculture that the hours in all areas of Liaoning are decreasing at maximal values of 40 h per decade (Gong *et al.*, 2010; Ye *et al.*, 2012; Ren *et al.*, 2005). Lighting is one of the main environmental factors for crop growth and development and it is very important to enhance yield and improve quality (Cui *et al.*, 2015). According to many studies, sunless weather or shading during the reproductive growth stage (especially the filling time) will reduce maize yield by a large margin (Early *et al.*, 1967; Gerakis & Tasopoulou *et al.*, 1980). Furthermore, the reduction was closely related to grain number per spike (Early *et al.*, 1967; Kiniry & Ritchie, 1985; Reed *et al.*, 1984; Hashemi-Dezfouli & Herbert, 1992). All of this has suggested that the deficiency of sunshine will significantly influence food security. Domestic and foreign scholars have conducted various experiments to this regard; however, the test materials used in these experiments were of more general varieties and inbred lines, which inevitably results in interference due to differences in the genetic background of experimental varieties. During years of breeding, we discovered and bred one pair of barren stalk (Shennong 98A) and non-barren stalk near-isogenic lines (Shennong 98B); the former was prone to barren stalk in low light conditions, while the latter was normal. This has provided a good foundation for the study of the photosynthetic characteristics of varieties under weak light condition.

The size of P_N is an important indicator of the photosynthetic capacity of plants. Light intensity reduction was the main reason that led to the decline of net photosynthetic rate (P_N), while the difference of weak light adaptation was determined by the photosensitivity of the varieties. Zhong *et al.* (2014) reported the genetic factor to be the main reason for the weak light sensitivity in some maize lines. The genetic defects of weak light sensitive varieties eventually led to a sharp decrease of yield. Li *et al.* (2010) suggested that the persistent change of the light intensity causes severe fluctuations of grain weight and grain number per spike. This study found that the net photosynthetic rate of the weak light sensitive species Shennong 98A was lower than that of Shennong 98B, regardless of natural light or shade stress conditions. 38% of shade stress was the boundary of light intensity that led to a significant difference between the low-light-sensitive line Shennong 98A and its near-isogenic line Shennong 98B Pn (Ward & Woolhouse, 1986). Some studies showed that the decrease of the maximum photosynthetic rate (P_N) under shading condition was not caused by stomatal limitation, since weak light did not reduce the CO_2 concentration in the cells (Ward & Woolhouse, 1986). The results of this study suggest that the decrease of photosynthetic rate under 18% shading was mainly caused by non-stomatal factors, while the main reason for the decreasing of the photosynthetic rate below 28% shading was the stomatal limitation transferring into a non-stomatal limitation.

AQY is a measurement unit of light energy conversion efficiency in photosynthesis, reflecting the photosynthetic capability of leaves in dim light conditions. The higher the AQY value, the stronger the capability will be (Cai, 2011; Liu *et al.*, 2011). Therefore, the higher AQY of 98B compared to 98A in low light means that 98B will adapt better. LCP and LSP reflect the capability of light utilization and represent the light requirement characteristics and capacity (Zhang *et al.*, 2014; Li *et al.*, 2007). Plants with smaller LCP and LSP better utilize the low photo flux density under limited lighting, which is related to the increasing chlorophyll content per leaf area and the decreasing respiration rate. Under light deficient conditions, plants reduce LCP and LSP to conduct photosynthesis, promote the accumulation of organic matter, and maintain the carbon balance to provide the required energy for growth. As a result, the weaker photosynthesis of 98A under low light levels insures growth; the higher LCP and LSP of 98B in dim light suggests better shade-tolerant ability and higher light utility efficiency.

Table 4. Effects on photosynthetic curves and related parameters of Shennong 98A and Shennong 98B under different shading treatments.

Shade intensity [%]	AQY		Pmax		LCP		LSP	
	Shennong 98A	Shennong 98B	Shennong 98A	Shennong 98B	Shennong 98A	Shennong 98B	Shennong 98A	Shennong 98B
CK	0.0341a	0.0395a	26.35a	27.17a	46.34a	52.62a	1707.52a	1901.66a
18%	0.0348a	0.0431b	25.41b	26.63b	46.88a	52.74a	1622.51b	1710.59b
28%	0.0426b	0.0418c	19.20c	22.76c	40.81b	51.11a	1391.88c	1446.75c
38%	0.0324c	0.0476d	17.52d	20.23d	44.24c	45.36b	1391.37c	1459.14c
60%	0.0365d	0.0399e	15.10e	17.38e	36.30d	39.26c	1358.57d	1357.73d
75%	0.0327c	0.0352f	14.14f	15.03f	36.31e	46.29d	1460.55e	1307.30e

Vertical bars denote SE (n = 5). Small letters indicatedifferences under different shade stresses at $p<0.05$, according to theleast significant difference (LSD) test

Effect of shading on chlorophyll fluorescence parameters of weak light sensitive cultivars: The chlorophyll fluorescence parameters are often used to evaluate crop photosynthetic efficiency and the effects of environmental stresses on crops (Van & Snel, 1900;Bolhar-Nordenkampf *et al*., 1989). Many researches reported that the change of chlorophyll fluorescence parameters are an important indicator (Flexas *et al*., 2002; Zarco-Tejada *et al*., 2013), while the chlorophyll fluorescence parameter is an important index to evaluate both uptake and utilization of nitrogen fertilizer and nitrogen deficiency in crops (Schahtl *et al*., 2005; Corp *et al*., 2009). Changes of chlorophyll fluorescence parameters were closely related to the process of photosynthesis, which further revealed the adaptability of maize under mild light stress (Krause & Weis, 1991). The maximum photochemical conversion efficiency (F_v/F_m) increased significantly in response to shading treatment. The changing trend of q_P and Φ_{PSII} was similar, Φ_{PSII} and q_P were increased significantly when shading stress increased from 18% to 38%, while Φ_{PSII} and q_P declined significantly under 60% and 75% shading stresses. The changing trend of NPQ was reversed to Φ_{PSII} and q_P. The above showed that light energy captured by the PSII antenna pigment was mainly used for photosynthesis under mild light stress, and to reduce the harm of weakening intensity on the photosynthetic system, to ensure normal photosynthesis. The ETR and q_P were significantly reduced when shading stress increased to 60% and 75%, the electron transfer declined almost to the stop state, and NPQ significantly increased, indicating that weak light stress was beyond the tolerance range. Previous studies showed differences in photosynthetic rate and fluorescence kinetic parameters under shading stress condition. This experiment arrived at similar conclusions. The main reason for the decline of net photosynthetic rate (P_N) was a decrease of light intensity, and the difference of adaptability to weak light was determined by the photosynthetic capacity of lines, i.e. the weak light physiological regulation mechanism of the shade-tolerant inbred was better than that of the weak light sensitive inbred.

Chlorophyll plays the role of absorbing, transferring, and conversing light during the photosynthesis process, while carotenoids participate in the important processes of light capture and photoprotection. Under different light conditions, plants typically adjust the ratio of both photosynthetic pigments in response to changes of environmental factors (Yu *et al*., 2011). Studies on chlorophyll content of *Ardisia violacea* (Zhang *et al*., 2014) and *Alhagi sparsifolia* (Xue *et al*., 2011) under shading conditions showed that the content of chlorophyll a, chlorophyll b, and chlorophyll (a+b) increased with increasing shading rates. This experimental data shows similar results. The chlorophyll content of 98B was higher than that of 98A after shading, which shows that 98B had the stronger ability to use weak light.

Conclusion

Chlorophyll a and total chlorophyll content of the two lines increased, and chlorophyll b content, Φ_{PSII} and q_P increased at first and then decreased, whileP_N and $G_s$showed the trend of gradually decreased. The change trend of NPQ was opposite to Φ_{PSII} and q_P. Compared the two inbred lines, photosynthesis and Chlorophyll fluorescence characters of non-barren stalk inbred line Shennong 98B were superior to barren stalk inbred line Shennong 98A. Under weak light environment, the photosynthetic efficiency of Shennong 98A was generally lower than that of Shennong 98B.We concluded that weak light resistant mechanism was associated with highly photosynthetic ratemechanism.

Acknowledgments

This work was supported by Cultivation Plan for Youth Agricultural Science and Technology Innovative Talents of Liaoning Province (2015041).

References

Bellasio, C. and H. Griffiths. 2014. Acclimation of C4 metabolism to low light in mature maize leaves could limit energetic losses during progressive shading in a crop canopy. *J. Exp. Bot.*, 65: 3725-3736.

Bolhar-Nordenkampf, H.R., S.P. Long, N.R. Baker, G. Oquist, U. Schreiber and E. Lechner. 1989. Chlorophyll fluorescence as a probe of the photosynthetic competence of leaves in the field: a review of current instrumentation. *Funct. Ecol.*, 3: 497-514.

Brown, I. 2013. Influence of seasonal weather and climate variability on crop yields in Scotland. *Int. J. Biometeorol.*, 57: 605-614.

Buykey, K.O. and R. Wells. 1991. Response of soybean photosynthesis and chloroplast membrane function to canopy development and mutual shading. *Plant Physiol*., 97: 245-252.

Cai, Z.Q. 2011. Shade delayed flowering and decreased photosynthesis, growth and yield of *Sacha Inchi* (*Plukenetia volubilis*) plants. *Inds. Crops & Prod.*, 34: 1235-1237.

Che, H.Z., G.Y. Shi, X.Y. Zhang, R. Arimoto, J.Q. Zhao, L. Xu, B. Wang and Z.H. Chen. 2005. Analysis of 40 years of solar radiation data from China, 1961-2000. *Geophy. Res. Lett*., 32: 2341-2352.

Corp, L.A., E.M. Middleton, P.K.E. Campbell, K.F. Huemmrich, Y.B. Cheng and S.T.D. Craig. 2009. Remote sensing techniques to monitor nitrogen driven carbon dynamics in field corn. Presented at the Society of Photo-Optical Instrumentation Engineers (SPIE) Conference Series.

Cui, H., J.J. Camberato, L.B. Jinand J.W. Zhang. 2015. Effects of shading on spike differentiation and grain yield formation of summer maize in the field. *Int. J. Biometeorol.*, 59: 1189-1200.

Cui, H.Y., L.B. Jin, B. Li, J.W. Zhang, B. Zhao, S.T. Dong and P. Liu. 2012. Effects of shading on stalks morphology, structure and lodging of summer maize in field. *Sci. Agric. Sin.*, 17: 3497-3505.

Demmig-Adams, B., C. Máguas, W.W. Adams, A. Meyer, E. Kilian and O.L. Lange. 1990. Effect of high light on the efficiency of photochemical energy conversion in a variety of lichen species with green and blue-green phycobionts. *Planta*, 180: 400-409.

Demming, A.B., W.W. Adams, D.H. Barker, B.A. Logan, D.R. Blowing and A.S. Verhoeven. 1996. Using chlorophyll fluorescence to assess the fraction of absorbed light allocated to thermal dissipation of excess excitation. *Physiolo. Plantarum*, 98: 253-264.

Early, E.B., W.O. Mcilrath, R.D. Seif and R.H. Hageman. 1967. Effects of shade applied at different stages of plant development on corn (*Zea mays* L.) production. *Crop Sci.*, 7: 155-156.

Ehleringer, J. and R.W. Pearcy. 1983. Variation in quantum yield for CO_2 uptake among C_3 and C_4plants. *Plant Physiol*., 73: 555-559.

Falbel, T.G., L.A. Staehelin and W.W. Adams. 1994. Analysis of xanthophyll cycle carotenoids and chlorophyll fluorescence in light intensity-dependent chlorophyll-deficient mutants of wheat and barley. *Photosynth. Res.*, 42(3): 191-202.

Farquhar, G.D. and T.D. Sharkey. 1982. Stomatal conductance and Photosynthesis. *Ann. Rev. Plant Physiol.*, 33: 317-345.

Flexas, J., J.M. Escalona, S. Evain, J. Gulías, I. Moya, C.B. Osmond and H. Medrano. 2002. Steady-state chlorophyll fluorescence (Fs) measurements as a tool to follow variations of net CO_2 assimilation and stomatal conductance during water-stress in C3 plants. *Physiologia Plantarum*, 114: 231-240.

Gerakis, P.A. and D.P. Tasopoulou. 1980. Effects of dense planting and artificial shading on five maize hybrids. *Agri. Meteorol*., 21: 129-137.

Gong, Q., H.Y. Wang, Y.F. Zhang, N. Lin, H. Chao, Z.Q. Gu, J.Y. Zhao and H.S. Yu. 2010. Analysis of changes in climate resources in Liaoning province in the context of global climate change. *Resour. Sci.*, 32: 671-678.

Govindjee. 2002. A role for a light-harvesting antenna complex of photosystem Ⅱ in photoprotection. *The Plant Cell*., 14: 1663-1668.

Hamamoto, H., Y. Shishido, T. Uchiumi and H. Kumakura. 2000. Effects of low light intensity on growth, photosynthesis and distribution of photoassimilates in tomato plants. *Environ. Control Biol.*, 38: 63-69.

Hashemi-Dezfouli, A. and S.J. Herbert. 1992. Intensifying plant density response of corn with artificial shade. *Agron. J.*, 84: 547-551.

Kiniry, J.R. and J.T. Ritchie. 1985. Shade-sensitive interval if kernel number of maize. *Agron. J.*, 77: 711-715.

Kiniry, J.R. and J.T. Ritchie. 1985. Shade-sensitive interval of kernel number of maize. *Agron. J*., 77: 711-715.

Krause, G.H. and E. Weis. 1991. Chlorophyll fluorescence and photosynthesis: The basics. *Annual Review of Plant Physiology*, 42: 313-349.

Li, C.H., Y.L. Zhao, G.H. Yang, L.M. Luan, Q. Wang and N. Li. 2007. Effects of shading on photosynthetic characteristics of different genotype maize. *Chinese J. App. Ecol.*, 18: 1259-1264.

Li, C.Q., H.M. Zheng, Y. Li and C.H. Li. 2010. Effect of planting density on the yield and development of maize ears. *Sci. Agri. Sin*., 43: 2435-2442.

Li, N., S.R. Guo, S. Shu and J. Sun. 2015. Effects of exogenous 24-epibrassinolde on leaf morphology and photosynthetic characteristics of tomato seedlings under low light stress. *Chinese J. App. Ecol.*, 26: 847-852.

Liu, J.F., W.J. Yang and Z.P. Jiang. 2011.Effects of shading on photosynthetic characteristics and chlorophyll fluorescence parameters in leaves of the endangered plant *Thuja sutchuenensis*. *Acta Ecologica Sinica*., 31: 5999-6004.

Lobell, D.B., M. Bänziger, C. Magorokosho and B. Vivek. 2011. Nonlinear heat effects on African maize as evidenced by historical yield trials. *Nature Climate Chang*, 1: 42-45.

Milne, B.F., Y. Toker, A. Rubio and S.B. Nielsen. 2015. Unraveling the intrinsic color of chlorophyll. *Angew. Chem. Int. Ed. Engl.*, 54: 2170-2173.

Pearcy, R.W. and J. Ehleringer. 2010. Comparative ecophysiology of C3 and C4 plants. *Plant Cell Environ*., 7: 1-13.

Reed, A.J., G.W. Singletary, J.R. Schussler, D.R. Williamson and A.L. Christy. 1984. Shading effects on dry matter and nitrogen partitioning,kernel number, and yield of maize. *Crop Sci.*, 28: 819-825.

Ren, G.Y., J. Guo, M.Z. Xu, Z.Y. Chu, L. Zhang, X.K. Zhou, Q.X. Li and X.N. Liu. 2005. Climate changes of China's mainland over the past half century.*Acta Meteorologica Sinica*., 63(6): 942-955.

Schahtl, J., G. Huber, F. X. Maidl, E. Sticksel, J. Schulz and P. Haschberger. 2005. Laser-induced chlorophyll fluorescence measurements for detecting the nitrogen status of wheat (*Triticum aestivum* L.) canopies. *Preci. Agric*., 6: 143-156.

Schreiber, U., U. Schliwa and W. Bilger. 1986. Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. *Photosynth. Res*., 10: 51-62.

Steel, R.G.D., J.H. Torrie and D.A. Dickey.1997. Principles and Procedures of Statistics. A Bio-Metrical Approach, 3rd ed. McGraw Hill Book Co., Inc., New York, USA.

Struik, P.C. 1983. Effects of short and long shadingduring different stages of growthon the development, productivity and quality of forage maize (*Zea may*s L.). *Netherlands J. Agri. Sci.*, 31: 101-124.

Tollenaar, M. and T.B. Daynard. 1978. Kernel growth and development at two positions on the ear of maize (*Zea mays* L.). *Can. J. Plant Sci*., 58: 189-197.

Ubierna, N., W. Sun, D.M. Kramer and A.B. Cousins. 2013. The efficiency of C4 photosynthesis under low light conditions in *Zea mays*, *Miscanthus*× *giganteus* and *Flaveria* bidentis. *Plant Cell Environ*., 36: 365-381.

Van, K.O. and J.F. Snel. 1990. The use of chlorophyll fluorescence nomenclature in plant stress physiology.*Photosynth. Res*., 25: 147-150.

Wang, Q., C.H. Li, S.P. Hao, Y.E. Zhang and J.F. Han. 2008. Effects of subsoil bulk density on late growth stage photosynthetic characteristics and grain yield of maize. *Chinese J. App. Ecol.*, 19(4): 787-793.

Ward, D.A. and H.W. Woolhouse. 1986. Comparative effects of light during growth on the photosynthetic properties of NADP-ME type C4 grasses from open an shaded habitats. I . Gas exchange, leaf anatomy and ultrastructure. *Plant Cell & Environ.*, 9: 261-270.

Xiao, D. and F.L. Tao. 2016. Contributions of cultivar shift, management practice and climate change to maize yield in North China Plain in 1981-2009. *Int. J. Biometeorol*., 60: 1111-1122.

Xue, W., X.Y. Li and J.T. Zhu. 2011. Effects of shading on leaf morphology and response characteristics of photosynthesis in *Alhagi sparsifolia*. *Chinese J. Plant Ecol.*, 35: 82-90.

Ye, M.Z., J.P. Guo, B. Yuan and J.F. Zhao. 2012. Thermal resources and maize temperature suitability in Northeast China under climate change.*Chinese J. App. Ecol.*, 23: 2786-2794.

Yu, Y.Y., D. Hu and E.H. Guo. 2011. Effects of urban shading on photosynthesis of *Euonymus japonicas*. *Acta EcologicaSinica*., 31: 5646-5653.

Zarco-Tejada, P.J., A. Catalina, M.R. González and P. Martín. 2013. Relationships between net photosynthesis and steady-state chlorophyll fluorescence retrieved from airborne hyperspectral imagery. *Remote Sens. Environ*., 136: 247-258.

Zhang, J., S. Dong, K. Wang, C. Hu and P.C. Liu. 2006. Effects of shading on the growth, development and grain yield of summer maize. *Chinese J. App. Ecol.*, 17(4): 657-662.

Zhang, Y., C. Liu and C. Yang. 2012. Analysis of heat-induced disassembly process of three different monomeric formsof the major light-harvesting chlorophyll a/b complex of photosystem II. *Photosynth. Res.*, 111: 103-111.

Zhang, Y., G.H. Xia, K. Ma, G.Y. Li, Y.C. Dai and C.Y. Yan. 2014. Effects of shade on photosynthetic characteristics and chlorophyll fluorescence of *Ardisia violacea*. *Chinese J. App. Ecol.*, 25: 1940-1948.

Zhao, M., S.K. Li, S.A. Wang and M.Y. Wang. 1999. Cluster analysis for photosynthetic characters of inbred lines of maize in China. *Acta Agronomica Sinica*., 25: 733-741.

Zhong, X.M., Z.S. Shi, F.H. Li and H.J. Huang. 2014. Photosynthesis and chlorophyll fluorescence of infertile and fertile stalks of paired near-isogenic lines in maize (*Zea mays* L.) under shade conditions. *Photosynthetica*, 52: 597-603.

Zhong, X.M., Z.S. Shi, F.H. Li, Z.B. Wang and H.W. Wang. 2011. Inducing effects of shade on genetic defect barren stalk of maize. *J. Maize Sci.*, 20: 58-63.

(Received for publication 7 September 2016)