Stomatal development and associated photosynthetic performance of capsicum in response to differential light availabilities

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Abstract

The mechanisms of capsicum growth in response to differential light availabilities are still not well elucidated. Hereby, we analyzed differential light availabilities on the relationship between stomatal characters and leaf growth, as well as photosynthetic performance. We used either 450–500 μmol m^{-2} s^{-1} as high light (HL) or 80–100 μmol m^{-2} s^{-1} as low light (LL) as treatments for two different cultivars. Our results showed that the stomatal density (SD) and stomatal index (SI) increased along with the leaf area expansion until the peak of the correlation curve, and then decreased. SD and SI were lower under the LL condition after three days of leaf expansion. For both cultivars, downregulation of photosynthesis and electron transport components was observed in LL-grown plants as indicated by lower light- and CO_2-saturated photosynthetic rate (P_{max} and $RuBP_{max}$), quantum efficiency of photosystem II (PSII) photochemistry (Φ_{PSII}), electron transport rate (ETR) and photochemical quenching of fluorescence (q_p). The observed inhibition of the photosynthesis could be explained by the decrease of SD, SI, Rubisco content and by the changes of the chloroplast. The low light resulted in lower total biomass, root/shoot ratio, and the thickness of the leaf decreased. However, the specific leaf area (SLA) and the content of leaf pigments were higher in LL-treatment. Variations in the photosynthetic characteristics of capsicum grown under different light conditions reflected the physiological adaptations to the changing light environments.

Additional key words: capsicum; chloroplast; low light; photosynthesis; Rubisco; stomata.

Introduction

Capsicum (*Capsicum annuum* L.) is regarded as one of the most important vegetable crops in the world. Due to its great demand for year-around production, capsicum is widely grown in greenhouses during winter and spring. Low photosynthetic photon flux density (PPFD) is the major factor limiting the greenhouse production. There is strong relation between the light-demand and the effects of growth irradiance on plant leaf photosynthesis and morphology. Photosynthesis is dependent on light for energy and for the induction of enzymatic processes. The architecture of plants is dependent on the quantity, direction, duration and quality of light (Ye 2007).

Generally, plants grown under high irradiance show high rates of photosynthesis, plants grown in low irradiance have lower net photosynthetic rate (P_N). In addition, the light-saturation point also increases with increasing irradiance during growth (Boardman 1977, Evans and Poorter 2001, Kyei-Boahen *et al*. 2003). Some observations reported that plants grown under low irradiance affected the height of the plant by increasing

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Abbreviations: CC – compensation CO_2 concentration; CE – carboxylation efficiency; Chl – chlorophyll; CP – compensation PPFD; E – transpiration rate; DM – dry mass; ETR – the electron transport rate; FM – fresh mass; g_s − stomatal conductance; NJ – Niujiao; NPQ – nonphotochemical quenching; P_{max} – PPFD-saturated photosynthetic rate; P_N – net photosynthetic rate; PPFD – photosynthetic photon flux density; PSII – photosystem II; q_p – photochemical quenching; Rubisco – ribulose-1,5-bisphosphate carboxylase/oxygenase; $RuBP_{max}$ – the maximum regeneration rate of ribulose-1,5-bisphosphate; SC – saturation CO_2 concentration; SD – stomatal density; SI – stomatal index; SLA – specific leaf area; SP – saturation PPFD; WUE – water use efficiency; ZJ-5 – Zhongjiao-5; Φ_{PSII} – quantum efficiency of PSII photochemistry.
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the length of the stem, and making the leaves wider and thinner (Stitt and Schulze 1994, Liu and Wang 2006). The researchers interpreted the change of the leaf development as an adaptive phenomenon to capture irradiance more efficiently. Stomata are structures in the epidermis and play an important role in determining the water and carbon cycle between the plants and the atmosphere (Miyashita *et al*. 2005). Among the various stomatal characters, stomatal density is an important eco-physiological parameter that affects gas exchange. Stomatal density is determined by stomata initiation during ontogenesis and by epidermal cell expansion at a later stage (Woodward 1987). It is assumed that the SD and SI vary along with the growth conditions (Woodward 1987, Hetherington and Woodward 2003). Stomatal characters are variable between species, habitats, areas and ages of the leaf.

The present knowledge on the effects of irradiance on stomatal development and associated photosynthetic capacity of capsicum are still insufficient. There is thus a great need to take into account more microscopic features related to the irradiance. The objective of this research was to determine: (*1*) how the development of stomata (SD, SI, size of stomata, epidermal cell density, *etc*.) is regulated in response to different light conditions for leaf growth starting from young to mature plants, (*2*) whether differences in the photosynthetic response to different light conditions and different capsicum genotypes exist, and (*3*) assess the capacity for photosynthetic acclimation of capsicum to different light environments. Accurate assessments of these effects are essential in order to maximize capsicum growth and yield through improved management practices, and will further contribute to the understanding of physiological mechanism of plants growth.

Materials and methods

Plant material and growth conditions: All the following experiments were conducted at China Agriculture University, Beijing (39.9°N 116.3°E). Two capsicum cultivars Zhongjiao-5 (ZJ-5, early maturing sweet pepper) and Niujiao (NJ, early maturing hot chili pepper) were used in this study. Seeds were soaked overnight and allowed to germinate on wet tissue paper in darkness. They were then sown in plastic pots (20 cm in diameter) with a mixture of field soil and vermiculite (2:1, v/v) in January 2007. After the seedlings had 5–6 leaves, the plants were transferred to environment-controlled cabinets, in which all the growth conditions were identical except for the light condition. The plants were grown under a 12 h-photoperiod with a 25°C during the day time, and 12 h for 18°C for night temperature regime and 70% relative humidity. All plants were well watered. There were two light treatments, high light (HL, control) and low light (LL). HL referred to a light intensity in the range of 450–500 μmol m^{-2} s^{-1} and LL provided a light intensity in the range of 80–100 μmol m^{-2} s^{-1}. The irradiance was provided by *Philips Master* fluorescent lamps (*Philips Lighting UK*, Guildford, UK). Measurements were performed after 30 days of treatment. Each treatment was replicated three times. We made three measurements on different individuals in each replicate.

Analysis of epidermal structure: The structure of the epidermis was examined using plant leaves developed from young to mature stages under HL- and LL-grown conditions. Leaf sections were painted on the abaxial surface with clear nail varnish as earlier described (Dami and Hughes 1995). Epidermal imprints were then stripped from the surface and examined by optical microscopy (*BX-50*, *Olympus*, Japan). A hemocytometer was used to calculate the densities of epidermal cells and stomata. The stomatal index was calculated as SI = 100 SD/(SD+ECD), where SD is stomata density and ECD is epidermal cell density as defined by Rengifo *et al*. (2002).

For scanning electron microscope observation, the leaf samples (2 × 2 mm) were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2), then transferred to 1% osmium tetroxide fixative for 30–40 min and washed several times with distilled water and then dehydrated first using 30% ethanol, followed by gradual series of increase in ethyl alcohol (30%, 50%, 70%) and finally with 100% ethanol for 20–30 min. Leaf samples were then transferred to propylene oxide for 20–40 min and washed with isoamyl acetate for 40 min and then oven-dried at 40°C overnight and subsequently the samples were coated with a very thin layer of gold for 5–10 min prior to scanning electron microscope analysis (*S-3400N, Hitachi*, Japan).

Photosynthetic gas exchange and chlorophyll (Chl) fluorescence measurements: Photosynthetic gas exchange was measured on fully expanded mature leaf in the morning from 09:00 to 11:00 h, 30 days after the treatment, using a portable photosynthesis system (*LI-6400*, *LI-COR*, Lincoln, NE, USA). The irradiance response curves were measured beginning at the highest irradiance and decreasing to 0 μmol m^{-2} s^{-1} PPFD (1,500; 1,200; 1,000; 800, 500, 300, 200, 150, 100, 50, 30, and 0 μmol m^{-2} s^{-1} PPFD). Each PPFD step lasted 3 min and data were recorded 5 times. The CO_2 and air temperature in the leaf chamber were maintained at 400 μmol mol^{-1} and 25°C respectively, and relative humidity was maintained at 70% during the measurements. Compensation PPFD (CP), saturation PPFD (SP) and PPFD-saturated photosynthetic rate (P_{max}) were obtained according to the nonrectangular hyperbola proposed by Thornley (2002). Leaf water-use efficiency (WUE) was calculated using instantaneous ratio of net photosynthetic rate (P_N) and

transpiration rate (E) with the PPFD under which plants were growing.

When the CO_2-response curves were determined, CO_2 concentration was about 100, 150, 200, 250, 300, 400, 500, 600, 800, and 1,000 μmol mol^{-1}, respectively. PPFD was kept at 1,000 μmol m^{-2} s^{-1}, air temperature in the leaf chamber was maintained at 25°C, and relative humidity was maintained at 70% during the measurements. The carboxylation efficiency (CE), compensation CO_2 (CC), saturation CO_2 (SC) and the maximum regeneration rate of ribulose-1,5-bisphosphate ($RuBP_{max}$) were estimated according to Farquhar and Sharkey (1982).

Chl fluorescence was measured on the same leaves that were used for photosynthesis measurements with a portable pulse amplitude modulation fluorometer *PAM-2100* (*Walz*, Effeltrich, Germany). Leaves were dark-adapted for at least 30 min prior to measurement. Initial fluorescence (F_0) was measured under a low ambient background light (< 0.1 μmol m^{-2} s^{-1}, 600 Hz) and then a 0.8-s saturating flash (8,000 μmol m^{-2} s^{-1}, 20 kHz) was applied to determine the maximal fluorescence (F_m) and the photochemical efficiency of PS II, (F_v/F_m). Then, "actinic light" (336 μmol m^{-2} s^{-1}) was applied and the steady-state fluorescence signal (F_s) was determined. F_m' was measured after another saturating pulse, the quantum efficiency of PSII photochemistry (Φ_{PSII}) was calculated as: $\Phi_{PSII} = (F_m' - F_s)/F_m'$ (Genty *et al*. 1989). After the "actinic light" was turned off, far-red irradiation (5 μmol m^{-2} s^{-1}, 3 s) was used to determine F_0', the electron transport rate (ETR) was calculated according to Schreiber *et al*. (1986): $ETR = \Delta F/F_m' \times PPFD \times \alpha \times \beta$, where PPFD is the photosynthetically active photon flux density, α is the leaf absorptance, and β is the distribution of absorbed energy between the two photosystems. β was assumed to be 0.5, and α was assumed to be 0.85. The q_p was used as an estimate of the proportion of open PSII reaction centers (Cornic 1994) and was calculated as: $q_p = (F_m' - F_s)/(F_m' - F_0')$. The non-photochemical quenching (NPQ), which is an estimate of the thermal energy dissipation, was estimated according to the model proposed by Demmig-Adams *et al*. (1996). $NPQ = F_m/F_m' - 1$.

Assays for Rubisco content: Mature leaf samples were homogenized in liquid nitrogen in a chilled mortar. Soluble protein was extracted by 1 ml of frozen extraction buffer (0.1 mM EDTA; 20 mM $MgCl_2$; 100 mM Tris-HCl, pH 7.5; 10 mM β-mercaptoethanol; 0.2 g polyvinylpolypyrrolidone). This was followed by centrifugation at 12,000 × *g* and 4°C for 30 min. Proteins were separated by SDS-PAGE on 4% (w/v) stacking gel and a 12% (w/v) resolving linear polyacrylamide gels using a *Mini-Gel System* (*C.B.S. Scientific*, CA, USA). Polypeptides were stained with Coomassie Brilliant Blue R-250.

Analysis of chloroplast ultrastructure: Mature leaf samples (2 × 2 mm) were preserved in 2.5% glutaraldehyde in 0.1 M phosphate buffer and then transferred to 1% osmium tetroxide to increase the fixation and washed several times with 0.1 M phosphate buffer and then dehydrated through series of acetone, and embedded in Spurr's epoxy resin. Thin sections were cut on an ultramicrotome (*Leica*, Germany) and then stained with 2.5% uranyl acetate followed by lead citrate, and then examined by transmission electron microscopy (*JEOL-1230*, Japan).

Analysis of leaf anatomical structure: Mature leaf samples (5 × 5 mm) were fixed in FAA (formaldehyde: acetic acid: 50% ethanol, 5:5:90), dehydrated and embedded in paraffin. 10 μm thick transverse sections were made with a *Minot*-type microtome. Following the paraffin elimination by immersion in xylol and washing in ethanol, sections were stained with safranine-fast green and examined by optical microscopy (*BX-50*, *Olympus*, Japan).

Determination of leaf pigment of mature leaves: Chl was extracted in 80% chilled acetone (v/v) and quantified with spectrophotometer (*UVICON-930*, *Kontron Instruments,* Zürich, Switzerland). Chl *a* was determined at wavelength 663 nm, Chl *b* at 646 nm and carotenoids at 470 nm (Lichtenthaler 1987).

Plant growth parameters and tissue biomass: The plants were collected after 30 days of treatment, then they were separated into leaves, stems, and roots to determine the fresh mass, then killed at 105ºC for 30 min, dried at 75ºC for 48 h, and weighed again. The following measurements were performed: root, stem, leaf biomass, and specific leaf area (SLA). SLA was defined as: SLA = LA/DM, where LA was the leaf area, DM was the dry mass of the leaf. Part of plants had grown under each treatment for more than 10 weeks for fruits set and anthesis.

Statistical analysis: Data were subjected to analysis of variance (*ANOVA*) and mean values were compared by the *Tukey* test ($p<0.05$) when a significant difference was detected. All the statistical analysis was performed using *SPSS 13.0 for Windows* (*SPSS*, Chicago, IL, USA).

Results

The effects of different light conditions on the relationship between stomatal characters and leaf area expansion

Stomatal density (SD): There was a strong correlation between the stomatal development and the growth of leaf (Fig. 1*A,B,E,F*). The SD increased along with leaf expansion and then decreased. Large differences in the response were found between the HL and LL growth conditions. The peak values of the SD for ZJ-5 and NJ grown under the HL condition were 469.54 mm^{-2} and 342.80 mm^{-2}, respectively. However, under the low light condition, the peak values of the SD decreased significantly for both cultivars (ZJ-5 at 290.09 mm^{-2}; NJ at 263.53 mm^{-2}). The highest SD of the HL- and LL-grown leaves for ZJ-5 appeared when the leaf reaches 60.20% and 50.85% of its final leaf size, respectively. For NJ, the highest SD of the HL- and LL-grown leaves appeared when the leaf reaches 59.82% and 40.34% of its final leaf size, respectively (Fig. 1). The values were obtained by the curve fitting.

Stomatal Index (SI): Initially, SI increased with the leaf expansion until the peak point and then decreased

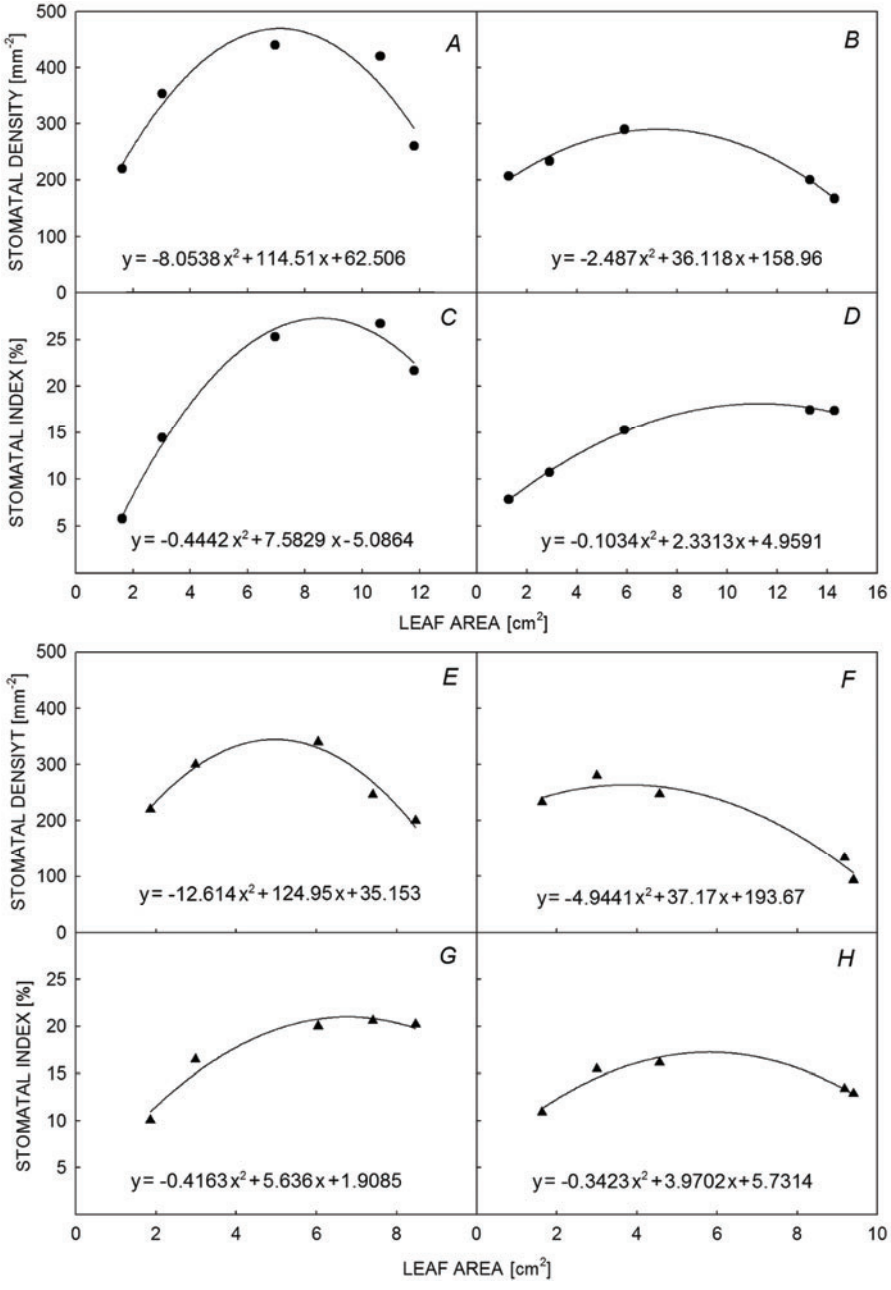

Fig. 1. The relationship between stomata density/index and leaf expansion of cv. ZJ-5 (*A−D*) and cv. NJ (*E−H*) grown under HL (*A,C,E,G*) and LL (*B,D,F,H*) conditions.

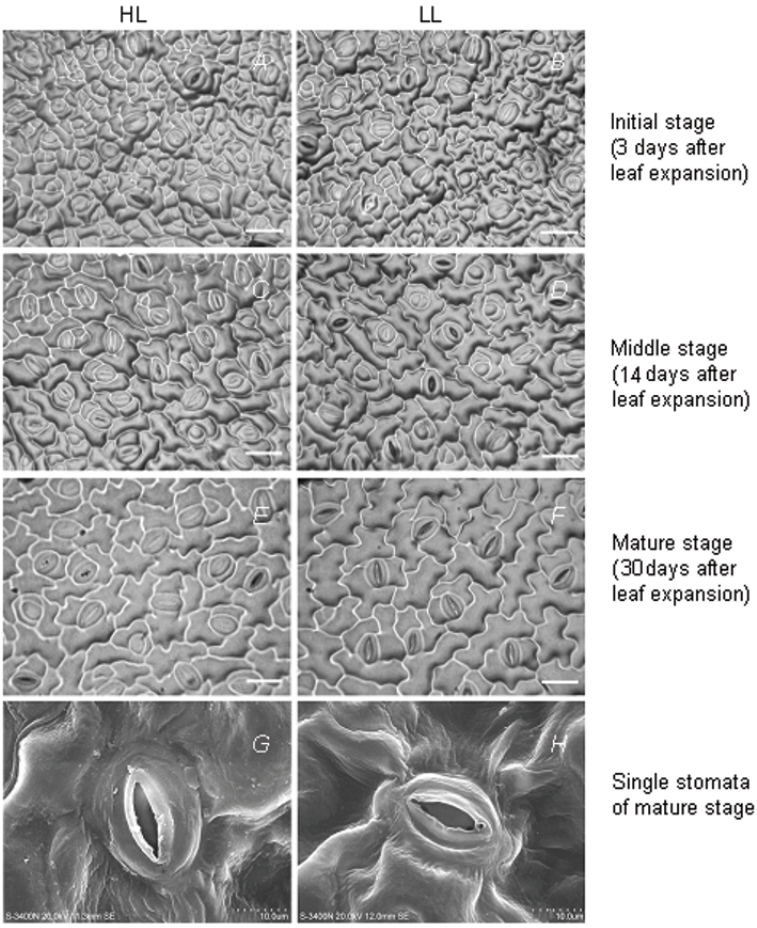

Fig. 2. A comparison of the effects of different light conditions on leaf epidermal structure using light microscope (*A*−*F*) and scanning electron microscope (*G,H*). Stomata and epidermal cells on the abaxial leaf epidermis of cv. ZJ-5 grown under HL- (*A,C,E,G*) and LL (*B,D,F,H*) conditions. Bar 40 μm (*A*−*F*), 10 μm (*G,H*).

(Fig. 1*C,D,G,H*); they showed patterns similarly to SD response for leaf areas. Under the HL condition, the peak values of SI for ZJ-5 and NJ were 27.28% and 20.98%, respectively (Fig. 1*C,G*). Under the LL condition, the peak values of SI decreased significantly for both cultivars (18.10% for ZJ-5 and 17.24% for NJ) (Fig. 1*D,H*). The highest SI of the HL- and LL-grown leaves for ZJ-5 appeared when the leaf reaches 78.94% and 72.27% of its final leaf size. For NJ, the highest SI of the HL- and LL-grown leaves appeared when the leaf reaches 79.92% and 61.69% of its final leaf size, respectively.

The shape and size of stomata: The stomata were formed in the early development stage of leaf; there were many small unfledged stomata on the epidermis of young leaves (Fig. 2*A,B*). The size of stomata and epidermal cells increased gradually with the leaf area expansion (Fig. 2). The stomata of the LL-grown leaves were less in number, but bigger in size (Table 1). The scanning electron microscope revealed that stomata of the HL-grown leaves mostly settled below the leaf surface, whereas under the LL condition, stomata were on the surface or above the leaf surface (Fig. 2*G,H*), this could be closely related the lower *E* (data not shown) and WUE under the LL condition.

Effects of different light conditions on photosynthesis parameters of mature leaf

***P*$_N$–PPFD curves**: When PPFD was <300 μmol m^{-2} s^{-1}, P_N increased steeply with PPFD, but gradually reached a plateau at higher PPFD (Fig. 3). For both cultivars, CP, SP, P_{max}, g_s, and WUE were higher under the high PPFD growth condition than those under the low PPFD growth condition (Table 2).

ZJ-5 had a lower CP but higher SP compared to those of NJ. For example, under the LL condition, ZJ-5 registered 14.99 μmol m^{-2} s^{-1} for CP and 1,089 μmol m^{-2} s^{-1} for SP, which were 52.01% and 133.61% of those required by NJ, respectively. As PPFD approached the SP, ZJ-5 and NJ produced significantly different P_N (11.74 and 7.56 μmol m^{-2} s^{-1}, respectively) (Table 2).

CO_2-response curves: The response curves in both cultivars differed for the HL and LL growth conditions (Fig. 3). The CC, SC, $RuBP_{max}$ and CE were significantly lower at the LL treatments (Table 2). Under the LL condition, ZJ-5 had a lower CC value than NJ, however, ZJ-5 produced higher $RuBP_{max}$ (16.93%) and CE (24.32%) than those of produced by NJ (Table 2).

Table 1. Effects of different light conditions on leaf epidermal structure of mature leaves of the two capsicum cultivars. Data represent the mean values ± SE of three replicates, and 20 visual fields of each replicate. The different letters represent statistical differences at $P<0.05$. HL – high light; LL – low light.

Parameters	ZJ-5 (sweet pepper)		NJ (hot pepper)	
	HL	LL	HL	LL
Epidermal cells density [mm^{-2}]	940±32^a	793±23^b	927±31^a	633±12^c
Stomatal density [mm^{-2}]	260±15^a	167±23^b	240±20^a	93±12^c
Stomatal length [μm]	30.45±1.24^a	33.43±1.24^a	32.33±0.89^a	35.83±1.29^a
Stomatal width [μm]	20.08±0.96^a	23.13±1.90^a	21.38±0.98^a	22.68±1.29^a
Stomatal index [%]	21.67±1.63^a	17.36±2.41^b	20.21±3.89^a	12.84±1.51^c

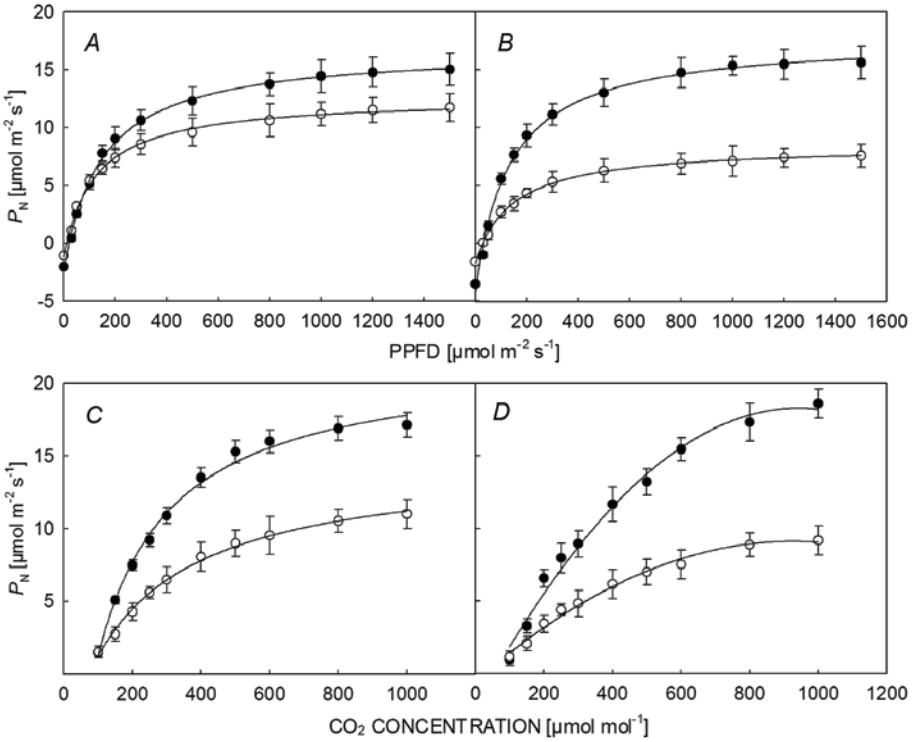

Fig. 3. The effects of light on the photosynthesis for cv. ZJ 5 (*A,C*) and cv. NJ (*B,D*) of mature leaves grown under HL (●) and LL (○). The light-response curves (*A,B*) and the CO_2-response curves (*C,D*) for photosynthesis are presented. Data represent the average ± SE of three replicates.

The values of Φ_{PSII}, ETR, and q_p decreased significantly, while NPQ increased under the LL condition, which could be observed through the evident deterioration of PSII photochemical energy storage (Table 2).

Rubisco content (SDS-PAGE) in mature leaf: The variation levels of the large and small subunit of the enzyme Rubisco under the HL- and LL conditions could be observed in Fig. 4. The content of Rubisco was down-regulated significantly in both large and small subunits under the LL condition. The band density was linearly correlated to the Rubisco content.

Ultrastructure of the chloroplast of the mature leaf: The internal structure of the chloroplast was significantly changed under the LL condition (Fig. 5). The number of the chloroplasts per cell profile was fewer, whereas the size of the chloroplast was bigger and the number of grana increased in the LL-treated plants, while the number of starch grain (per chloroplast) and the size of starch grain decreased in the both cultivars (Table 3).

Effects of light on the plant phenotype, plant biomass, and pigment content: The total dry mass of the plants declined significantly for plants grown under the low-PPFD condition (Table 4). The root/shoot ratio of the plants was higher at the HL-treatment, while it decreased significantly at the LL-treatment (Table 4), indicating a shift of biomass allocation from root to shoot with the increased survival rate of the plantlets during the acclimation.

Decreasing PPFD caused the increase of specific leaf

area (SLA) (Table 4), which increased the photosynthetically available radiation captured by the leaves. The LL-grown leaves contained higher Chl and carotenoids (Table 4). The thickness of leaf became thinner while treated with LL, which could be observed by light-microscopy analysis. The LL-condition reduced the number of fruits/plant and the anthesis date was delayed.

Discussion

Stomata play an important role in regulation of gas exchange between the interior of the leaf and the atmosphere. In leaves, the pattern of stomatal distribution is highly variable between species. Changes in the environment appear to act by modulating the developmental and patterning pathways to determine stomatal frequency. Stomatal characters have been found to be affected for example by CO_2 concentration (Woodward and Kelly 1995, Lin *et al*. 2001), ozone (Pääkkönen *et al*. 1998), ultraviolet radiation (Negash and Björn 1986), drought stress (Bosabalidis and Kofidis 2002) and by the growth altitude (Hultine and Marshall 2000). The stomatal responses to environment may be species-dependent and nonuniform across leaves.

Table 2. Comparison of photosynthetic characteristics of mature leaves of two capsicum cultivars grown under HL- and LL conditions. Data are the means ± SE of three replicates. The different letters in the same column represent statistical differences at $P<0.05$. HL – high light; LL – low light; CC – compensation CO_2 concentration; CE – carboxylation efficiency; CP – compensation PPFD; ETR – electron transport rate; g_s – stomatal conductance; NPQ – non-photochemical quenching; P_{max} – PPFD-saturated photosynthetic rate; q_p – photochemical quenching; $RuBP_{max}$ – the maximum regeneration rate of ribulose-1,5-bisphosphate; SC – saturation CO_2 concentration; SP – saturation PPFD; WUE – water use efficiency; Φ_{PSII} – quantum efficiency of PSII photochemistry.

Parameters	ZJ-5 (sweet pepper)		NJ (hot pepper)	
	HL	LL	HL	LL
CP [μmol m^{-2} s^{-1}]	23.87±3.54^b	14.99±1.26^c	37.99±4.12^a	28.82±2.34^b
SP [μmol m^{-2} s^{-1}]	1390±29^a	1089±19^b	1057±23^b	815±17^c
P_{max} [μmol m^{-2} s^{-1}]	15.02±1.24^a	11.74±1.78^b	15.06±1.12^a	7.56±0.99^c
g_s [mol m^{-2} s^{-1}]	0.209±0.003^a	0.088±0.004^b	0.178±0.003^a	0.076±0.002^b
WUE[μmol $mmol^{-1}$]	2.19±0.02^a	1.80±0.09^b	2.83±0.05^a	1.89±0.06^b
CC [μmol mol^{-1}]	60.12±3.25^b	47.38±2.54^c	78.24±2.96^a	50.64±1.96^c
SC [μmol mol^{-1}]	1040±29^a	837±27^b	1090±22^a	690±18^c
$RuBP_{max}$ [μmol m^{-2} s^{-1}]	19.11±2.59^a	10.29±1.16^b	21.60±1.88^a	8.80±1.99^b
CE [mol m^{-2} s^{-1}]	0.050±0.001^a	0.028±0.001^b	0.049±0.002^a	0.022±0.002^b
Φ_{PSII}	0.579±0.020^a	0.418±0.057^b	0.622±0.029^a	0.411±0.021^b
ETR [μmol m^{-2} s^{-1}]	73.02±2.43^a	52.59±7.16^b	78.36±3.65^a	51.73±2.73^b
q_p	0.800±0.019^a	0.570±0.061^b	0.826±0.036^a	0.566±0.037^b
NPQ	0.274±0.009^a	0.305±0.014^a	0.238±0.029^a	0.265±0.047^a

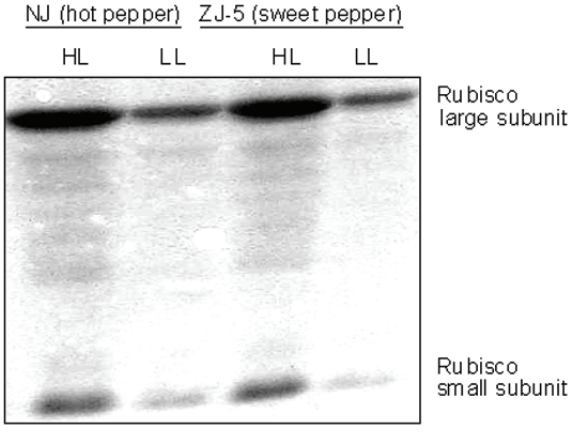

Fig. 4. The relationship between growth irradiance and Rubisco content of mature leaves, Coomassie-stained SDS-PAGE of Rubisco for cv. ZJ-5 and cv. NJ. Equal amounts of proteins (30 μg) were spotted on each gel for electrophoresis.

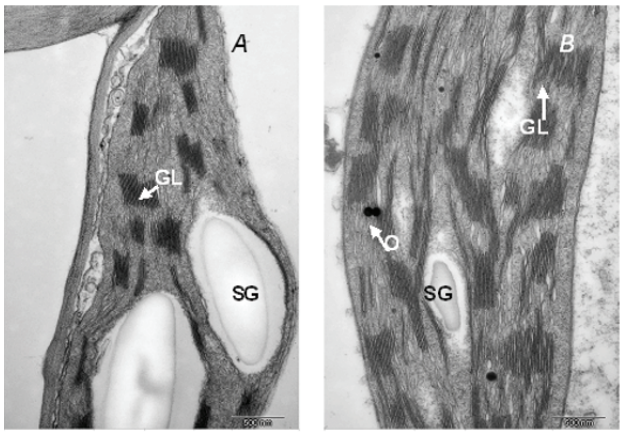

Fig. 5. A comparison of the ultrastructure of chloroplasts in capsicum plants cv. ZJ-5 of mature leaves grown under HL- (*A*) and LL (*B*) conditions. GL – lamellae; SG – grain; O – osmium granule. Bar 500 nm.

Table 3. Effects of different light conditions on the ultrastructure of chloroplast in mature leaves of capsicum. Data represent the average ± SE of three replicates, and 20 visual field of each replicate. The different letters represent statistical differences at $P<0.05$. HL – high light; LL – low light.

Parameters	ZJ-5 (sweet pepper)		NJ (hot pepper)	
	HL	LL	HL	LL
Chloroplast [number cell profile^{-1}]	14.4±1.4^a	11.4±2.8^b	16.3±2.0^a	12.2±1.6^b
Grana [number chloroplast^{-1}]	20.0±2.1^b	25.7±2.1^a	18.7±1.6^b	24.6±2.5^a
Starch grain [number chloroplast^{-1}]	3.30±0.96^a	1.50±0.71^b	3.00±0.78^a	1.50±0.86^b
Chloroplast length [μm]	5.95±0.32^b	7.29±0.55^a	5.46±0.37^b	6.82±0.37^a
Chloroplast width [μm]	2.54±0.44^a	2.97±0.11^a	2.45±0.27^a	2.74±0.23^a
Starch grain length [μm]	1.96±0.17^a	0.99±0.12^b	1.61±0.09^a	0.80±0.15^b
Starch grain width [μm]	1.06±0.13^a	0.49±0.10^b	0.89±0.16^a	0.40±0.10^b

Table 4. The effects of different light conditions on plant physiological parameters of mature leaves of capsicum. Data represent the average ± SE of three replicates. The different letters represent statistical differences at $P<0.05$, the same below. Part of plants had grown for more than 10 weeks for anthesis and fruits set. Chl – chlorophyll; DM – dry mass; FM – fresh mass; HL – high light; LL – low light; SLA – specific leaf area.

Parameters	ZJ-5 (Sweet pepper)		NJ (Hot pepper)	
	HL	LL	HL	LL
Total plant DM [g]	4.90±1.02^a	2.40±0.57^b	5.00±1.20^a	2.70±0.69^b
Root: shoot ratio [g g^{-1}(DM)]	0.69±0.03^a	0.33±0.01^b	0.61±0.03^a	0.29±0.01^b
SLA [cm^2 mg^{-1}]	0.30±0.01^b	0.52±0.02^a	0.31±0.01^b	0.46±0.02^a
Leaf thickness [μm]	147.9±7.4^a	112.1±7.3^b	145.3±7.1^a	109.2±7.5^b
Chl (*a*+*b*) [mg g^{-1}(FM)]	0.749±0.016^b	2.766±0.019^a	0.873±0.013^b	2.892±0.013^a
Carotenoids [mg g^{-1}(FM)]	0.180±0.010^b	0.356±0.001^a	0.209±0.003^b	0.421±0.002^a
Anthesis [d]	80±4^b	91±5^a	72±3^b	85±5^a
Fruits [number plant^{-1}]	14.0±2.4^a	3.5±1.3^b	11.8±2.1^a	2.5±1.2^b

We investigated the effects of differential light conditions (HL and LL) on the relationship between stomatal characters and the leaf area expansion and found that stomata were formed in the early developmental stage of leaf. The SD and SI increased along with the leaf expansion until the peak values, and then decreased (Fig. 1). The decrease of SD after the peak values could largely be explained by the increase in epidermal cell expansion and by the enlargement of the leaf area. The lower SD and SI were observed on the surface of leaves grown under the LL condition after three days of leaf expansion (Figs. 1, 2). There is a positive relation between irradiance and both SD and SI. Low light may directly affect the initiation of the number of stomata during ontogenesis. SD is influenced by epidermal cell size, whereas SI is a direct measure of a proportion of epidermal cells differentiated into stomata, which may be modified by low light (Ferris *et al.* 2002). The reduction in the SI was mainly caused by the reduction in SD (Table 1). In an investigation of the mechanism of the effect of light intensity on the SD and SI of the model plant *Arabidopsis thaliana*, Lake *et al*. (2001) found that it was mature leaves that detected light first and consequently transmitted a long-distance signal to control stomatal development in developing leaves. Young leaves, however, seemed to lack the capacity to respond to light intensity. The differences in sugar content and plant hormone concentrations between mature and young leaves may be the sources of the signal transmitted from mature to young leaves (Coupe *et al*. 2006), which supports the idea that there is an ecologically important link between the responses evoked by light and by carbon dioxide. It is apparent that further work is required to determine where the signal integrates specifically into the stomatal patterning and developmental pathways.

The present results also suggested that both regeneration of RuBP (as indicated by $RuBP_{max}$) and the Rubisco amount and/or activity (as indicated by CE) decreased by the low-light treatment. The photosynthetic capacity of both cultivars declined significantly under the LL condition. It was evident from the decrease of the proportion of light energy used for photochemistry and the increase of the proportion of excess light energy under the LL condition. Our results corroborated the conclusions of earlier investigations (Krause 1988, Chow *et al*.1990, Lee *et al*. 1999, Souza *et al*. 2004, Chen and Cheng 2007, Chen *et al.* 2008).

The two cultivars differed significantly in response to the irradiance in the current study. ZJ-5 showed a higher P_{max} than that of NJ when grown under LL (Fig. 3*A,B*).

This was the case despite Rubisco being slightly less abundant in ZJ-5 (Fig. 4). It could well have been due to higher SD and SI at maturity. The higher SD and SI could have given rise to a higher intercellular CO_2 which could explain the higher P_{max}. This phenomenon was similar to that observed by Wang *et al*. (2007a) for apricot, where the cultivars exhibited different adaptation abilities to low irradiance probably due to the genetic background effect.

Sun- and shade leaves differ in a number of functional traits. As irradiance decreases, growth and biomass accumulation of plants decrease, plants allocates more biomass to leaves, the shade leaves are larger and thinner, and have higher leaf pigments (Table 4). These results had been well debated in many other previous studies (Rijkers *et al*. 2000, Terashima *et al*. 2001, Wang *et al*. 2007b, Chang *et al*. 2008). It means that phenotypic plasticity should optimize the plant functioning in the given environment (Guan *et al*. 2002, Duan *et al*. 2005).

In conclusion, our study revealed a strong correlation between the stomata characteristics and the growth of the leaf. The SD and SI increased along with the leaf expansion until the peak values, and then decreased. Compared to the HL condition, the SD and SI of leaves were lower after three days of leaf expansion under the LL condition. The low irradiance also reduced the synthesis of the photosynthates as well as decreased the Rubisco content in the mesophyll cells and also the membranes of the thylakoids suffered from significant changes. The photosynthetic capacity of both cultivars declined significantly under the LL condition. The combination of tolerance mechanisms conferred capsicum a high degree of plasticity in response to differential light conditions.

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