The Responses of Morphological Trait, Leaf Ultrastructure, Photosynthetic and Biochemical Performance of Tomato to Differential Light Availabilities

FU Qiu-shi1,3, ZHAO Bing1, WANG Xue-wen1, WANG Yu-jue1, REN Shu-xin2 and GUO Yang-dong1

1 College of Agriculture & Biotechnology, China Agricultural University, Beijing 100193, P.R.China
2 School of Agriculture, Virginia State University, Petersburg, VA23806, USA
3 Department of Horticulture, Cornell University, Ithaca, NY14853, USA

Abstract

The whole-plant morphology, leaf ultrastructure, photosynthesis as well as enzyme activities of two tomato cultivars (Meifen-2 and Hongsheng) to differential light availabilities (450-500 μmol m⁻² s⁻¹, 75-100 μmol m⁻² s⁻¹) were examined in controlled environment. The results showed that the plant biomass and root/shoot ratio decreased and the specific leaf area increased significantly under the low light condition. There was a significant increase in malondialdehyde (MDA) concentration, superoxide dismutase (SOD) and peroxidase (POD) activities and decrease in soluble sugar and protein contents in LL-grown plants. For both cultivars, downregulation of photosynthesis and electron transport components were observed in LL-grown plants, the inhibition of the photosynthesis under the LL condition could be partially explained by the decrease of stomata density and by the changes of chloroplast.

Key words: chlorophyll fluorescence, chloroplast, photosynthesis, stomata, tomato

INTRODUCTION

Tomato (Lycopersicon esculentum Mill.) is regarded as one of the most important horticultural crops in the world. Due to its great demand for year-around production, tomato is widely grown in the greenhouses during winter and spring. Low photosynthetic photon flux density (PPFD) is the major factor limiting the greenhouse production.

Many physiological processes in plants are affected by irradiance, which is one of the most important environmental factors affecting plant survival, growth, reproduction, and distribution (Evans and Poorter 2001; Zhang et al. 2003; Fu et al. 2010). Some observations reported that plants grown under low irradiance, the height of the plant and the length of the stem increased, the leaf became wider and thinner (Anderson 1986). The researchers interpreted the change of the leaf development as an adaptive phenomenon to capture irradiance more efficiently. In contrast to this, plants grown under high irradiance decrease their specific leaf area (SLA), they absorb a large amount of photons and sustain high biomass accumulation (Vats et al. 2002). Low light stress also causes oxidative stress, changing the activities of various antioxidant enzymes (Sanità di Toppi and Gabbrielli 1999). It is found that low light enhances lipid peroxidation in plant cells, reflected by increased malondialdehyde (MDA) concentration (Chaoui et al. 1997). However, plants have evolved several mechanisms to prevent damage from reactive oxygen species (ROS), one of them is to use the enzymatic antioxidant system that includes superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and ascor-
bate peroxidase (APX), scavenging active oxygen species (AOS) (Bowler et al. 1992).

Photosynthesis is widely used as a tool for indicating environmental stress and selection of growth conditions suitable for different species (Pastenes et al. 2003). The response of leaf photosynthesis to irradiance has been reported for many horticultural crops including apple (Mierowska et al. 2002), potato (Havaux and Davaud 1994), pepper (Fu et al. 2010), and cucumber (Zhou et al. 2004). Generally, plants grown in low irradiance have lower net photosynthetic rate ($P_A$), in addition, the light-saturation point also increases with increasing irradiance during growth (Boardman 1977). It has been commonly observed that the capacity of plants to grow and develop in regimes differing from their original habitats depends on their ability for photosynthetic acclimation to changing environment (Pearcy 1977; Wolfgang and Thomas 1992).

The present knowledge on the effects of irradiance on morphological, anatomical, and photosynthetic performance of tomato is still insufficient. In this study, we compared whole-plant morphology, leaf gas exchange, chlorophyll fluorescence parameters, chloroplast ultrastructure, and stomatal characteristic together with several enzyme activities of tomato under different irradiance levels, providing a comprehensive study to better understand the physiological mechanisms that enable plants to adapt to different light conditions and maintain growth, development, and productivity during stress periods.

**MATERIALS AND METHODS**

**Plant materials and growth conditions**

All the following experiments were conducted at China Agricultural University, Beijing (Lat/Lon: 39.9° N/116.3° E). Tomato cultivars of Meifen-2 and Hongsheng (cherry tomato) were selected for the study. Seeds were sown in plastic pots (10-cm diameter) with the sterilized mixture of peat and vermiculite (3:1, v/v) in a greenhouse and germination was performed in the dark. After the fifth leaf (counted from the cotyledons) had completely developed, the seedlings of similar dimensions were transferred to a controlled-environment cabinet at a day/night temperature of 22/18°C and a relative humidity of 70-80% during a 12-h photoperiod. The plants were classified into two groups according to the treatment. One group of plants was grown at 450-500 μmol m$^{-2}$ s$^{-1}$ PPFD, and was identified as high light (HL). While another group of plants was subjected to 75-100 μmol m$^{-2}$ s$^{-1}$ PPFD, and was identified as low light (LL). The irradiance was provided by Philips Master fluorescent lamps (Philips Lighting UK, Guildford, UK). Plants were well watered three times a wk with Hoagland’s nutrient solution. 30 plants of each cultivar per treatment (HL and LL) were arranged in a completely randomized block design. Each treatment was replicated three times.

**Plants growth parameters and tissue biomass**

Five plants were evaluated after 4 wk of treatment. The stem diameter was measured by vernier caliper. The total leaf area (LA) was determined using leaf area meter (LI-3100, Li-COR Company, USA). The plants were separated into leaves, stems, and roots. These plant samples were then immediately weighed to determine the fresh weight, then killed at 105°C for 30 min, dried at 75°C for 48 h, and weighed. Specific leaf area (SLA) was defined as: SLA=LA/DW, where LA was the leaf area, DW was the dry weight of the leaf.

**Determination of pigments, soluble sugar, and protein content**

The fourth fully expanded leaves of tomato plants were collected for pigment analysis. Chlorophyll (Chl) was extracted in 80% cold acetone (v/v) and quantified with spectrophotometer (UVICON-930, Kontron Instruments, Zurich, Switzerland). The supernatant was read at 663 and 647 nm for Chl \textit{a} and Chl \textit{b}, respectively, and at 470 nm for carotenoids (Car) content. The pigment contents were calculated using the formulae of Lichtenthaler (1987).

Soluble sugars were extracted from 0.5 g fresh material with 80% ethanol, using five extraction steps. The remaining pellet was boiled in 3% (v/v) HCl for 3 h in order to hydrolyze starch. In both extracts, soluble sugar content was determined colorimetrically using anthrone as a reagent according to Fales (1951). The soluble protein content was determined using
bovine serum albumin as standard. The soluble proteins were extracted by grinding leaves (0.5 g fresh weight) in a mortar with 1 mmol L⁻¹ sodium phosphate buffer (pH 7.0) containing 10 mmol L⁻¹ dithiothreitol (DTT), 1 mmol L⁻¹ phenylmethylsulphonyl fluoride (PMSF), 5 mmol L⁻¹ 2-mercaptoethanol and 1% (w/v) Polyclar AT (Sigma-Aldrich, Saint Louis, MO, USA).

**Gas exchange measurements**

In each treatment, three plants were selected randomly and the third leaf from the top of each plant was used for determining photosynthesis by an Infra-Red Analyzer (LI-6400 System, Li-COR, Lincoln, NE, USA). Measurements were carried out in the morning, and different parameters of photosynthesis such as \( P_N \), stomatal conductance \( (g_s) \), intercellular \( \mathrm{CO}_2 \) concentration \( (C_i) \), ambient \( \mathrm{CO}_2 \) concentration \( (C_a) \) and transpiration rate \( (E) \) were determined after plants were treated with different irradiance for 4 wk. Intrinsic water use efficiency (WUE) was calculated by dividing \( P_N \) by \( g_s \), and stomatal limitation value \( (L_s) \) was calculated using the following formula: \( L_s = 1 - C_i / C_a \) according to Yin et al. (2006). Attached last fully expanded leaves of the primary tiller of HL and LL grown plants were used to obtain light-response curves for whole-leaf photosynthesis. Leaf temperature was fixed to 25°C, and 50% relative humidity. The light-response curves for photosynthesis were obtained via step-wise increases in irradiance from darkness to 1500 \( \mu \text{mol} \text{m}^{-2} \text{s}^{-1} \) at 350 \( \mu \text{mol} \text{mol}^{-1} \text{CO}_2 \).

**Chlorophyll fluorescence measurements**

Chlorophyll fluorescence parameters were measured on attached leaves using a portable pulse amplitude modulation fluorometer (PAM-2100, Walz, Effeltrich, Germany). Leaves were dark-adapted for 30 min before starting the experiment. Initial fluorescence \( (F_o) \) was obtained by illuminating the leaf with a weak modulated light, superimposing a saturating flash of white light \( (1 \text{s}; 4500 \mu \text{mol} \text{m}^{-2} \text{s}^{-1}) \) yielded maximum chlorophyll fluorescence \( (F_m) \). The maximum quantum yield for electron transport by open photosystem II (PS II) centers (photochemical efficiency) was calculated as \( F_o / F_m = (F_m - F_o) / F_m \). The steady-state \( (F_s) \) and the maximal \( (F_m^*) \) fluorescence levels were determined on the same leaves. Afterwards, a far-red pulse \( (3 \text{s}) \) was provided in order to measure the minimal level in the light-adapted state \( (F_o^*) \). The following parameters were calculated: (1) The quantum efficiency of PS II photochemistry \( \Phi_{PSII} = (F_m^* - F_o^*) / F_m^* \); (2) The electron transport rate \( \text{ETR} = \Phi_{PSII} \times PPFD \times 0.84 \times 0.5 \), where PPFD is the photosynthetically active photon flux density, 0.84 is the leaf absorptance, and 0.5 is the distribution of absorbed energy between the two photosystems (Schreiber et al. 1986); (3) the photochemical quenching coefficient was calculated as \( q_p = (F_m^* - F_o^*) / (F_m^* - F_o) \), and the non-photochemical quenching coefficient as \( NPQ = (F_m - F_m^*) / F_m^* \); (4) the fraction of excitation energy that is utilized for photochemistry \( (P) \) was estimated as: \( P = F_o^* / F_m^* \times q_p \); (5) the total fraction of absorbed light energy that is dissipated thermally within the PS II antennae \( (D) \) was calculated as: \( D = 1 - F_o^* / F_m^* \); (6) the excess excitation energy \( (E_x) \) defined as a fraction of absorbed light neither going to \( P \) nor \( D \) was calculated as: \( E_x = F_o^* / F_m^* \times (1 - q_p) \), where \( P \), \( D \), and \( E_x \) were estimated according to the model proposed by Demmig-Adams et al. (1996).

**Analysis of stomatal structure and chloroplast ultrastructure**

The structure of the stomata was examined in the third fully expanded leaves of HL and LL grown plants using scan electron microscope. For scanning electron microscope observation, the test samples were prepared according to the method of Fu et al. (2009). Briefly, the leaf samples (0.2 cm×0.2 cm) were fixed with 2.5% glutaraldehyde in 0.1 mol L⁻¹ phosphate buffer (pH 7.2), then transferred to 1% osmium tetroxide fixative for 30°C 40 min. The samples were dehydrated first using 30% ethyl alcohol and then washed with isoamyl acetate for 40 min and then oven dried at 40°C overnight and subsequently gold coated for 5-10 min prior to scanning electron microscope (S-3400N, HITACHI, Japan).

Materials were cut from the third fully expanded leaves from the apex of the HL and LL grown plants.
Fresh leaf pieces (0.2 cm×0.2 cm) were placed in a fixative solution consisting of 3 % glutaraldehyde in 0.2 mol L⁻¹ phosphate buffer (pH 7.2) and gently vacu-umed (1/2 atmosphere) for 4 h; then kept at 4°C overnight. The leaf samples were then transferred to 1% osmium tetroxide fixative for 30-40 min, and dehydration in ethanol series (10-70%) and acetone (70-100%). The samples were then 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).

Evaluation of lipid peroxidation

For the measurement of lipid peroxidation, the thiobarbituric acid (TBA) test, which determines MDA as an end product of lipid peroxidation was used (Hodges et al. 1999). Fresh leaves (0.5 g) were homogenized in 4 mL 1% trichloroacetic acid (TCA) and centrifuged at 10 000×g for 10 min, 2 mL of 20% TCA containing the 0.6 % (w/v) TBA was added to the 1 mL of supernatant and the mixture was boiled at 100°C for 30 min. The reaction was stopped by placing the tubes in an ice bath. The samples were centrifuged at 10000×g for 5 min, and the absorbance of the supernatant was read at 532 nm. A correction for non-specific turbidity was made by subtracting the absorbance value taken at 600 nm. The amount of MDA-CTBA complex (red pigment) was calculated from the extinction coefficient of 155 mmol L⁻¹ cm⁻¹.

Enzyme extraction and assays

The crudely enzymatic extracts of each part were prepared in 0.05 mol L⁻¹ phosphate buffer (pH 7.8) by grinding with a pestle and mortar under frozen condition using liquid nitrogen. The homogenate was filtered through one layer of nylon and centrifuged at 12 000×g for 10 min, and the supernatants were used for the SOD and POD assays. All operations were performed at 4°C.

SOD activity was determined by the nitro blue tetrazolium (NBT) method (Giannopolitis and Ries 1977). The reaction buffer contained 50 mmol L⁻¹ potassium phosphate buffer (pH 7.8), 10 μmol L⁻¹ EDTA, 13 mmol L⁻¹ methionine, 75 μmol L⁻¹ NBT, 2.0 μmol L⁻¹ riboflavin and the required amount of enzyme extract. Test tubes containing the reaction solution were irradiated under fluorescent light tubes for 15 min. Identical tubes which were not illuminated, served as blanks. The reaction began when the fluorescent lamp was turned on and stopped 5 min latter when the lamp was turned off (van Rossun et al. 1997). The absorbance of irradiated and non-irradiated solution was measured at 560 nm. The SOD activity was expressed as units mg⁻¹ protein.

POD activity was measured as the change of absorbance of 470 nm due to guaiacol oxidation according the method described by Polle et al. (1994). The reaction mixture (3 mL final volume) was composed of 50 mmol L⁻¹ potassium phosphate buffer (pH 7.0), 2.7 mmol L⁻¹ guaiacol, 2 mmol L⁻¹ H₂O₂ and enzyme extract.

Statistical analyses

The data were analyzed using statistical program (ver. 8.0; SAS Institute Inc., Cary, NC, USA). Duncan’s multiple range test (DMRT) at 5% level was performed.

RESULTS

Effects of LL and HL conditions on plant phenotype and plant biomass

When plants grown under the LL condition, the leaf number decreased, while the total LA increased. The plant total DW declined significantly at LL stress and the reduction was 22.26% of Meifen-2 (HL, 5.93 g; LL, 4.61 g) and 39.8% of Hongsheng (HL, 6.86 g; LL, 4.13 g), respectively (Table 1). Decreasing PPFD caused the increase of SLA, with the result that photosynthetic available radiation captured by the leaves increased. At LL treatment, the plant diameter/height ratio decreased by 25 and 23.08% for Meifen-2 and Hongsheng, respectively (Table 1). Decreasing PPFD caused the increase of SLA, with the result that photosynthetic available radiation captured by the leaves increased. At LL treatment, the plant diameter/height ratio decreased by 25 and 23.08% for Meifen-2 and Hongsheng, respectively (Table 1). The root/shoot ratio of plants decreased at LL treatment (Table 1).

Leaf pigment, soluble sugar, and protein contents

The LL stress altered the Chl and Car contents in two tomato cultivars. Compared to the HL condition, the
Chl $a$, Chl $b$, and Car content of two cultivars grown under LL condition increased significantly (Table 2). In contrast, the Chl $a/b$ ratio decreased during acclimation because the content of Chl $b$ increased more than that of Chl $a$. Under LL condition, the Chl/Car ratio increased from 8.02 (HL) to 8.11 and 7.93 (HL) to 8.21 for cultivar Meifen-2 and Hongsheng, respectively. Compared with HL plants, the amount of soluble sugars was drastically decreased by LL treatment, the soluble protein contents of Meifen-2 and Hongsheng decreased 20.92 and 30.18%, respectively (Table 3).

**Table 1** Changes in plant height, plant dry mass (DM), leaf area (LA) and other leaf parameters of tomato acclimated under HL (450-500 μmol m$^{-2}$ s$^{-1}$) and LL (75-100 μmol m$^{-2}$ s$^{-1}$) treatments on 4 wk

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Meifen-2</th>
<th>Meifen-2</th>
<th>Hongsheng</th>
<th>Hongsheng</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (cm)</td>
<td>35.63±1.493 b</td>
<td>44.67±0.517 a</td>
<td>56.17±2.843 a</td>
<td></td>
</tr>
<tr>
<td>Stem diameter (mm)</td>
<td>5.81±0.237 a</td>
<td>5.43±0.299 b</td>
<td>5.19±0.594 b</td>
<td></td>
</tr>
<tr>
<td>Diameter/height ratio</td>
<td>0.16±0.015 a</td>
<td>0.12±0.009 b</td>
<td>0.10±0.017 b</td>
<td></td>
</tr>
<tr>
<td>Number of leaves</td>
<td>14.00±0.820 a</td>
<td>12.70±1.530 b</td>
<td>15.70±1.530 b</td>
<td></td>
</tr>
<tr>
<td>Total LA (cm$^2$)</td>
<td>770.80±50.03 b</td>
<td>860.90±33.9 a</td>
<td>962.20±103.4 a</td>
<td></td>
</tr>
<tr>
<td>SLA</td>
<td>0.31±0.022 b</td>
<td>0.49±0.0038 a</td>
<td>0.66±0.075 a</td>
<td></td>
</tr>
<tr>
<td>Total DW (g)</td>
<td>5.93±1.292 a</td>
<td>6.86±1.948 a</td>
<td>4.13±0.965 b</td>
<td></td>
</tr>
<tr>
<td>Root/shoot ratio</td>
<td>0.17±0.024 a</td>
<td>0.13±0.015 a</td>
<td>0.10±0.010 b</td>
<td></td>
</tr>
</tbody>
</table>

Data are the means±SE of three replicates. The different letters represent statistical differences at $P<0.05$. The same as below.

**Table 2** Changes in chlorophyll (Chl) contents, carotenoid (Car) contents, Chl $a/b$ and Chl/Car ratio in leaves of tomato acclimated under HL (450-500 μmol m$^{-2}$ s$^{-1}$) and LL (75-100 μmol m$^{-2}$ s$^{-1}$) treatments on 4 wk

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Meifen-2</th>
<th>Meifen-2</th>
<th>Hongsheng</th>
<th>Hongsheng</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chl $a$ (mg g$^{-1}$ FW)</td>
<td>1.86±0.108 b</td>
<td>2.22±0.167 a</td>
<td>1.76±0.080 b</td>
<td>2.33±0.277 a</td>
</tr>
<tr>
<td>Chl $b$ (mg g$^{-1}$ FW)</td>
<td>0.62±0.027 b</td>
<td>0.78±0.127 a</td>
<td>0.54±0.059 b</td>
<td>0.79±0.107 a</td>
</tr>
<tr>
<td>Car (mg g$^{-1}$ FW)</td>
<td>0.31±0.020 b</td>
<td>0.37±0.011 a</td>
<td>0.29±0.001 b</td>
<td>0.38±0.038 a</td>
</tr>
<tr>
<td>Chl/Car ratio</td>
<td>8.02±0.221 b</td>
<td>8.11±0.405 a</td>
<td>7.93±0.409 b</td>
<td>8.21±0.120 a</td>
</tr>
<tr>
<td>Chl $a/b$ ratio</td>
<td>3.05±0.050 a</td>
<td>2.85±0.218 b</td>
<td>3.26±0.190 a</td>
<td>2.95±0.016 b</td>
</tr>
</tbody>
</table>

**Table 3** Changes in soluble sugar content, soluble protein contents, MDA contents, SOD activities and POD activities of tomato acclimated under HL (450-500 μmol m$^{-2}$ s$^{-1}$) and LL (75-100 μmol m$^{-2}$ s$^{-1}$) treatments on 4 wk

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Meifen-2</th>
<th>Meifen-2</th>
<th>Hongsheng</th>
<th>Hongsheng</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluble sugar (mg g$^{-1}$FW)</td>
<td>1.41±0.019 a</td>
<td>0.83±0.060 b</td>
<td>2.02±0.042 a</td>
<td>0.95±0.003 b</td>
</tr>
<tr>
<td>Soluble pro (mg g$^{-1}$FW)</td>
<td>1.53±0.121 a</td>
<td>1.21±0.147 b</td>
<td>1.69±0.138 a</td>
<td>1.18±0.052 b</td>
</tr>
<tr>
<td>MDA (μmol g$^{-1}$ (FW))</td>
<td>10.52±0.367 b</td>
<td>14.16±0.702 a</td>
<td>7.58±0.168 b</td>
<td>9.42±0.352 a</td>
</tr>
<tr>
<td>SOD (U mg$^{-1}$ FW)</td>
<td>8.85±0.720 b</td>
<td>12.82±0.060 a</td>
<td>6.96±0.541 b</td>
<td>10.92±1.078 a</td>
</tr>
<tr>
<td>POD (U g$^{-1}$ FW min$^{-1}$)</td>
<td>1.89±0.008 b</td>
<td>2.56±0.160 b</td>
<td>1.95±0.094 b</td>
<td>2.38±0.051 a</td>
</tr>
</tbody>
</table>

MDA concentration and enzyme activities

Lipid peroxidations in leaf samples were measured to assess the membrane damage. Under LL conditions, the MDA concentration of Meifen-2 and Hongsheng increased from 10.52 (HL) to 14.16 and 7.58 (HL) to 9.42, respectively (Table 3). The response of SOD activity and POD activity to LL stress varied with genotype: compared to HL condition, SOD activities in leaves of Meifen-2 and Hongsheng increased by 44.86 and 56.89%, respectively, whereas the activity of POD increased 35.45 and 22.05% for Meifen-2 and Hongsheng, respectively (Table 3).

Leaf gas exchange measurements

In this study, the $P_N$ was significantly inhibited by LL stress. $P_N$ for tomato leaves grown under HL condition was 7.52 and 9.98 μmol m$^{-2}$ s$^{-1}$ for Meifen-2 and Hongsheng, after 4 wk of LL treatment, $P_N$ values were...
reduced by approximately 49.2 and 52.2%, respectively (Fig. 1-A). We observed that this decrease was accompanied by a noticeable decline of $g_s$ (Fig. 1-B). However, the $C_i$ increased in LL-grown plants (Fig. 1-C). The change of leaf blade transpiration varied with genotypes under LL intensities (Fig. 1-D). Compared with the HL condition, $L_s$ was decreased obviously under LL stress (Fig. 1-E). The calculated WUE coefficients correlated positively with the light intensity during plant cultivation. Compared to the HL condition, WUE decreased significantly after LL treatment (Fig. 1-F).

Compared with the HL treatment, the response curves of $P_N$ and $g_s$ to PPFD for plants grown under the LL condition were lower (Fig. 2-A, B, C and D). When PPFD was <400 μmol m$^{-2}$ s$^{-1}$, the $P_N$ response from all the plants was rapid, which along with the time response becomes gradually at a plateau (Fig. 2-A, B).

**Chlorophyll fluorescence measurements**

The $F_{v}/F_{m}$ ratio indicates the potential quantum efficiency of PS II of dark-adapted leaves. $\Phi_{PSII}$, $q_P$, and ETR are related to photochemical processes, while NPQ is related to non-photochemical processes. When tomato plants were exposed to LL, the values of $F_{v}/F_{m}$ did not change significantly for both cultivars (Table 4). The values of $\Phi_{PSII}$, ETR, and $q_P$ decreased significantly, while NPQ increased under the LL condition (Table 4). The estimated energy partitioning of absorbed light to various pathways indicated that the P decreased significantly in response to decreased PPFD, where P was more sensitive to PPFD, while the D and $E_x$ increased rapidly under the LL condition (Table 4).

**Leaf stomatal structure**

Most of stomata distributed on the abaxial surface (Fig. 3-A, B, C, and D). The average stomatal area (length×
The Responses of Morphological Trait, Leaf Ultrastructure, Photosynthetic and Biochemical Performance of Tomato

Comparing to the epidermal cells, the majority of stomata were protuberant on the abaxial surface of leaves grown under the LL condition (Fig. 3-D and E).

Ultrastructure of chloroplast

The structure of chloroplast in the third leaf grown under HL and LL conditions were described in Fig. 4. The internal structure of the chloroplast was significantly changed under the LL condition: the size of the chloroplast was bigger, the number of grana increased...
and grana had more thylakoids. Also, compared to the controlled plants, the number of the chloroplast per palisade cell was fewer (7.45±0.76 and 8.02±0.42 for Meifen-2 and Hongsheng), whereas the number of the chloroplast was (11.53±1.39) and (12.12±0.87) for Meifen-2 and Hongsheng, respectively under HL condition. The number of starch grain (per chloroplast) and the size of starch grain decreased in both cultivars under the LL condition (Fig. 4-C, D). The number of plastoglobuli (per chloroplast) became large increase and the lamellae arranged disorderly (Fig. 4).

**DISCUSSION**

Our study indicates that the capacity of photosynthetic system of tomato to resist photoinhibition and to recover photosynthetic function under low PPFD was lower compared to that of the plants acclimated at high PPFD. In our study, $P_n$ and $g_s$ decreased whereas $C_i$ increased under LL treatment, which indicates that non-stomatal limitations occurred under LL condition. These results were consistent with previous studies (Farquhar and Sharkey 1982; Kirschbaum and Pearcy 1988; Jung and Steffen 1997; Baligar et al. 2008). Leonados et al. (1996) and Law and Crafts-Brandner (1999) reported a similar relationship between leaf temperature and stomatal conductance working with cotton and wheat. Low $L_s$ values indicate a higher efficiency of the carbon assimilation at the carboxylation sites and the physiological plasticity of the photosynthetic apparatus in order to acclimate to environmental stress conditions, including water deficit and soil flooding.

Chlorophyll fluorescence continues to be a mainstay in studies of photosynthetic regulation and plant responses to the environment due to its sensitivity, convenience, and non-intrusive quality (Wang et al. 2007). The efficiency of photosynthetic electron transport depends on the coordinated interaction of PS II and PS I in the electron-transport chain. Each photo-
system is associated with distinct pigment-protein complex, which absorbs the light in different wavelengths. The adjustment in photosystem stoichiometry in chloroplast can correct the unbalanced absorption of light by two photosystems and optimize electron transport, thereby improving photosynthetic efficiency (Chow et al. 1990). Kitajima and Hogan (2003) indicated that the LL plants had a higher ratio of PS II/PS I which ensured that the supply of electrons from PS II was sufficient to keep pace with the rate of excitation of PS I so that light reaching PS I was efficiently used. Our results were in line with earlier findings with this respect (Jung and Steffen 1997). The $F_v/F_m$ ratio of HL and LL plants were close to 0.8 which is typical of healthy, non-photoinhibited leaves (Maxwell and Johnson 2000). This suggests that photochemical efficiency of PS II was not or only slightly affected by the LL stress. The values of $\Phi_{PS II}^*$, ETR and $q_p$ decreased, while NPQ increased significantly. NPQ has been described as the most common form of protection against low light and is associated with xanthophyll cycle activity (Maxwell and Johnson 2000). The $q_p$ value provides an estimate of the fraction of oxidized PS II quinone acceptor or "open" PS II centers. This quenching of Chl $a$ fluorescence is mainly caused by the use of energy for photosynthetic electron transport. Photochemical quenching decreases while non-photochemical quenching increases in lower irradiance, which resulted in a lower photosynthetic capacity for tomato growing under LL conditions.

Stomata plays an important role in determining the water and carbon cycle between the plants and the atmosphere. Stomatal density affects gas exchange, stomatal conductance and instantaneous water-use efficiency (Woodward and Bazzaz 1988). We found that the stomatal density and stomatal index decreased on the abaxial surface of tomato leaves grown under the LL condition. This might indicate that the lower stomatal density under low irradiance could largely be explained by enlarging of LA, and also due to decrease in stomatal initiation. Considering the role of stomata in various physiological processes, the parallel changes in the reduction of stomatal density may have important consequences for the response of low irradiance (Wang and Kellomaki 1997).

The ultrastructure observations were focused particularly on the chloroplasts, because the membranes of thylakoids suffered from the greatest changes during adverse environmental conditions. Our results showed that the chloroplasts were significantly affected by LL condition. The observed inhibition of photosynthesis rate may be related to the changes of chloroplast. Under HL condition, plantlet chloroplasts had well developed grana and starch granules (Wetzstein and Sommer 1982). The accumulation of starch grains in chloroplasts from HL leaves were obviously (Fig. 4-A and C). Plastoglobulis are a characteristic of chloroplast, sometimes related with weak irradiance stress.

In conclusion, the morphological acclimation of tomato to different levels of irradiation was correlated with changes in SLA, total DW, and diameter/height ratio. The observed inhibition of the photosynthesis under the LL condition could be explained by the decrease of stomata density, and the changes of chloroplast. A multitude of physiological, morphological, and anatomical characteristics collectively conferred tolerance to low light.

Acknowledgements
This work was partly supported by the grants to Prof. Guo Yangdong (2009CB119000) and the Chinese Universities Scientific Fund (2009-2-06).

References
Chow W S, Melis A, Anderson J M. 1990. Adjustments of
photosystem stoichiometry in chloroplasts improve the quantum efficiency of photosynthesis. Proceedings of the National Academy of Sciences of the USA, 87, 7502-7506.


(Managing editor WENG Ling-yun)
学霸图书馆

www.xuebalib.com

本文献由“学霸图书馆-文献云下载”收集自网络，仅供学习交流使用。

学霸图书馆（www.xuebalib.com）是一个“整合众多图书馆数据库资源，提供一站式文献检索和下载服务”的24小时在线不限IP图书馆。

图书馆致力于便利、促进学习与科研，提供最强文献下载服务。

图书馆导航：

图书馆首页 文献云下载 图书馆入口 外文数据库大全 疑难文献辅助工具