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ARTICLE

Impact of Light Variation on Development of Photoprotection, Antioxidants, and Nutritional Value in *Lactuca sativa* L.

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Lettuce plants were grown at low (LL), middle (ML), and high light (HL) conditions to examine the relationship between photoacclimatory plasticity, light energy utilization, and antioxidant capacity. With the increase in light intensity from LL to ML, the energy flux via ΔpH- and xanthophylls-regulated thermal dissipation, fluorescence and constitutive thermal dissipation, and electron transport for photorespiratory carbon oxidation all increased significantly. However, plants at HL exhibited reduced electron transport for photosynthetic carbon reduction and decreased maximal photochemical efficiency of photosytem II (PSII) as compared to that at ML. Increasing light level significantly increased the alternative electron transport, $O_2^{\bullet-}$ production rate, and H_2O_2 and malondialdehyde (MDA) contents followed by increased ferric reducing antioxidant power (FRAP) and activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR). Moreover, plants exposed to HL showed higher nutritional value as indicated by the high contents of ascorbate, glutathione, carotenoids, and α-tocopherol. It was concluded that absorption of excess photon energy at high light was associated with increased antioxidant capacity and that produce quality could be improved by short-term exposure to suboptimum irradiance.

KEYWORDS: Lettuce; photosynthesis; energy allocation; reactive oxygen species; antioxidant enzymes; ascorbate; α-tocopherol; glutathione; carotenoid; nutritional quality

INTRODUCTION

Many environmental factors including light, temperature, moisture, and nutrient supply have significant influences on plant growth and development and, eventually, the yield and produce quality. Fluctuations in microclimate environments result in absorption of photon energy that exceeds the capacity of plants for utilization of excitation energy through photosynthetic electron transport, thus leading to photooxidative damage of the cell components due to the formation of reactive oxygen species (ROS). Meanwhile, plants have developed various mechanisms to regulate the efficiency of absorption, transfer, and utilization of excitation energy to balance the energy, thereby minimizing photooxidative damage (*1*).

An array of photoprotection mechanisms are known to play important roles in removing excess energy absorbed in plant or ROS, including light-dependent or -independent thermal dissipation and fluorescence energy dissipation, photorespiration, cyclic electron transports around photosystems, and alternative electron transport such as the water−water cycle (*1*). Studies elucidated that many enzymatic and nonenzymatic compounds were involved in these processes to scavenge the excess energy or ROS in the photosynthetic apparatus (*1*−*3*). ROS-scavenging enzymes in plants include superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), ascorbate peroxidase (APX, EC 1.11.1.11), monodehydroascorbate reductase (MDHAR, EC 1.6.5.4), dehydroascorbate reductase (DHAR, EC 1.8.5.4), and glutathione reductase (GR, EC 1.6.4.2), whereas nonenzymatic compounds include ascorbate (AsA), glutathione (GSH), α-tocopherol, and carotenoids such as xanthophyll cycle pigments. Enzymes such as SOD and other enzymes are able to scavenge superoxide generated in photosystem I (PSI), whereas xanthophyll cycle pigments can dissipate the excess energy in the form of heat and quench both triplet chlorophyll (Chl) and singlet oxygen. Generally, there are increases in the activities of these ROS-scavenging enzymes and/or the contents of these phytochemicals in plants after exposure to stressful conditions (*1*−*3*). In addition to their functions in plants, ascorbate, glutathione, α-tocopherol, and carotenoids possess health-promoting properties and play important roles in the prevention of diseases including cancer, heart disease, and other chronic diseases in humans (*4*, *5*). With the recognition of the

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health benefits of antioxidant phytochemicals, enhancement of phytochemicals through manipulation of plant growth environments may increase the nutritional value of vegetables, fruits, and cereal grains (*5*).

In recent years, plant biologists, agriculturists, and plant breeders have shown increasing interest in antioxidant improvement of food crops. Selective engineering of plants with enhanced antioxidant content is a promising method; however, this technique has not been used in commercial production due to various reasons. Cultural practices are also considered to be potential approaches to increase antioxidant contents (*6*). It has been shown that plants could produce higher levels of antioxidants in response to high light and stress temperatures (*1*−*3*). On the other hand, constant exposure to stress conditions can result in reduction of crop productivity. Alternatively, it seems that short-term exposure to an environmental stress may be feasible to improve the nutritional values of produce without significant yield loss because the response of antioxidant metabolism is often much faster than that of the biomass production.

Lettuce (*Lactuca sativa* L.) is an important leafy vegetable mainly consumed fresh in salads and a good dietary source of phytochemicals such as phenolic compounds and vitamins A, C, and E as well as minerals such as calcium and iron, which are essential in preventing diseases and promoting health and wellness in people (*7*). Previous studies have demonstrated the impact of different production environments on phytochemical antioxidants in lettuce (*8*, *9*). It is considered to be a convenient model crop to investigate the relationship among light stress tolerance, photoacclimatory plasticity, and nutritional quality of vegetables. In the present study, we grew lettuce plants at three light regimens to test the hypothesis that absorption of excess photon energy at high light is associated with improvement of antioxidant capacity. Antioxidant phytochemicals were also determined to assess produce quality under short-term exposure to a suboptimum growth light.

MATERIALS AND METHODS

Plant Materials and Treatments. Experiments were carried out in the spring of 2006 in a greenhouse at Zhejiang University. Loose leaf lettuce (*L. sativa* L. var. *crispa* L. cv. Italian Champion) seeds were sown in a mixture of soil and perlite (1:1, v/v) in plug trays (cell volume = 50 mL) at 25 °C. Seven days after seeding, the seedlings at two-leaf stage were then transferred to containers (40 × 25 × 15 cm, six seedlings per container) with continuously aerated full-strength Enshi nutrient solutions. Prior to the light treatment, all plants were grown under the same light condition. Ten days after hydroponic culture, the containers were randomly assigned to low light (LL), middle light (ML), and high light (HL) irradiance treatment, respectively. Full natural sunlight served as the ML treatment. The LL treatment was carried out by covering plants with two layers of polyethylene shade net at 2 m above the ground between 8:00 a.m. and 5:00 p.m., whereas the HL treatment was applied by using normal daylight plus artificial light between 8:00 a.m. and 5:00 p.m. with four high-pressure sodium lamps that had an emission spectrum around 550−650 nm (Philips, Shanghai, China; 250 W). Photosynthetic photon flux density (PPFD) at midday was typically 200−350, 700−900, and 1000−1200 μmol m^{-2} s^{-1} for LL, ML, and HL treatments, respectively, measured with a light quantum sensor (LI-6400, Li-Cor Inc., Lincoln, NE). Each treatment had 48 plants with 4 replicates, and each 12 plants were positioned under a separate bank of light as a replicate. During the experiment, the temperature in the greenhouse was maintained at 27/20 °C (day/night), and the air around the plants was continuously circulated by fans to minimize temperature changes arising from net covering and lamps. At 18 days after light treatments, gas exchange and Chl *a* fluorescence measurements were conducted on one fully expanded young leaf from four different plants. Leaves from each treatment were sampled, frozen quickly in liquid nitrogen, and stored at −86 °C or freeze-dried for the subsequent biochemical analyses, and the residual leaves were oven-dried at 80 °C for 3 days for the determination of the dry weight of plants.

Gas Exchange, Chl *a* Fluorescence, and Photosynthesis Analyses. Gas exchange and Chl *a* fluorescence were determined simultaneously with a portable infrared gas exchange analyzer equipped with a Chl fluorometer attachment (LI-6400, Li-Cor Inc., Lincoln, NE), on the day of harvest. The net photosynthetic rate (P_n), steady state fluorescence yield (F_s), and light-adapted maximum fluorescence (F_m') were measured at both 9 a.m. and 12 p.m. After the measurements of the irradiance-adapted parameters, the plants were placed in a dark room and remained there for >30 min. Then a saturating pulse (8000 μmol m^{-2} s^{-1}) was applied over 0.8 s, and the maximum photochemical efficiency of PSII (F_v/F_m) was recorded.

Energy Flux Determination. The quantum efficiency of photochemical energy dissipation (Φ_{PSII}, $1 - F_s/F_m'$), light-dependent (Φ_{NPQ}, $F_s/F_m' - F_s/F_m$) and light-independent thermal dissipation, and fluorescence energy dissipation ($\Phi_{f,D}$, F_s/F_m) at 12 p.m. on the day of harvest were calculated according to the method of Hendrickson et al. (*10*) with $\Phi_{PSII} + \Phi_{NPQ} + \Phi_{f,D} = 1$. The rate of energy dissipation via each process (J_{PSII}; J_{NPQ}; $J_{f,D}$) was calculated by multiplying the respective quantum efficiency (Φ) by the assumed proportion of absorbed quanta used by PSII reaction centers, irradiance, and leaf absorption, respectively (*10*, *11*). Utilization of photons absorbed by the PSII antennae in photosynthetic electron transport and thermal dissipation was then assessed from the quantum efficiency and flux of each process.

The rate of oxygenation by Rubisco (V_o) and the rate of carboxylation by Rubisco (V_c) were estimated following the method of Miyake and Yokota (*11*), respectively. Under atmospheric conditions, the electron fluxes in the two cycles (photosynthetic carbon reduction and photorespiratory carbon oxidation) can be expressed as $J_c = 4 \times V_c$ and $J_o = 4 \times V_o$, respectively. An alternative flux, J_a, caused by electrons that are not used by the carboxylation and oxygenation by Rubisco in the total electron flux driven by PSII (J_{PSII}), can be estimated from $J_{PSII} - (J_c + J_o)$ (*11*).

Leaf Pigment Analyses. Chl was extracted with 80% (v/v) acetone and measured by spectrophotometric absorbance at 663 nm for Chl *a* and 645 nm for Chl *b*. Pigments from the xanthophyll cycle pool (V, violaxanthin; A, antheraxanthin; Z, zeaxanthin) in the frozen leaves were extracted with 80% acetone, filtered through a 0.45 μm membrane, and analyzed by an HPLC system equipped with a Shimadzu LC-10 AT series pumping system (Shimadzu Corp., Kyoto, Japan). A Spherisorb C18 column (5 μm, 250 mm × 4.0 mm) was used with a flow rate of 1.5 mL min^{-1} (*12*). Elution was carried out using acetonitrile/methanol (75:25, v/v) (A) and methanol/ethyl acetate (70:30, v/v) (B) as the mobile phase. The gradient used was as follows: Start with 100% A for 7 min, increase to 100% B within 2 min. After 100% B had been maintained for 23 min, the column was allowed to re-equilibrate for 5 min in 100% A prior to the next injection. Ten microliters of sample was injected, and the pigments were detected by their absorbance at 445 nm. The de-epoxidation form for the xanthophyll cycle was expressed as $(A + Z)/(V + A + Z)$.

Reactive Oxygen Species and Lipid Peroxidation. The $O_2^{\bullet -}$ production rate was measured by analyzing the nitrite formation from hydroxylamine in the presence of $O_2^{\bullet -}$ (*13*). Each 0.5 g of frozen leaf segment was homogenized with 3 mL of 65 mM potassium phosphate buffer (pH 7.8) and centrifuged at 5000*g* for 10 min. The incubation mixture contained 0.9 mL of 65 mM phosphate buffer (pH 7.8), 0.1 mL of 10 mM hydroxylamine hydrochloride, and 1 mL of the supernatant. After incubation at 25 °C for 20 min, 17 mM sulfanilamide and 7 mM α-naphthylamine were added to the incubation mixture. Ethyl ether in the same volume was added and centrifuged at 1500*g* for 5 min. The absorbance in the aqueous solution was read at 530 nm. H_2O_2 content in lettuce leaves was measured by monitoring the absorbance of the titanium−peroxide complex at 415 nm, using the method of Brennan and Frenkel (*14*). Leaf tissue was homogenized in cold acetone in the ratio of 1 g of tissue to 2 mL of acetone. After centrifugation at 12000*g* for 5 min, the pellet was discarded, and 0.5 mL of the extract was collected. Titanium reagent (50 μL of 20% titanium tetrachloride in concentrated HCl, v/v) was added to 0.5 mL of the extract, followed by the addition of 0.1 mL of NH_4OH (25%) to precipitate the peroxide−titanium complex. After 5 min of centrifugation at 10000*g*, the supernatant was discarded and the

precipitate was repeatedly washed in 1 mL of acetone and centrifuged for 5 min at 10000*g*. The precipitate was solubilized in 1 mL of 2 N H_2SO_4 and brought to a final volume of 2 mL. The absorbance of the obtained solution was read at 415 nm against a water blank. For the measurement of lipid peroxidation in leaves induced by high light, the thiobarbituric acid (TBA) test, which determines malonaldehyde (MDA) as an end-product of lipid peroxidation, was used (*3*). Each 0.5 g of frozen leaf segment was homogenized in a potassium phosphate buffer (pH 7.8). The homogenate was centrifuged at 12000*g* for 20 min, and 1 mL of the supernatant was incubated in boiling water for 30 min. The reaction was stopped by placing the reaction tubes in an ice bath, the samples were then centrifuged at 1500*g* for 10 min, and the absorption of the supernatant was read at 532 nm. The value for nonspecific absorption at 600 nm was subtracted. The amount of MDA−TBA complex was calculated from the extinction coefficient 155 mM^{-1} cm^{-1}.

The histochemical detection of H_2O_2 and $O_2^{\bullet-}$ in lettuce leaves was performed using methods as previously described (*15*, *16*). Briefly, for the analysis of H_2O_2 an endogenous peroxidase-dependent in situ histochemical staining was conducted, in which leaf disks were vacuum-infiltrated with 0.1 mg mL^{-1} of 3,3′-diaminobenzidine (DAB) in 50 mM Tris−acetate buffer (pH 5.0) and incubated at 25 °C in dark for 24 h. The histochemical detection of $O_2^{\bullet-}$ in lettuce leaves was performed by infiltrating leaf disks directly with 0.1 mg mL^{-1} *p*-nitro blue tetrazolium (NBT) in 25 mM K-HEPES buffer (pH 7.6) and incubating at 25 °C in the dark for 2 h. In both analyses, leaf quarters were rinsed in 80% (v/v) ethanol for 10 min at 70 °C, mounted in lactic acid/phenol/water (1:1:1, v/v/v), and photographed.

Antioxidant Capacity Test. Each 1.0 g of frozen leaf sample was extracted with 10 mL of HPLC grade methanol. Ferric reducing antioxidant power (FRAP) assay was performed as described previously (*17*). The fresh FRAP reagent was produced by mixing 300 mM acetate buffer (pH 3.6), 20 mM ferric chloride, and 10 mM 2,4,6-tripyridyl-*S*-triazine dissolved in 40 mM HCl with a 10:1:1 (v/v/v) ratio. The mixture was incubated at 37 °C for 10 min. Prior to the analysis, the initial absorbance of 3 mL of FRAP reagents (blank) was recorded at 593 nm using a UV−vis spectrophotometer (UV-2401PC, Shimadzu Corp., Kyoto, Japan). A 100 μL lettuce extract was then added into the cuvettes containing 3 mL of FRAP reagent and mixed thoroughly. After incubation for 4 min at ambient temperature (30 °C), the absorbance values at 593 nm were recorded. FRAP values were calculated from $FeSO_4$ standard curves and expressed as millimoles per 100 grams of fresh weight (FW).

Enzyme Assays. Each 0.5 g of frozen leaf sample was homogenized in 3 mL of 25 mM HEPES buffer (pH 7.8) containing 0.2 mM EDTA, 2 mM ascorbate, and 2% (w/v) PVP. The homogenate was centrifuged for 20 min at 12000*g*, and the supernatant obtained was used for enzyme analysis. All operations were performed at 0−4 °C. SOD activity was measured in a reaction mixture containing 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 13 mM methionine, 75 μM nitroblue tetrazolium (NBT), 2 μM riboflavin, and the enzyme aliquot (*18*). Riboflavin was added as the last component, and the reaction was initiated by placing the tubes under two 15 W fluorescent lamps. The reaction was terminated after 15 min by removing the reaction tubes from the light source. Nonilluminated and illuminated reactions without enzyme served as calibration standards. The absorbance was recorded at 560 nm, and 1 unit of SOD activity was defined as the amount of enzyme required to cause a 50% inhibition of the rate of NBT photoreduction. CAT activity was assayed in a reaction mixture containing 25 mM phosphate buffer (pH 7.0), 10 mM H_2O_2, and the enzyme aliquot (*19*). The reaction was detected at 240 nm, employing an extinction coefficient for H_2O_2 of 39.4 mM cm^{-1}. APX activity was measured in a reaction mixture containing 25 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA, 1 mM H_2O_2, 0.25 mM AsA, and the enzyme aliquot (*20*). The reaction was followed at 290 nm (E = 2.88 mM^{-1} cm^{-1}). GR activity was measured in a reaction mixture containing 25 mM potassium phosphate buffer (pH 7.8), 0.12 mM NADPH, 0.5 mM GSSG, and the enzyme aliquot (*21*). The reaction was followed at 340 nm (E = 6.22 mM^{-1} cm^{-1}). DHAR activity was measured in a reaction mixture containing 25 mM potassium phosphate buffer (pH 7.0), 3 mM GSH, 0.25 mM DHA, and the enzyme aliquot (*22*). The reaction was followed at 265 nm (E = 14 mM^{-1} cm^{-1}). MDHAR activity was measured in a reaction mixture containing 25 mM potassium phosphate buffer (pH 7.8), 0.2 mM EDTA, 0.1 mM AsA, 0.5 U of AsA oxidase, 0.1 mM NADH, and the enzyme aliquot (*23*). The reaction was followed at 340 nm (E = 6.22 mM^{-1} cm^{-1}). All enzyme activities were measured spectrophotometrically. All reactions were developed at 25 °C in 3 mL quartz cuvettes. Data were expressed as specific activity with protein content determined using the method of Bradford (*24*).

Determination of Ascorbate and Glutathione Contents. Frozen leaf samples (0.5 g) were extracted with 5% (w/v) trichloroacetic acid (TCA) for the assays of the ascorbate and glutathione contents. The contents of AsA and DHA were determined according to the method of Sgherri et al. (*25*). The reaction mixture for total ascorbate contained a 0.2 mL aliquot of supernatant, 0.5 mL of 150 mM phosphate buffer (pH 7.4) containing 5 mM EDTA, and 0.5 mL of 10 mM DTT. After incubation for 10 min at room temperature, 0.15 mL of 0.5% *N*-ethylmaleimide was added to remove excess DTT. AsA was determined in a similar reaction mixture, except that 0.3 mL of H_2O was added instead of DTT and *N*-ethylmaleimide. Color developed in both reaction mixtures after the addition of the following reagents: 0.6 mL of 10% (w/v) TCA, 0.6 mL of 44% (v/v) orthophosphoric acid, 0.6 mL of 4% (w/v) α,α′-dipyridyl in 70% (v/v) ethanol, and 0.3% (w/v) $FeCl_3$. After vortex mixing, the mixtures were then incubated at 40 °C for 40 min, and the color produced was read at 525 nm. The concentration of DHA was estimated from the difference between total ascorbate and AsA. GSH and GSSG contents were measured by the 5,5′-dithiobis(nitrobenzoic acid) (DTNB)−GR recycling procedure (*26*). Total glutathione was determined by spectrophotometry at 412 nm at 25 °C, using 0.1 U of baker's yeast GR, 6 mM DTNB, and 2 mM NADPH. GSSG was determined according to the same method after removal of GSH by 2-vinylpyridine derivatizations. GSH was determined by subtraction of GSSG from the total glutathione content.

α-Tocopherol Analysis. α-Tocopherol content of leaf tissues was measured by HPLC according to the method described by Munné-Bosch and Alegre (*27*). Freeze-dried leaves (1 g) were extracted with 5 mL of methanol containing 50 mg L^{-1} citric acid and 50 mg L^{-1} isoascorbic acid. The extract was centrifuged at 1500*g* for 3 min at 3 °C, and the supernatant was transferred into a volumetric flask. The extraction procedure was repeated four times with 5 mL of methanol each time. The collected supernatants were passed through a 110 μm pore size cellulose nitrate filter (Schleicher & Schuell, Dassel, Germany). The extract was then concentrated through vacuum evaporation to eliminate the methanol, degassed with nitrogen, and stored at −20 °C.

Prior to injection, the extract was dissolved in 4 mL of acetonitrile and centrifuged at 1500*g* for 3 min at 3 °C. α-Tocopherol was separated at room temperature on a 5 μm column (250 × 4.0 mm; ODS Hypersil, Knauer, Berlin, Germany) using acetonitrile/distilled water/2 M citric acid (98:2:0.2, v/v/v) as an eluant at a flow rate of 1.1 mL min^{-1}. UV detection was carried out at 295 nm (SPD-10A, Shimadzu Corp., Kyoto, Japan), and fluorescence was measured at an excitation wavelength of 295 nm and an emission wavelength at 340 nm (RF-10AXL, Shimadzu). One hundred microliters of sample was injected, and duplicates were run for each extract. α-Tocopherol was identified by coelution with an authentic standard from Sigma and by its characteristic UV to fluorescence peak area ratio.

Carbohydrate Analysis. The freeze-dried, ground samples were used for the determination of carbohydrate content. Hexose, sucrose, and starch were determined colorimetrically at 490 nm using a modified phenol−sulfuric acid method (*28*). One hundred milligrams of sample was extracted in 2 × 10 mL volumes of 80% ethanol (80:20, v/v, ethanol/water), centrifuged, and adjusted to 25 mL in volumetric flasks for spectrophotometric determination of hexose and sucrose. The pellets from centrifugation were oven-dried at 60 °C for 48 h. Starch was measured by hydrolyzing the dried pellet for 3 h in 5 mL of 3.6% HCl at 100 °C, centrifuging the extract, and adjusting the volume to 25 mL for spectrophotometric determination of the resultant sugars in the extract also at 490 nm. Hexose, sucrose, and starch concentrations were expressed as milligrams per gram of dry weight (DW).

Statistical Analyses. Measurements on randomly selected samples from four replicates were analyzed by one-way analysis of variance using the MIXED procedure in SAS 8.0 (SAS Institute, Cary, NC). Multiple comparisons between treatment means were conducted using Tukey's test at the $P < 0.05$ level.

Table 1. Growth and Photosynthetic Characteristics of Lettuce Plants Grown under Low Light (LL), Medium Light (ML), and High Light (HL)[a]

		P_n (μmol of CO_2 m^{-2} s^{-1})					
treatment	DW (g $plant^{-1}$)	9:00 a.m.	12:00 a.m.	F_v/F_m	MDA (μmol g^{-1} of FW)	Chl (mg g^{-1} of FW)	Chl *a*/*b*
LL	0.97 ± 0.05 c	6.2 ± 0.8c	7.8 ± 0.2 c	0.85 ± 0.006 a	3.83 ± 0.18 c	0.81 ± 0.05 a	2.17 ± 0.07 b
ML	4.61 ± 0.32 b	9.8 ± 0.9b	23.3 ± 0.4 a	0.84 ± 0.005 a	4.16 ± 0.07 b	0.71 ± 0.03 a	3.13 ± 0.13 a
HL	5.99 ± 0.23 a	12.8 ± 0.9a	20.9 ± 0.4 b	0.75 ± 0.006 b	4.91 ± 0.09 a	0.49 ± 0.01 b	3.25 ± 0.19 a

[a] P_n, net photosynthetic rate; F_v/F_m, maximum photochemical efficiency of PSII; MDA, malondialdehyde; Chl, chlorophyll; DW, dry weight; FW, fresh weight. Results are expressed as the mean value ± standard deviation (n = 4). Values in the same column followed by different letters are significantly different at the 5% level.

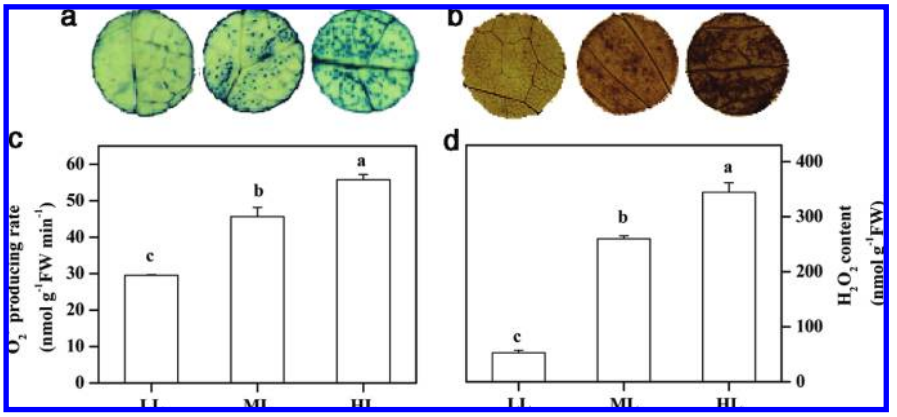

Figure 1. In situ detection of leaf (**a**) $O_2^{\bullet-}$ and (**b**) H_2O_2 and quantitative measurements of (**c**) $O_2^{\bullet-}$ and (**d**) H_2O_2 level in lettuce leaves after 18 days of exposure to low light (LL), medium light (ML), and high light (HL). Data are the means of four replicates with standard deviation shown by vertical bars. Bars topped by different letters are significantly different at the 5% level.

Table 2. Energy Flux in Lettuce Leaves at Low Light (LL), Medium Light (ML), and High Light (HL)[a]

	energy flux (μmol e^- m^{-2} s^{-1})					
treatment	J_{NPQ}	$J_{f,D}$	J_{PSII}	J_c	J_o	J_a
LL	0.8 ± 0.1 c	11.7 ± 0.6 c	47.3 ± 0.4c	37.5 ± 0.2 c	8.1 ± 0.1 c	1.7 ± 0.1 c
ML	41.1 ± 3.0 b	58.6 ± 0.8 b	140.2 ± 2.7b	100.7 ± 1.6 a	27.9 ± 0.5 b	11.6 ± 0.6 b
HL	182.3 ± 15.6 a	178.3 ± 16.5 a	159.2 ± 8.5 a	89.0 ± 2.6 b	32.5 ± 1.3 a	37.7 ± 4.7 a

[a] J_{NPQ}, energy flux via ΔpH- and xanthophylls-regulated thermal dissipation; $J_{f,D}$, energy flux via fluorescence and constitutive thermal dissipation; J_{PSII}, energy flux via photochemical energy dissipation; J_c, electron transport for photosynthetic carbon reduction; J_o, electron transport for photorepiratory carbon oxidation; and J_a, alternative electron transport. Results are expressed as the mean value ± standard deviation (n = 4). Values in the same column followed by different letters are significantly different at the 5% level.

RESULTS AND DISCUSSION

Irradiance, Plant Growth, and Photosynthetic Characteristics. In this study, lettuce plants were grown under three light levels. Different levels of light significantly affected plant growth. LL plants had much lower biomass accumulation as compared to ML, whereas HL increased biomass accumulation by 29.9% (**Table 1**). LL plants exhibited lowest P_n at both 9:00 a.m. and 12:00 p.m. on the date of harvest. HL plants exhibited higher P_n than ML plants at 9:00 a.m. but lower P_n at 12:00 p.m. The decrease in P_n for HL plants at 12:00 p.m. was probably due to photoinhibition as indicated by the decrease in F_v/F_m after prolonged exposure to high light because the light intensity for HL treatment at midday was higher than the light saturation point (i.e., 900 μmol m^{-2} s^{-1}) of lettuce. This was in agreement with early observations that photoinhibiton occurred in plants of soybean and rice after exposure to high light (*29*, *30*). Consistent with the photoinhibition, HL plants exhibited lower Chl content and higher degree of lipid peroxidation as indicated by an increase of MDA content, a measure of oxidative stress in plants. With the increase in light level, the Chl *a*/*b* ratio also increased. Increased Chl *a*/*b* ratio has been suggested as an acclimation of the photosynthetic system to high light habitats in order to avoid photodamage (*2*).

ROS and Allocation of Energy Flux. Plants cannot utilize all of the light adsorbed, and the excess radiation adsorbed can potentially result in the production of ROS such as superoxide and H_2O_2. In this study, NBT staining and DAB staining were used to examine in situ whether the increasing light level induced an accumulation of $O_2^{\bullet-}$ and H_2O_2, respectively. As observed in **Figure 1**, the staining intensity in both methods increased with light level, indicating the high $O_2^{\bullet-}$ and H_2O_2 levels in HL leaves. In line with the staining results, spectrophotometric analysis further confirmed the effect of light level on $O_2^{\bullet-}$ production rates and H_2O_2 levels in leaves. $O_2^{\bullet-}$ production rate and H_2O_2 content in HL plants increased by 22.1 and 32.4%, respectively, compared with ML plants. Higher ROS levels generated at high light conditions were also observed in plants of rice (*30*).

Photosynthesis is one of the main processes contributing to elevated generation of ROS. To determine whether HL-induced accumulation of $O_2^{\bullet-}$ and H_2O_2 paralleled the changes of photosynthetic electron transport, we examined the effect of light intensity on the allocation of photons absorbed by PSII antennae. As light intensity increased, J_{PSII}, $J_{f,D}$, and J_{NPQ} all significantly increased (**Table 2**). Among the three parameters, J_{NPQ} showed the most significant changes in response to light intensity. The significant increase in J_{NPQ} suggested that xanthophylls cycle, and presumably the lutein cycle operated at higher efficiency

under HL conditions because light-dependent thermal dissipation is mainly driven by the xanthophylls cycle and presumably the lutein cycle (*1*, *2*). J_{PSII} is composed of J_c, J_o, and J_a. ML plants showed the highest value of J_c, consistent with the change in P_n measured at 12 p.m. However, HL markedly activated two O_2-dependent photoprotection processes, that is, photorespiration (electron flux expressed as J_o) and alternative electron transport (electron flux expressed as J_a). HL treatment increased J_o and J_a by approximately 1.2- and 3.2-fold, respectively, in comparison to the ML treatment. The increase in J_o was in accordance with previous findings that photorespiration worked as a protection mechanism under HL conditions (*29*). The sharp increase in J_a in ML and HL plants suggested that ML and HL exposure resulted in an increased antioxidant metabolism such as the water−water cycle as found in chilled plants (*3*). Antioxidant metabolism such as the water−water cycle is accompanied by ROS generation. In compliance with this, higher J_a was always accompanied by a higher $O_2^{\bullet-}$ production rate and H_2O_2 content in our study (**Figure 1**).

Antioxidant and Food Quality. To investigate whether the increase in excess excitation energy in photosynthesis up-regulated the antioxidant capacity of lettuce, the FRAP assay that directly measures antioxidants or reductants in a sample was used in the present study. The FRAP assay could not work with thiols such as glutathione because the reduction potentials for thiols generally are below that of the Fe^{3+}/Fe^{2+} half-reaction. However, because only limited amounts of glutathione in plants are absorbed by human, and almost no other antioxidant thiols are present in dietary plants, the FRAP method has been thought to be suitable for assessment of total antioxidants in plants (*31*). It was found that FRAP was enhanced with the increase in light level, and FRAP for HL plants was 2.18-fold of that of LL plants (**Figure 2**). The FRAP value for ML lettuce plants in our study is lower than those of chili pepper, kale, and cabbage, but higher than those of tomato, asparagus, broccoli, and cucumber, indicating that lettuce is a food source with relatively higher antioxidant capacity (*31*). Ascorbate, glutathione, tocopherol, and carotenoids are the major antioxidants present in stressed plants (*1*−*3*). Consistent with other studies (*2*, *3*), increase of ascorbate (AsA and DHA) and glutathione contents in lettuce grown under HL conditions was observed in this experiment (**Table 3**). However, HL treatment led to declines in ratios of AsA/(AsA + DHA) and GSH/(GSH + GSSG), suggesting that prolonged exposure to HL resulted in an impaired redox homeostasis. Meanwhile, HL plants had higher levels of total carotenoids, xanthophyll-cycle pigments (V + A + Z), de-epoxidation state of the xanthophyll cycle (A + Z/V + A + Z), and α-tocopherol as compared with ML and LL plants (**Table 4**). These results are generally in agreement with previous findings that ascorbate, glutathione, α-tocopherol, and carotenoids play a critical role in the antioxidation and stress tolerance (*1*−*3*). The essential role of the xanthophyll cycle (V + A + Z) and the de-epoxidation of the xanthophyll cycle in thermal dissipation (NPQ) are well-established (*1*). The correlation between NPQ and xanthophyll cycle (V + A + Z) as well as the de-epoxidation of the xanthophyll cycle suggested that the increased xanthophyll cycle and the de-epoxidation of the xanthophyll cycle were important strategies utilized by plants for photoprotection, especially under high light stresses (**Tables 2** and **4**).

Similar to the changes in FRAP, activities of antioxidative enzymes (SOD, CAT, APX, MDAR, DHAR, and GR) all significantly increased with the elevation of light level. For example, HL-grown lettuce exhibited higher APX activity than ML-grown lettuce by 35.4% (**Figure 3**). Accordingly, antioxidative enzymes played an important role in scavenging the ROS in HL-grown plants.

To determine the light effects on nutritional composition of lettuce, carbohydrates such as hexose, sucrose, and starch were also measured in plants after exposure to different light levels (**Figure 4**). The contents of hexose, sucrose, and starch in HL plants increased by 153.1, 82.9, and 48.2%, respectively, compared with ML plants. Hexose, sucrose, and starch contents in LL plants, however, were not significantly different from that in ML

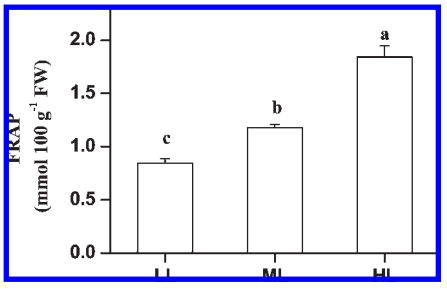

Figure 2. Effects of 18 days of exposure to low light (LL), medium light (ML), and high light (HL) on the ferric reducing antioxidant power (FRAP) in lettuce leaves. Data are the means of four replicates with standard deviation shown by vertical bars. Values followed by different letters are significantly different at the 5% level.

Table 3. Ascorbate and Glutathione Contents in Lettuce Leaves at Low Light (LL), Medium Light (ML), and High Light (HL)[a]

	ascorbate (μmol g^{-1} of FW)			glutathione (nmol g^{-1} of FW)		
treatment	AsA	DHA	AsA/(AsA + DHA)	GSH	GSSG	GSH/(GSH + GSSG)
LL	4.22 ± 0.15 b	1.96 ± 0.21 b	0.68 ± 0.03 a	151.0 ± 3.6 c	13.72 ± 0.50 c	0.91 ± 0.002 a
ML	4.34 ± 0.25 b	2.70 ± 0.30 b	0.62 ± 0.30 ab	180.8 ± 10.8 b	20.06 ± 0.88 b	0.90 ± 0.002 a
HL	6.42 ± 0.39 a	5.21 ± 0.19 a	0.55 ± 0.01 b	225.5 ± 11.9 a	44.71 ± 0.62 a	0.83 ± 0.008 b

[a] AsA, reduced ascorbate, DHA, oxidized ascorbate; GSH, reduced glutathione; GSSG, oxidized glutathione. Results are expressed as the mean value ± standard deviation (n = 4). Values in the same column followed by different letters are significantly different at the 5% level.

Table 4. Carotenoids and α-Tocopherol Contents in Lettuce Leaves at Low Light (LL), Medium Light (ML), and High Light (HL)[a]

treatment	carotenoids (mg g^{-1} of FW)	xanthophyll cycle pigment (mmol mol^{-1} of Chl)	(A + Z)/(V + A + Z)	α-tocopherol (μmol g^{-1} of FW)
LL	0.12 ± 0.01 b	49.6 ± 2.5 c	0.13 ± 0.01 c	0.23 ± 0.01 c
ML	0.15 ± 0.03 b	77.7 ± 3.2 b	0.25 ± 0.01 b	0.44 ± 0.02 b
HL	0.25 ± 0.02 a	89.2 ± 2.6 a	0.40 ± 0.01 a	0.59 ± 0.03 a

[a] V, violaxanthin; A, antheraxanthin; Z, zeaxanthin. Results are expressed as the mean value ± standard deviation (n = 4). Values in the same column followed by different letters are significantly different at the 5% level.

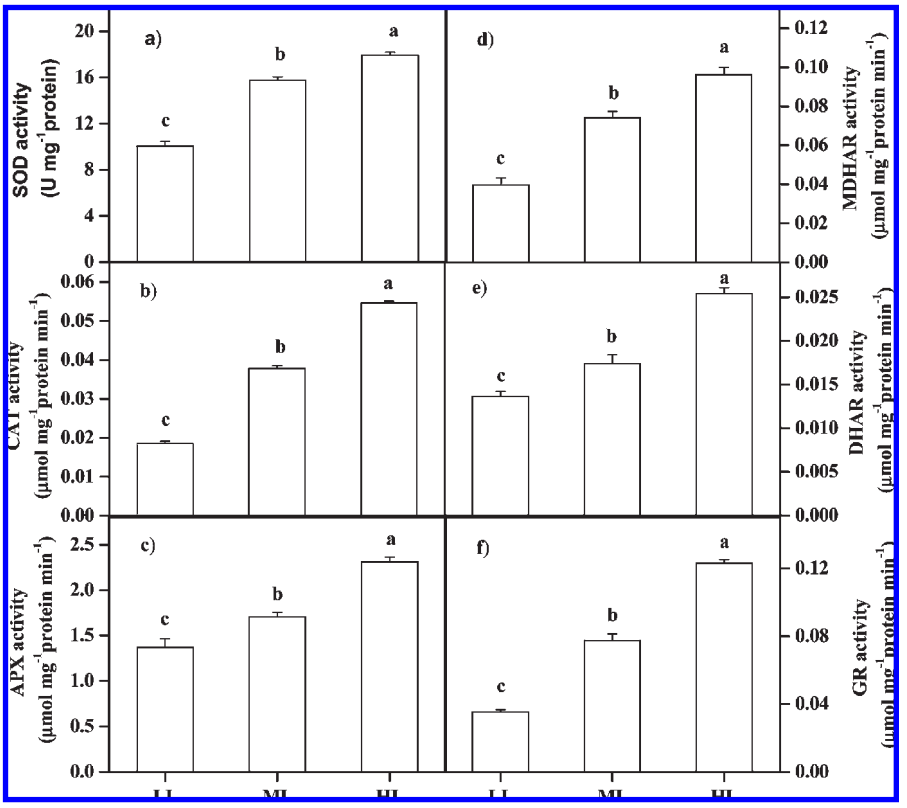

Figure 3. Effects of 18 days of exposure to low light (LL), medium light (ML), and high light (HL) on the activities of (**a**) SOD, (**b**) CAT, (**c**) APX, (**d**) MDHAR, (**e**) DHAR, and (**f**) GR in lettuce leaves. Data are the means of four replicates with standard deviation shown by vertical bars. Values for the same parameter topped by different letters are significantly different at the 5% level.

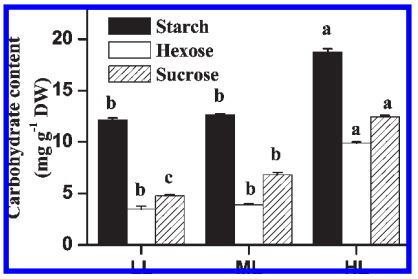

Figure 4. Effects of 18 days of exposure to low light (LL), medium light (ML), and high light (HL) on the contents of starch, hexose, and sucrose in lettuce leaves. Data are the means of four replicates with standard deviation shown by vertical bars. Values for the same parameter topped by different letters are significantly different at the 5% level.

plants except that sucrose content was decreased in LL plants. It has been shown that plants tend to accumulate high levels of carbohydrates under high light (*32*). The increases of carbohydrates in HL plants in this study were also consistent with the higher rate of photosynthesis in HL treatment.

It is noteworthy that ascorbate, glutathione, α-tocopherol, and carotenoids not only are the antioxidants required for plant growth, particularly under environmental stresses, but are also of great importance to human health owing to their potent antioxidant properties. The significance of ascorbate, glutathione, and α-tocopherol in human health has been well recognized (*4*, *5*). Carotenoids such as zeaxanthin and lutein are implicated in the protection of human vision and other aspects of human health (*5*). Meanwhile, lettuce leaf extracts contained compounds with high specific peroxyl radical scavenging activities (*7*). The primary phenolic compounds in the leaf tissue extracts are mono- and dicaffeoyltartaric acid (CTA and DCTA), mono- and dicaffeoylquinic acid (CQA and DCQA), quercetin 3-malonylglucoside (QMG), quercetin 3-glucoside (QG), cyanidin 3-malonylglucoside (CMG), and an unknown phenolic ester (UPE), which are sensitive to environmental factors (*7*). This provides us a feasible approach to increase their contents by modifying the growth environment, and our results also support this feasibility. Further work is warranted to investigate the changes in the individual phenolic compounds of lettuce in response to environmental stimuli. Bioavailability and health benefits of antioxidant phytochemicals also deserve in-depth study. In addition, caution should be taken when antioxidant capacity is used as the main parameter for the nutritional value of lettuce and other leafy vegetables because antioxidative metabolism in plants may be up-regulated by certain toxic chemicals.

In conclusion, results from the present study demonstrated that a high light level caused an increase of excess excitation energy and ROS generation in lettuce plants. Furthermore, plants developed enhanced total antioxidant power with an implication of health-promoting effects when exposed to high light intensity. Photoprotection mechanisms in plants may be used to increase antioxidant phytochemicals to improve the nutritional quality of vegetables such as lettuce by applying supplemental lights to crops grown under controlled production environment.

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