Effects of different light intensities on anti-oxidative enzyme activity, quality and biomass in lettuce

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Abstract

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Recently, the worldwide demand for romaine lettuce (*Lactuca sativa* L.) has been increasing. Thus, regulation measures of supplementary or shading light are often used in the production of lettuce in some regions. However, inconsistent results on the light saturation point of lettuce from previous studies do not facilitate the regulation of light intensity. In the present study, the effects of different light intensities on anti-oxidative enzyme activity, yield and quality of lettuce were investigated. The results reveal the following: (1) judged by the dynamics of anti-oxidative enzyme activity, there was no light stress to occurr in the 100, 200 and 400 μmol/m^{2}s treatments, a mild light stress occurred in the 600 μmol/m^{2}s treatment, and a serious light stress occurred in the 800 μmol/m^{2}s treatment; (2) increased light intensity gradually reduced the contents of soluble protein and nitrate in lettuce, whereas the content of soluble sugar remarkably increased. The biomass of a single plant of lettuce in the 600 μmol/m^{2}s treatment was the highest and second highest in the 400 μmol/m^{2}s treatment but was the lowest in the 100 μmol/m^{2}s treatment. No significant difference in the biomass of single plant was observed between the 400 and 600 μmol/m^{2}s treatments. Based on these results, the range of 400 μmol/m^{2}s to 600 μmol/m^{2}s is a recommendable light intensity for lettuce production.

Keywords: environmental stress; superoxide radicals; yield; vegetable

Recently, the worldwide demand for romaine lettuce (*Lactuca sativa* L.) has been increasing because of its crisp texture, pleasant aroma and flavor, fresh appearance, as well as richness in phytochemicals, such as phenolic compounds. Lettuce constitutes an important part of greenhouse production. Among various environmental factors, light conditions is one of the most important variables affecting the growth and development of plants (Inada, Yabumoto 1989). Therefore, many studies focused on the effects of different light conditions on growth and nutritional quality (Knight, Mitchell 1983a, b; Glenn et al. 1984; Gaudreau et al. 1995; Hunter, Burritt 2004, 2005; Li, Kubota 2009). Overall, these studies suggested that high light intensity usually promotes the growth of lettuce and decreases nitrate concentration in lettuce (Blom-Zandstra et al. 1988; Gaudreau et al. 1995). However, the growth-promoting effect only works well within a certain range of light intensity, and that is, light intensity must be lower than the light saturation point. With respect to the light saturation point of lettuce, different studies have conflicting results. Some studies showed that

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the saturation point of lettuce is as high as 889 to 932 μmol/m^2s (Knight, Mitchell 1983a, b). In contrast, other studies revealed that the saturation point of lettuce is only 500 to 520 μmol/m^2s (Glenn et al. 1984).

In the production practice of lettuce, the major limiting factor for winter greenhouse production is the low natural light level in higher latitudes. Consequently, a supplementary lighting system is often used to maintain the winter production of lettuce in some regions, especially in Quebec, Canada, where almost all lettuce growers use artificial lighting systems. In lower latitudes, such as South China, high-intensity light during late spring and early autumn often results in photoinhibition and plant injury. Consequently, most lettuce growers eliminate the adverse effects of high-intensity light on lettuce by taking various shade measures. Thus, maximizing economic benefits by optimizing light intensity regulation is important for lettuce growers in high and low latitudes. The light saturation point should be one of the most important parameters upon which optimal light intensity regulation is based. However, conflicting results on light saturation point of lettuce cannot facilitate light intensity regulation in lettuce production. Hence, other parameters that can be used to guide light intensity regulation must be established.

Environmental stress results in the production and accumulation of reactive oxygen species (ROS), such as superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^-) (Mittler 2002). ROS causes oxidative damage to membrane lipids and proteins (Molassiotis et al. 2006), thus plants evolved several mechanisms to prevent damage from ROS. One of these defensive mechanisms is use of the enzymatic antioxidant system that includes superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD). The level of malondialdehyde (MDA), a decomposition product of polyunsaturated fatty acids produced during the peroxidation of membrane lipids, is often used as an indicator of oxidative damage (Mittler 2002). In other words, the levels of SOD, CAT, POD, and MDA signify whether lettuce plants are under environmental stress, or the extent to which plants are under such stress. Studies on the anti-oxidative enzyme activity of lettuce grown under the drought or salinity stress were reported (Eraslan et al. 2007; Pei, Bie 2007). However, little information on the anti-oxidative enzyme activity of lettuce grown under the light stress is reported. When lettuce plants are grown under very strong light conditions, excess light energy results in the production and accumulation of ROS. Consequently, lettuce plants was induced to be under light stress, inevitably leading to changes in the levels of SOD, CAT, POD and MDA. In the present paper, a recommendable light intensity for lettuce production can be proposed by comparing dynamics of the levels of SOD, CAT, POD and MDA, quality, as well as the biomass of lettuce grown under different light intensity conditions.

MATERIAL AND METHODS

Plant materials and growth conditions. All experiments were conducted in a phytotron chamber in the Jiangsu University, China, during the spring 2010. Loose green leaf lettuce cultivar, Lvling (obtained from Nanjing Lvling Seed Co., Ltd., Nanjing, China), was used as plant materials in the experiment. This cultivar is currently widely cultivated throughout China. On 11 March, uniform-sized seedlings of romaine lettuce at the 10-leaf stage were transplanted into individual plastic pots (20 × 25 cm) filled with perlite. One seedling was planted in each pot. The seedlings in pots were then transferred into the phytotron chamber on 21 March. The phytotron chamber was illuminated with high-pressure sodium lamps and sunlight dysprosium lamps of 400 W. A photoperiod of 14/10 h (light/dark), temperature of 20/16°C (day/night), and air relative humidity of 70% were maintained throughout the experiment. Treatments with four replicates (four pots) consisted of five light intensity levels of 100, 200, 400, 600 and 800 μmol/m^2s. Different light intensity levels were obtained by placing pots on shelves of different heights in the same phytotron chamber, in which the same temperatures from upper to lower chambers were guaranteed using a fan vertically blowing air onto the lettuce plants. The lettuce plants were irrigated daily with a complete nutrient solution containing (mg/l): N 150; P 50; K 150; Mg 50; Ca 150; Fe 5; Mn 0.5; Cu 0.03 and Zn 0.05 (Gul et al. 2005).

Measurement methods. Anti-oxidative enzyme activities were measured on 21 March, 24 March, 28 March and 5 April, respectively. Yield and quality were measured on 7 April.

Enzyme extraction and assay. Enzyme extraction procedures were carried out at 0 to 4°C. Samples from fresh matured leaves (0.5 g) were homogenized in an ice cold mortar in 5 ml of 50mM sodium phosphate buffer (pH 7 for CAT and pH 7.8 for SOD and POD) containing 1mM EDTA-Na_2. The supernatant was used for the determination of SOD, CAT and POD after centrifugation (1,000 g, 20 min). The SOD activity was assayed by the nitroblue tetrazolium (NBT) method (Giannopolitis, Ries 1977). CAT activity was measured spectrophotometrically (Dhindsa et al. 1981). POD activity was measured using the method of Maehly and Chance (1954).

MDA content. The extent of lipid peroxidation in terms of MDA formation was measured according to the method of Esterbauer and Cheeseman (1990).

Determination of quality and biomass. On 7 April, the contents of soluble sugar, soluble protein and nitrate in fresh matured leaves of lettuce were determined using the methods of anthrone-sulfuric acid colorimetry, Commassie brilliant blue G-250 coloration (Li 2007), and UV-spectrophotometry (Cai 2005, Shanghai Precision Scientific Instrument Co., Ltd., Shanghai, China), respectively. The lettuce plants were harvested, and the aboveground biomass of a single plant (fresh weight) of lettuce under different light intensity treatments was determined.

Statistical analyses. Results of the activities of CAT, SOD and POD, and the contents of MDA, soluble sugar, soluble protein, and nitrate, as well as the yields, that resulted from lettuce plant specimens that underwent the various light intensity treatments, were presented as means with standard errors. Statistical analyses, one-way ANOVA and Duncan's multiple range tests were performed at $\alpha = 0.05$ significance level using the SPSS statistical software package (IBM Co., NY, USA).

RESULTS AND DISCUSSION

Dynamics of the levels of SOD, CAT, POD and MDA in different treatments

As a major scavenger of ROS, SOD catalyzes the dismutation of superoxide to hydrogen peroxide and oxygen. However, H_2O_2 is also toxic to cells and has to be further scavenged by CAT, POD, or both, to water and oxygen (Sairam et al. 2005). The content of MDA is often used as an indicator of oxidative damage (Mittler 2002).

With the extension of treatment duration, the dynamics of the levels of SOD, CAT, POD and MDA for lettuce grown under different light intensity conditions are expressed in Fig. 1. The levels of SOD, CAT and POD in lettuce grown in the 100, 200 and 400 μmol/m^2s treatments were relatively low and did not change drastically throughout the experiment. The contents of MDA in lettuce grown in the above three treatments were also low, only with a slight increase in the early experiment and a slight decrease in the late experiment (Fig. 1d). Moreover, no significant differences in the levels of SOD, CAT, POD and MDA were observed among the 100, 200 and 400 μmol/m^2s treatments, respectively.

For the 600 μmol/m^2s treatment, Fig. 1 reveals that the levels of SOD, CAT, POD and MDA initially drastically increased, peaked three days after treatment, and then gradually decreased until the late experiment. The levels of SOD, CAT, POD and MDA in the 600 μmol/m^2s treatment were more than those in the 100, 200 and 400 μmol/m^2s treatments. Significant differences were observed in the above parameters between the 600 μmol/m^2s treatment and the 100, 200 and 400 μmol/m^2s treatments on 3, 7 and 15 days after treatment (excluding the parameters of CAT and POD 15 days after treatment), respectively.

For the 800 μmol/m^2s treatment, Fig. 1 indicates that the levels of SOD, CAT and POD initially very drastically increased, peaked three days after treatment, and then rapidly decreased until the late experiment. Each one was lower than the corresponding value in the other four light intensity treatments in the late experiment (15 days after treatment). Significant differences in SOD and POD were observed, but none in CAT between the 800 μmol/m^2s treatment and the other four light intensity treatments. SOD and POD also exhibited more sensitivity to strong light than CAT. The content of MDA increased rapidly in the early experiment and then rose slowly to the maximum until the late experiment. MDA content was significantly higher than the corresponding value in the other four light intensity treatments throughout the experiment.

From the above dynamics of anti-oxidative enzyme activity, the following deductions can be drawn. No stress resulting from light intensity occurred in the 100, 200 and 400 μmol/m^2s treatments. In the 600 μmol/m^2s treatment, a certain amount of excess light energy led to the production of ROS and a relatively substantial increase in MDA content. The activities of SOD, CAT and POD then

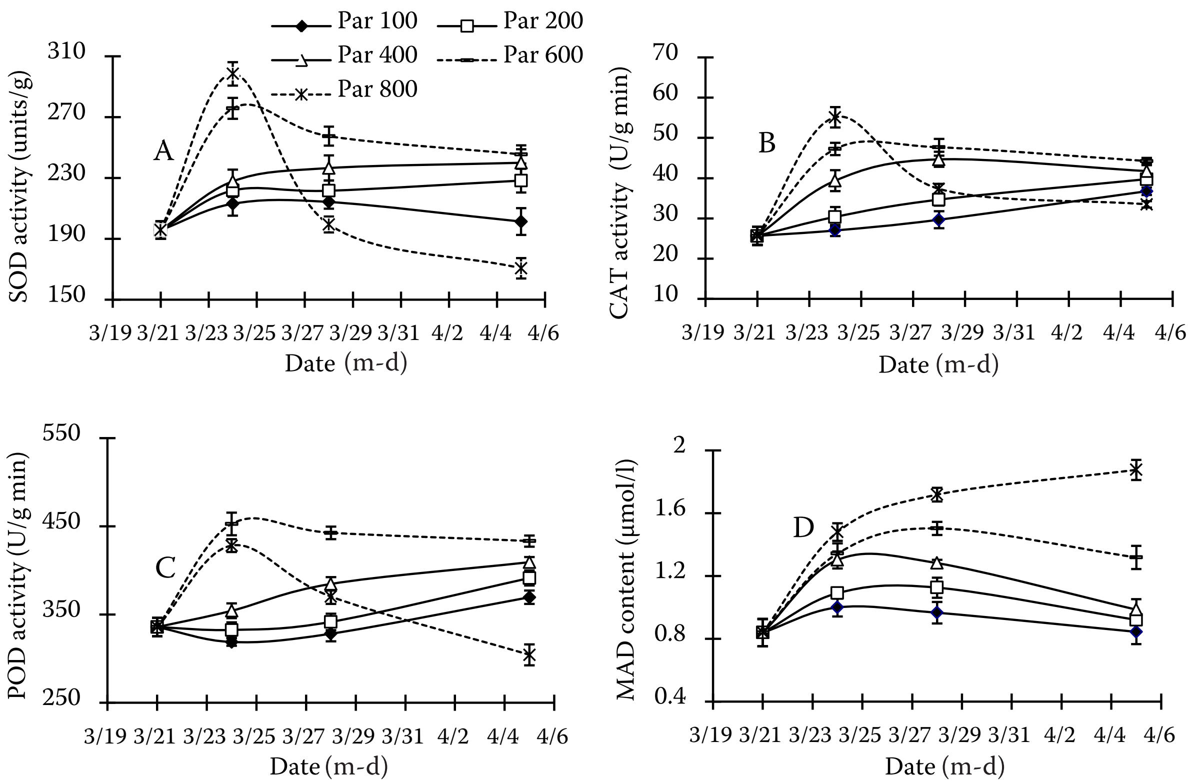

Fig. 1. Dynamics of the levels of SOD, CAT, POD and MDA under different light intensity treatments

increased and remained at a higher level throughout the experiment. Thus, a certain proportion of ROS was scavenged timely and the content of MDA decreased to a level that was still significantly higher than that in the 100, 200 and 400 μmol/m^{2}s treatments. All these findings showed that a mild stress resulting from light intensity occurred in lettuce grown under the 600 μmol/m^{2}s light conditions. In the 800 μmol/m^{2}s treatment, a large number of excess light energy led to the production and accumulation of a mass of ROS and a substantial increase in the content of MDA. The activities of SOD, CAT and POD increased rapidly in the early experiment, but then decreased drastically with the extension of treatment duration. On the other hand, the content of MDA continuously increased. All these results demonstrated that the strong light intensity of 800 μmol/m^{2}s led to a serious damage to the enzymatic anti-oxidant system, rendering it unable to scavenge ROS timely. Therefore, lettuce was under serious light stress during this treatment.

Effect of different light intensities on quality and biomass

Light conditions have an important effect on the quality and yield of vegetables. The present study examined the effects of different light in-

Table 1. Quality and biomass of above-ground part of lettuce under different light intensity treatments

Light intensity (μmol/m^{2}s)	Soluble protein (mg/g)	Soluble sugar (mg/g)	Nitrate (mg/g)	Biomass of above-ground part (g/plant, FW)
100	6.87 ± 0.23^a	2.85 ± 0.17^c	3.35 ± 0.12^a	127.98 ± 8.32^c
200	7.08 ± 0.42^a	3.64 ± 0.29^c	2.19 ± 0.10^b	145.65 ± 7.53^b
400	6.96 ± 0.35^a	4.64 ± 0.31^{bc}	1.03 ± 0.06^c	158.45 ± 6.21^{ab}
600	6.75 ± 0.27^a	4.96 ± 0.25^b	0.84 ± 0.07^c	162.89 ± 7.13^a
800	5.86 ± 0.18^b	5.77 ± 0.21^a	0.51 ± 0.04^d	135.56 ± 5.76^c

means ± SE based on five replicates for quality and yield are presented; means within a column followed by the same letter are not significantly different at the level of 5%

tensity conditions on the quality and yield of lettuce plants grown under otherwise similar environmental conditions. The results are shown in Table 1. The contents of soluble protein and nitrate in lettuce gradually reduced with increased light intensity, whereas the content of soluble sugar remarkably rose. The soluble protein in the 800 μmol/m^{2}s treatment was the lowest, and was significantly lower than that in the other four light intensity treatments. No significant differences were observed among the 100, 200, 400 and 600 μmol/m^{2}s treatments. Although weak light facilitated the synthesis of soluble protein, this promotion was weakened under a very low light, such as 100 μmol/m^{2}s. The maximum and minimum of soluble sugar appeared in the 800 μmol/m^{2}s and 100 μmol/m^{2}s treatments, respectively. Among the five different light intensity treatments, the soluble sugar in the 800 μmol/m^{2}s treatment was significantly higher than that in other four light intensity treatments. No significant differences were observed between the 400 and 600 μmol/m^{2}s treatments, as well as between the 100 and 200 μmol/m^{2}s treatments. Maximum and minimum amounts of nitrate appeared in the 100 and 800 μmol/m^{2}s treatments, respectively. A significant difference was observed between the nitrate content of the 800 μmol/m^{2}s and any other treatments, whereas no significant difference was observed between the nitrate content of the 400 and 600 μmol/m^{2}s treatments. The above-ground biomass of a single plant (FW) of lettuce under different treatments was determined. The results show the following above-ground biomass of a single plant grown under different light intensity treatments (μmol/m^{2}s): 600 > 400 > 200 > 800 > 100. A significant difference was observed between the above-ground biomass of a single plant under the 600 μmol/m^{2}s, as well as 100, 200 or 800 μmol/m^{2}s treatments, whereas no significant difference was observed between the biomass of a single lettuce plant in the 600 μmol/m^{2}s and 400 μmol/m^{2}s treatments.

CONCLUSIONS

In agricultural production, quality, yield, and cost are the three most important criteria by which the optimization of environmental factors is conducted. Thus, these three factors should always be fully taken into account. In the present study, the following conclusions were obtained through the investigation of the effects of different light intensities on the anti-oxidative enzyme activity, quality, and above-ground biomass of a single plant of lettuce, the following conclusions were obtained.

When light intensity was between 100 and 400 μmol/m^{2}s the activities of SOD, CAT and POD, as well as the content of MDA remained at low levels with only a minor range of variation. No photoinhibition occurred in the lettuce. When light intensity reached 600 μmol/m^{2}s, the activities of SOD, CAT and POD initially increased rapidly and then decreased slightly to relatively high levels, accompanied with a moderate content of MDA, which showed that a mild light stress had occurred in the lettuce. The activities of SOD, CAT and POD initially increased rapidly and then decreased drastically to very low levels, when light intensity reached 800 μmol/m^{2}s. In contrast, the content of MDA continued to rise until the late experiment, which showed that a serious light stress occurred.

Despite the occurrence of mild stress in the 600 μmol/m^{2}s treatment, the above-ground biomass was still the highest, but the difference between the above-ground biomass was not significant in the 400 and 600 μmol/m^{2}s treatments. Although nitrate content was lowest and soluble sugar content was highest in the 800 μmol/m^{2}s treatment, the soluble protein was lowest and the yield was the second lowest in this treatment due to serious light stress. Based on these results, the range of 400 to 600 μmol/m^{2}s is a recommendable light intensity for lettuce production.

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