

Effects of Light Intensity during Acclimatization on Antioxidative Enzyme Activities and Growth in Mericlone Plantlets of a Cattleya Hybrid

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Mericlone plantlets of *Brassolaeliocattleya* Sanyan Ruby ‘Shinmei’ with one leaf transplanted into pots with sphagnum moss were acclimatized at 230 (high), 83 (intermediate) and 50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (low light intensity) for 4 weeks in growth cabinets under a 16-h photoperiod and 25°C. The main objective was to determine the antioxidative enzyme activities and the growth of the potted mericlone plantlets. At the intermediate light intensity superoxide dismutase (SOD) that disproportionates superoxide to hydrogen peroxide, and catalase (CAT) reduced activities in both leaves and roots. However, CAT in roots increased 6 h after the onset of acclimatization and then remained constant. Ascorbate peroxidase (APX) activity with participation of ascorbate in roots and CAT activity in roots did not change significantly. At the high light intensity, activities of SOD and APX of leaf and of the three antioxidative enzymes of roots increased. After 4 weeks, foliar SOD and APX activities were markedly higher under the high light intensity than under the intermediate one. Under the low light intensity, these three enzyme activities in both leaves and roots increased. Activities of foliar SOD and CAT decreased after 2 weeks and then remained constant as the activity of APX increased. The three enzymes in the roots maintained higher activities under the low light intensity than under the intermediate one; CAT activity declined rapidly 2 weeks later and reached a level similar to those at the intermediate light intensity. After 4 months of acclimatization, leaves under the high light intensity were shorter than those under intermediate and low ones; roots under the low light intensity were shorter than those of other light intensities. These findings indicate that high light intensity affects leaves of cattleya mericlone plantlets more than their roots, while low light intensity has a reverse effect. The mericlone plantlets were difficult to acclimatize quickly to high light conditions, while they were easy under low light. The antioxidative enzymes are also shown to be possible indexes for light acclimatization.

Key Words: acclimatization, antioxidative enzymes, cattleya, light intensity, mericlone.

Introduction

Like temperature and moisture, light is one of the significant factors to affect acclimatization of orchid mericlones. To adapt orchids to various growing environments each species is recommended to be cultured under a suitable light condition (Mastalerz, 1977; Northen, 1970). Especially for the acclimatization of mericlones and seedlings grown by aseptic cultures, more care is required than for open cultured plants. Thus, a light intensity of 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ is the optimum on the basis of growth and weight of seedlings (Islam et al., 1998). Light is important for photosynthesis but also responsible for the occurrence of leaf scorch when plants are exposed to sudden high intensities (Northen, 1970).

During photosynthesis, light energy absorbed by leaves combines CO_2 with H_2O while releasing O_2 ; the

e^- produced in Photosystem II (PSII) finally reduces CO_2 via the electron transport system. However, all of e^- are not used for assimilation; the excess e^- reduces O_2 to form active oxygen species (AOS) such as superoxide (O_2^-) and H_2O_2 . AOS are harmful to plants and cause damage to various plant body membranes. These damages that easily occur at high light intensities, high temperatures and/or low concentrations of CO_2 , are hereby referred to as photoinhibition. Under these conditions, oxygenase activity of Rubisco also induces photorespiration; that in the peroxisomes glyoxylate pathway produces H_2O_2 by oxidation (Scandalios, 1994). Therefore, plants furnish AOS scavenging systems in which superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) are involved (Anderson et al., 1983; Asada, 1992; Halliwell, 1974).

Li et al. (2001a) reported the effects of high light intensities on SOD, APX and CAT activities in leaves of potted cattleya and cymbidium plants that have different photosynthetic pathways and ecological distributions. However, no such information is available

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on orchid seedlings and mericlone plantlets. Cultural circumstances such as light and temperature may affect roots more than leaves because of transplantation of plantlets from the flasks. We determined that roots of cattleya and cymbidium mericlone plantlets were more easily affected than their leaves by acclimatization temperatures. Increases in foliar SOD activity under lower temperatures indicate intensity of thermal stresses and adaptability of mericlone plantlets to environmental damages (Li et al., 2004a, b). The aim of this study was to clarify the effects of light intensity on AOS scavenging enzymes and to obtain more information about indexes of light acclimatization.

Materials and Methods

1. Acclimatization of mericlone plantlets

Mericlone plantlets of *Brassolaeliocattleya* Sanyang Ruby 'Shinmei' were purchased from a cattleya nursery and grown at 25°C and 50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ light intensity. Single-leaf (6 cm long) plantlets grown in flasks were washed free of the cultural medium and transplanted into plastic pots (15 cm in diameter) with sphagnum moss. Large community pots with 30 plantlets each were transferred to growth cabinets kept at 25°C and 50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (low light intensity), 83 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (intermediate light intensity) and 230 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (high light intensity) provided by fluorescent lamps. Their acclimatization was started on June 25, 2001 under a 16-h photoperiod from 8:00 to 24:00. The plantlets were irrigated daily and fertilized with a nutrient solution (N: P₂O₅: K = 195: 79: 570 ppm) as required.

2. AOS scavenging enzyme extraction and assay

Leaves and roots (about 2 cm of root apex was removed) were excised at the onset of experiment (0 h) and after 6, 12, 24 and 48 h. In addition, samples were prepared after 1, 2, 3 and 4 weeks for enzyme extraction. Three samples, weighing 1 g (FW), were frozen with liquid nitrogen and stored at -20°C. Growth of acclimatized seedlings was also determined at the end of the experiment. Samples were macerated in 4 mL of 50 mM phosphate buffer (pH 7.0) with a mortar and pestle and the mixture was filtered through 2 layers of gauze. Filtrates were centrifuged at 10000 rpm for 20 min at 5°C with a cooling centrifuge (Tomy MX-150, Japan). The supernatants assayed for enzyme activities were used as crude enzyme extracts. SOD, APX, and CAT were determined as described previously (Li et al., 2004b).

3. Growth measurement

After 4 months acclimatization 12 mericlone plantlets under each treatment were collected at random for growth analysis. Leaf length and width and root length and numbers, including new leaves and roots were measured. Leaf width was determined at the middle widest part of each leaf.

Results

1. SOD

1) Activity changes within 48 h

SOD activities in both leaves and roots increased significantly in all treatments at the onset of the experiment (Fig. 1a, c). The highest leaf values were attained after 6 h the high light intensity treatment, whereas the maximum in roots occurred at the low light intensity; the rates declined in both organs. At the intermediate light intensity the SOD activity of the roots increased after 6 h and thereafter maintained nearly constant.

2) Activity changes within 4 weeks

After 1 week, SOD activities of both leaves and roots maintained the same values as those after 48 h in the three light intensities (Fig. 1b, d). Subsequently, SOD activity in leaves under the low and intermediate light intensities and roots under the high and intermediate light intensities decreased gradually. However, the activity in leaves increased at the high light intensity. After 4 weeks SOD activity in leaves was higher at the high light intensity than at the intermediate and the low ones, but that of roots remained low at the intermediate and high light intensities.

2. APX

1) Activity changes within 48 h

APX activity in leaves decreased at the low and intermediate light intensities (Fig. 2a). However, it recovered to the initial value at the low light intensity while it remained low at the intermediate intensity. At the high light intensity APX activity decreased slightly after 12 h and then reverted to the initial level. Roots

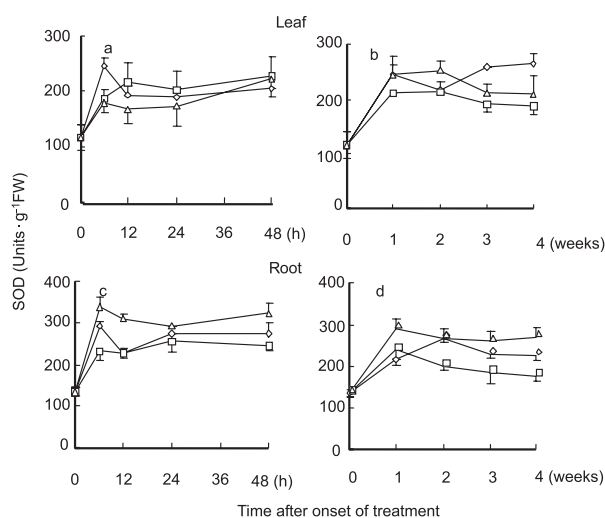


Fig. 1. Effect of acclimatization light intensity (\diamond 230 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (high); \square , 83 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (intermediate); \triangle , 50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (low)) on SOD activity in leaves (a, b) and roots (c, d) of mericlone plantlets of a cattleya hybrid. Vertical bars represent SD ($n=3$).

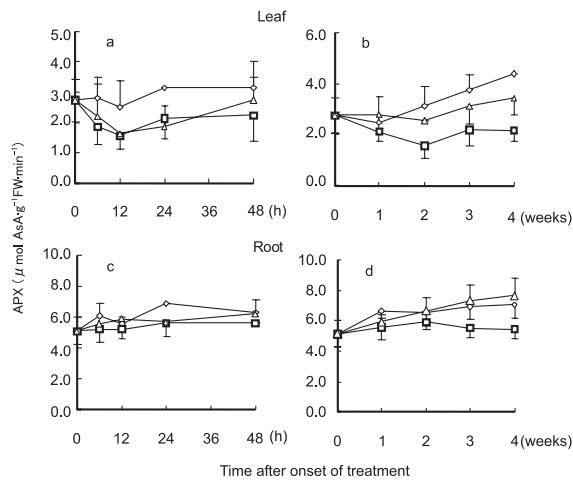


Fig. 2. Effect of acclimatization light intensity (\diamond , $230 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (high); \square , $83 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (intermediate); \triangle , $50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (low)) on APX activity in leaves (a, b) and roots (c, d) of mericlone plantlets of a cattleya hybrid. Vertical bars represent SD ($n=3$).

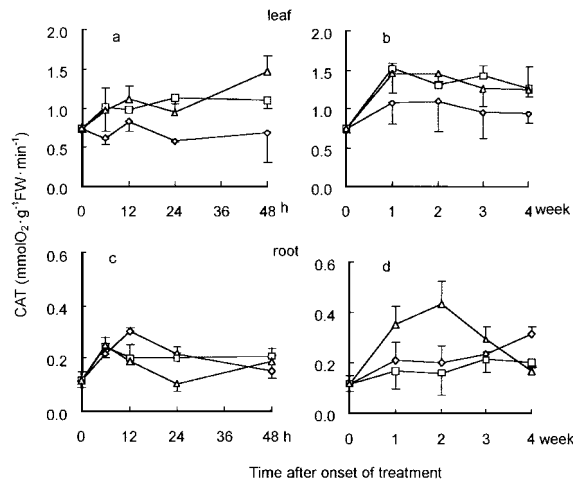


Fig. 3. Effect of acclimatization light intensity (\diamond , $230 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (high); \square , $83 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (intermediate); \triangle , $50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (low)) on CAT activity in leaves (a, b) and roots (c, d) of mericlone plantlets of a cattleya hybrid. Vertical bars represent SD ($n=3$).

showed no distinctive change in APX activity for 48 h at the three light intensities (Fig. 2c).

2) Activity changes within 4 weeks

APX activity in leaves and roots increased at the low and high light intensities after 3 and 4 weeks (Fig. 2b, d), whereas that in leaves at the intermediate light intensity decreased after 2 weeks and then reverted to the initial level after 3 weeks. Thus, APX activities in both leaves and roots at the low and high light intensities after 4 weeks were higher than those at the intermediate ones.

Table 1. Growth of mericlone plantlets of a cattleya hybrid after 4 months acclimatization as affected by light intensity.

Light intensity ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	Leaf		Root	
	Length (cm)	Width (cm)	Length (cm)	Numbers
230 (high)	4.03 a ^z	1.37 a	5.02 b	7.0 a
83 (intermediate)	5.95 b	1.45 a	6.02 c	9.1 a
50 (low)	5.12 b	1.42 a	4.21 a	5.1 a

^z Different letters within columns indicate significant differences by Fischer's new multiple range test at $P \leq 0.05$.

3. CAT

1) Activity changes within 48 h

CAT activity in leaves increased at the low and intermediate light intensities, especially at the former; it changed little at the high light intensity. CAT activity in roots rose in the three treatments for 12 h from the onset of the treatment and then fell to near its initial level (Fig. 3a, c).

2) Activity changes within 4 weeks

After one week CAT activity increased markedly in leaves at the low and the intermediate light intensities and then gradually decreased. At the high light intensity, CAT activity increased slightly but the change was not significant. In roots, CAT activity increased at the low and the high light intensities; the former was greater than that of the latter for 2 weeks and then decreased to the initial level. CAT activity at the intermediate light intensity remained constant (Fig. 3b, d).

4. Growth of mericlone plantlets

Leaves of the plantlets at the intermediate and the low light intensities were longer than those at the high light intensity at the end of the experiment (Table 1). Roots were the longest at the intermediate light intensity, but the number of roots did not significantly differ among light treatments. The results indicate that newly developed roots, which were clearly distinguishable from old parts by neck formation and fresh root color, were longer under the intermediate light intensity.

Discussion

Photooxidation occurs not only in the low latitude regions with strong solar radiation, but also in regions and under the conditions where light intensity is low and plant growth is inhibited. Plants produce cells with low molecular reductants, SOD, APX, CAT etc. to effectively scavenge harmful AOS. This AOS scavenging system is essential for plants to cope with changes in the growing environments for the maintenance of photosynthetic function (Mano and Asada, 1999). Light intensity, as well as temperature (Li et al., 2004a) affected SOD, APX and CAT in both leaves and roots in cattleya mericlone plantlets; those of leaves were affected intensively by the high light intensity, whereas roots were affected by the low light intensity. Moreover,

after 4 months of culture, the leaves at the intermediate and low light intensities were longer than those at the high light intensity. Also, root length at the intermediate light intensity was longer than those at low or high light intensity. These results suggest that antioxidative enzymes may affect plant growth mediated by scavenging AOS formed by light stress.

SOD and APX are present in chloroplasts, mitochondria, microbodies and cytoplasm; CAT is only present in microbodies. SOD that catalyzes O_2^- to H_2O_2 and O_2 , APX that oxidizes ascorbate with H_2O_2 , and CAT that decomposes H_2O_2 to O_2 and H_2O , are associated predominantly with guard systems for AOS in cells (Fridovich, 1986). The induction of these enzymes are synchronized with each other under some environmental stresses (Morita and Tanaka, 1999). Upon removing plantlets from the culture flasks, SOD activity in acclimatizing leaves in all three light intensities increased within 6 hr after the onset of the treatment. Similarly, increased foliar SOD activities were observed under adverse temperatures (Li et al., 2004a). The highest rates occurred at the high light intensity, whereas, at the low and the intermediate light intensities, SOD activity increased after 1 week of acclimatization and then declined, but the enzyme activities remained high under the high light intensity. This suggests that excessive light energy induces an increase in AOS in chloroplasts, because SOD is activated by the presence of O_2^- (Fridovich, 1974). Under the high light intensity APX is associated with water-water cycle (Asada, 1999) in which excessive e^- transforms H_2O to H_2O_2 via O_2^- ; subsequently, H_2O is reconstituted to dissipate excess light energy. However, APX was not activated and CAT reduced the activity, suggesting an increase in H_2O_2 . As H_2O_2 inactivates SOD (Fridovich, 1986), this enzyme activity decreased during 48 h. However, because both SOD and APX activities were high after 2 weeks of acclimatization, the water-water cycle worked well and plants adapted to the high irradiance. Under the high light intensity CAT activity in leaves was lower than that at the low and intermediate light intensities which differed from findings of Li et al. (2001a) who worked with potted cattleya plants; however the results were similar to those with potted cymbidiums. Cattleyas undergo classulacean acid metabolism (CAM) but the acid cycle depends on plant age and environmental factors. Besides, CAT activity in mericlone plantlet leaves in this study was higher than that of potted cattleya plants which was low similar to the CAM plants because no or low photorespirations occurred (Li et al., 2001a, b). Therefore, it seems that the mericlone plantlets did not initiate CAM during acclimatization and performed as C_3 plants. Under the high light intensity CAT was not activated. Unlike the temporary increases in activities reported by Li et al. (2004a), Feierabend et al. (1992) showed that photoinactivation of CAT and photosystem II is general phenomena of the initial photodisorder that

results in irreversible inactivation of CAT by O_2^- (Kono and Fridovich, 1982). Furthermore, as H_2O_2 can affect photosynthesis (Kaiser, 1976), the presumed accumulation in peroxisomes forms high-reactive hydroxyl radical ($OH\cdot$) through Harber-Weiss reaction (Kono, 1988). This reaction may decrease photosynthetic products by breaking down chlorophylls and microbodies.

Under the low light condition, SOD activity, which increased in leaves after the onset of the treatment, decreased after 3 weeks. APX increased after 2 weeks of acclimatization similar to the result obtained with leaves of potted cattleya plants that was not affected by a short exposure of sunlight, but stimulated by shading (Li et al., 2001a, b). The changes in SOD and APX may be responsible for increased chlorophyll content under the low light intensity. Although chlorophyll contents were not determined in this study, they increased in potted cattleya plants under the low light intensity; the changes correlated well with SOD and APX activities (Li et al., 2001a) of which one isozyme is located in chloroplasts (Asada 1999; Fridovich, 1986). However, low light intensity did not seem to affect photorespiration because no difference in CAT activity between the intermediate and the low light intensities was observed.

Exposed roots that grew in sphagnum moss produced almost no chlorophyll, so that they produced little photoassimilates. When the effects of the high and low light intensities are compared, the latter affected more SOD and APX activities. Mericlone plantlets in flasks can absorb sugars in the media and grow smoothly, but when suddenly transferred to an autotrophic condition by transplantation, they encounter nutritional shortage at the low light intensity that causes possible stress. This supposition is partially supported by the decreased root number and length at the low light intensity after 4 months of acclimatization. Root length was also affected by the high light intensity. The high SOD, APX and CAT activities at both low and high light intensities during acclimatization compared with those at the intermediate light intensity are partly attributed to the damaged roots during transplantation.

In conclusion, root damage during transplantation and changes in light intensities during acclimatization induced much stress that increased SOD activity, but the levels of SOD activation are useful to evaluate degrees of stresses imposed to plantlets. Cattleya mericlone plantlets require a longer period to become acclimatized to strong light as shown by the increasing foliar SOD and APX activities after 4 weeks of acclimatization. They have the potential to tolerate low light intensity that allows the antioxidative enzymes to adapt to changes depending on plant species.

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カトレア交配種メリクロン苗の抗酸化酵素活性と生長に及ぼす順化光強度の影響

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カトレア交配種メリクロン苗をフラスコからミズゴケでポットに植え替え、植物育成用蛍光灯で光量を 50 (弱光), 83 (中光), 230 (強光) $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ に設定した 25 °C, 16 時間日長のグロースキャビネット内で、4 週間順化し、葉と根の抗酸化酵素活性の変化および生育量について調べた。中光区では葉と根で生じたスーパーオキシドを過酸化水素に分解する SOD 活性および根の過酸化水素を分解する CAT 活性は 6 時間後に増大した後、安定状態となった。葉と根で生じた過酸化水素をアスコルビン酸により分解する APX 活性および根の CAT 活性はほとんど変化しなかった。これに対して強光区では葉の CAT 活性は変化せず、葉の SOD と APX および根の 3 酵素の活性は増大した。弱光区では葉、根ともに 3 酵素

の活性は増大した後、葉の SOD, CAT 活性が処理後 2 週間以降、低下して安定状態となり、APX 活性は上昇を継続した。根の SOD, APX, CAT 活性は弱光下で中光区より高く推移した。ただし、根の CAT 活性は処理 2 週間後から著しく低下して処理終了時には中光区と同等となった。順化 4 か月後、強光区の葉長は中、弱光区より有意に短くなった。弱光区の根長は強光、中光区より短かった。中光区の葉長と根長は強、弱光区より長かった。これらの結果は強光が葉に、弱光が根に大きく影響すること、カトレアメリクロン苗は強光に対して早期の適応が困難であること、弱光への適応能力は有することなどを示すとともに、これらの酵素が光環境への順化の指標となり得ることを示している。