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Effects of light intensities on antioxidant enzymes and malondialdehyde content during short-term acclimatization on micropropagated *Phalaenopsis* plantlet

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Abstract

We investigated the effects of light stress on the activities of antioxidant enzymes, such as superoxide dismutase (SOD), dehydro ascorbate reductase (DHAR), monodehydro ascorbate reductase (MDHAR), ascorbate oxidase (AO), glutathione reductase (GR), guaiacol peroxidase (G-POD), catalase (CAT), glutathione *S* transferase (GST), lipoxygenase (LOX), lipid peroxidation (LP), leaf protein content and photosynthetic efficiency (Fv/Fm) in order to evaluate their role during acclimatization in *Phalaenopsis* orchids. Six months old in vitro grown plantlets were exposed to low light (LL-60 μmol m^{-2} s^{-1}), intermediate light (IL-160 μmol m^{-2} s^{-1}) and high light (HL-300 μmol m^{-2} s^{-1}) photosynthetic photon flux density (PPFD), respectively under controlled condition. Plantlets exposed to HL intensity had lower level of Fv/Fm ratio than the LL grown plantlets during acclimatization. Regarding antioxidants enzymes, SOD activity increased in leaves with increasing light intensity but light stress had no significant effect in roots. DHAR and MDHAR activities increased in LL and IL but decreased at HL. The CAT activity increased in both leaves and roots with increasing light intensity. While G-POD activity increased in roots, POD activity was not detected in leaves. No significant change in GR activity has been found at IL and HL, though it decreased significantly at LL compared to in vitro grown plantlets. There was an increase in AO activity in leaves of about 50% at HL compared to in vitro grown plantlets, whereas no changes in roots were observed. GST activity showed pronounced stimulation in both leaves and roots of the plantlets exposed to HL compared to in vitro grown ones. Total leaf protein content increased in light stressed plantlets compared to in vitro

Abbreviations: AO, ascorbate oxidase; APX, ascorbate peroxidase; C, indicates in vitro grown plantlets; CAT, catalase; CDNB, 1-chloro-2,4-dinitrobenzene; DTNB, 5,5′-dithiobis (2-nitrobenzoic acid); DHA, dehydro ascorbate content; DHAR, dehydro ascorbate reductase; Fv/Fm, photosynthetic efficiency; G-POD, guaiacol peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; GST, glutathione *S* transferase; HL, high light; IL, intermediate light; LL, low light; LP, lipid peroxidation; LOX, lipoxygenase; MDA, malondialdehyde; MDHA, monodehydro ascorbate content; MDHAR, monodehydro ascorbate reductase; NBT, nitro blue tetrazolium; PMSF, phenylmethylsulfonyl fluoride; PVP, poly vinyl pyrrolidone; PPFD, photosynthetic photon flux density; ROS, reactive oxygen species; SOD, superoxide dismutase; TBA, 2-thiobarbituric acid

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grown plantlets. LP and LOX increased during light stress compared to in vitro grown plantlets suggesting that LOX mediated lipid peroxidation contributed to the oxidative damage occurring in the present study. These results suggest that increase in enzyme activities were an adaptive response of the plantlets to higher amounts of reactive oxygen species (ROS) generated during acclimatization under light stress.

Keywords: Antioxidative enzymes; Fv/Fm; Lipid peroxidation; Lipoxygenase; Light intensity; *Phalaenopsis*

1. Introduction

Plants are exposed to a range of fluctuating light intensities (photosynthetic photon flux-PPF) in natural environments, which can lead to depression in photosynthetic efficiency (photoinhibition) mainly due to oxidative damage to the photosystem (PS) II (Powles, 1984). At low light intensity, an increase in photosynthetic carbon fixation has occurred, which varies depending on growth and light intensity, may lead to different susceptibilities to photoinhibition (Powles, 1984). However, above a certain threshold, carbon fixation becomes saturated and photosynthesis is incapable of using all of the energy absorbed by the plants. Under these conditions of excess light absorption, the chloroplast lumen becomes acidic in nature, reduces the electron transport chain, and excitation energy accumulates within chloroplast. Excess excitation energy (EEE) could result in an increase in the singlet and triplet forms of chlorophyll and singlet oxygen, which are toxic in nature. Depletion of $NADP^+$ pool under EEE causes an increase in the rate of electron flow from the donor side of photosystem I (PSI) to oxygen, generating reactive oxygen species (ROS) such as superoxide and hydrogen peroxide (Asada, 1999). The main source of ROS generation in plants is chloroplast (Asada, 2000). Furthermore, once initiated, lipid peroxidation (LP) becomes autocatalytic, resulting in massive membrane photodestruction (Niyogi, 1999). If these ROS are not removed immediately, ROS can cause damage to the cellular and molecular machinery, protein modification and lipid peroxidation. To minimize the ROS effects, plants have evolved various antioxidative enzymes and low molecular weight non-antioxidative substances such as ascorbate, glutathione, α-tocopherol. The enzymatic antioxidants namely, SODs convert superoxide to H_2O_2 and oxygen, and exist in three isoforms e.g., Cu/Zn SOD, Fe-SOD and MnSOD. Besides, ascorbate-glutathione cycle enzymes such as ascorbate peroxidase (APX), dehydro ascorbate reductase (DHAR), monodehydro ascorbate reductase (MDHAR) and glutathione reductase (GR) constitute the defensive system against the ROS (Asada, 1999). Superoxide radicals generated on the acceptor side of PSI are detoxified by a series of membrane-associated and stromal enzymes, including superoxide dismutase (SOD) and ascorbate peroxidase (Asada, 1999).

In vitro propagation is an efficient method to produce large amount of uniform plantlets. However, micropropagated plantlets are associated with several physiological and anatomical abnormalities during in vitro growth such as low photosynthesis, non-proper functioning of stomata, malfunctioning of water housekeeping systems mainly due to high humidity inside the culture vessel (Kozai, 1991; Grout and Aston, 1978; Capellades et al., 1990). Once transferred to ex vitro, micropropagated plantlets are (easily) susceptible to photoinhibition because of lack of well-developed physiological systems mentioned above. Therefore, acclimatization of micropropagated plantlets to ex vitro is a crucial step to cope with the new environment for better growth and development. These environmental changes associated with in vitro plantlets can be improved in ex vitro by controlling the light intensity and with improved number of air exchange (Amâncio et al., 1999; Hahn and Paek, 2001). However, to protect the plants from certain environmental changes plants modify their physiological condition using antioxidants systems. More or less, ascorbate-glutathione cycle plays an important role to protect the plant from such immediate adverse condition. Huylenbroeck et al. (2000) reported the existence of antioxidative enzymes in micropropagated plantlets during acclimatization. Therefore, a better understanding of the effects of light stress on antioxidant enzymes