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Photosynthetic pigments, morphology and leaf gas exchange during ex vitro acclimatization of micropropagated CAM *Doritaenopsis* plantlets under relative humidity and air temperature

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Abstract

The effect of relative humidity (RH) and temperature on CO_2 assimilation (An), stomatal conductance (Sc), transpiration rate (Tr), chlorophyll content, fresh and dry weight, leaf length, leaf area, leaf width, formation of new root and survival rate have been assayed in *Doritaenopsis* in growth chamber after 1 month of acclimatization. Reduced growth was observed at below and above 25 °C whereas it was increased with increasing humidity. Relative water content (RWC) was decreased at 50% and 70% humidity after second day of transfer and recovered completely with the progression of acclimatization. RWC also reduced at high temperature but recovered slowly and a gradual decrease of RWC was observed at 15 °C. A visual symptom of severe leaf tip burn was observed at 50–70% humidity and at 35 °C during acclimatization. At 15 °C and 50% humidity sudden decrease of photosynthetic efficiency (F_v/F_m) was observed, which could not recover in temperature treated plantlets during acclimatization period. Chlorophyll content increased with increasing humidity and at 15 and 35 °C chlorophyll content was decreased compared to 25 °C. Chlorophyll a/b ratio was unchanged while total chlorophyll/carotenoids ratio was increased from low to high temperature. Exposure of plantlets to high temperature led to a noticeable decrease in An, Sc and Tr, and at 15 °C they were more decreased whereas significant differences were not observed in the parameters tested under humidity after 25 days of acclimatization. During daytime at 15 °C, increase in An, Sc and Tr indicates the plantlets adaptability in the new environment. The peroxidase activity remained unaffected in all humidity stress whereas low temperature increased the peroxidase activity

Abbreviations: An, CO_2 uptake; CAM, crassulacean acid metabolism; DW, dry weight; FW, fresh weight; LHC, light harvesting complex; POD, peroxidase; F_v/F_m, photosynthetic efficiency; PPFD, photosynthetic photon flux density; RH, relative humidity; RWC, relative water content; Sc, stomatal conductance; Tr, tanspiration rate; VPD, vapour pressure deficit

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compared to high temperature. These finding suggests that photosynthetic properties was greatly affected by air temperature conditions with a reduction of An, Sc and Tr at 15 and 35 °C compared to humidity stress that played a greater role in limiting photosynthesis.

Keywords: Acclimatization; CO_2 assimilation; Chlorophylls; *Doritaenopsis*; CAM; Humidity; Photosynthesis; Relative water content; Temperature

1. Introduction

In vitro grown plantlets are considered to have low photosynthetic efficiency, which fails to provide a positive carbon balance. Therefore, sugar is required as a carbon and energy source for their mixotrophic growth (Grout and Ashton, 1978). Kozai et al. (1990) suggested increasing in photosynthetic efficiency of in vitro plantlets would be helpful during acclimatization to ex vitro conditions. Development of photosynthetic properties are very important for in vitro plantlets, which could be improved by altering their environmental growth conditions, such as increasing light intensity, humidity, air temperature or CO_2 concentration. Among different factors, the effects of humidity and temperature on plantlets growth are often neglected by growers. Variations of humidity between 1 and 0.2 kPa vapour pressure deficit (VPD) have small effects on the physiology and development of horticultural crops (Grange and Hand, 1987). Nevertheless, some authors observed that low VPD and temperature positively affects dry matter accumulation, but they did not record any physiological disorders in plantlets (Bakker, 1984; Yoshida, 1981). Humidity and temperature play an important role in the growth and development of plantlets metabolism during acclimatization. High humidity often causes shoot elongation, increase fresh weight, leaf area but may cause plantlets diseases such as mildew and botrytis as well as bacterial infection where moisture forms on the leaf surfaces (Kranz, 1996; Remigio et al., 2003). However at high and low temperatures growth and development of plant species are greatly reduced (Yoshida, 1981). High humidity level also reduces transpiration rate (moisture loss from the leaves). Since transpiration is essential not only to cool leaf surface temperature but creates a suction effect resulting in water and mineral uptake and transportation within the plantlets. Thus, the deficiency in water supply could induce physiological and morphological changes in plantlets affecting the growth and survival rate (Tenhunnen et al., 1987). The physiological response to humidity and temperature has been studied in many crops (Lahav and Trochoulias, 1982; Menzel and Simpson, 1988). Relatively very little information is available on *Doritaenopsis* physiology and morphology under stress conditions. *Doritaenopsis* are low light orchids and will grow and flower reliably in natural or artificial light as low as 300–500 foot-candles (fc). However, 1000–1500 fc is the preferred level. The ideal temperature range is 20–24 °C, however, they can tolerate higher temperatures better, than lower temperatures. Humidity and water are important to *Doritaenopsis* plantlets, the recommended humidity being between 50% and 80%. Having a monopodial growth habit, *Doritaenopsis* rarely produce offshoots in nature. They are proliferated by ex vitro top-cuttings, but cloning rate is extremely low, therefore propagation through tissue culture is desirable.

Doritaenopsis take up atmospheric CO_2 predominantly at night and have distinct four phases of CO_2 assimilation (Osmond, 1978). Carbon dioxide enters the mesophyll leaf tissue through the open stomata at night (Phase I of CAM) and in the reaction catalyzed by phosphoenol/pyruvate carboxylase (PEPC) in the cytosol it combines with phosphoenol pyruvate (PEP) to form oxolaacetate. Thus, in CAM plantlets the fixation of CO_2 to malic acid takes place at night. On illumination, the stomata close and malic acid is remobilized and decarboxylated. Temperature is one of the most important factors affecting plant growth and physiology (Berry and Björkman, 1980). Increasing temperature may substantially increase carbon assimilation in some plant assuming other environmental factors do not limit assimilation. However, low temperature induces stomata closure and inactivation of photosynthetic cycle enzymes, which reduced utilization of light energy in carbon metabolism and induced photoinhibition. The inactivation of photosynthesis is associated with PS II and is very sensitive to heat