



# Carbon assimilation of petunia cuttings in a non-disturbed rooting environment: Response to environmental key factors and adventitious root formation

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## ABSTRACT

Considering that adventitious root formation (ARF) relies on adequate supply of carbon, the objectives of this study were (I) to evaluate how CO<sub>2</sub> assimilation contributes to the carbon balance of petunia cuttings and (II) to study the extent to which CO<sub>2</sub> assimilation depends on the progress of ARF and environmental key factors. CO<sub>2</sub> gas exchange and dry matter production of *Petunia hybrida* ‘Mitchell’ cuttings were monitored in a specifically designed multiple open chamber system using plastic covered rooting trays as measuring cuvettes connected to an infrared CO<sub>2</sub> sensor. Excised cuttings were rooted for two weeks in a growth chamber at 22/20 °C (day/night) at a photosynthetic photon flux density (PPFD) of 100 μmol m<sup>-2</sup> s<sup>-1</sup> and a CO<sub>2</sub> concentration of approximately 400 ppm. An increase in dry matter of 186 mg per cutting, or 158% of the initial dry matter, reflected high carbon assimilation. Whilst the shoot dry matter of cuttings increased substantially in the very first week root dry matter growth was not observed until day seven after planting. The short-term response of CO<sub>2</sub> gas exchange to environmental conditions revealed that net photosynthesis ( $P_N$ ) enhanced with increasing PPFD, with a maximum  $P_N$  of 7.8 μmol m<sup>-2</sup> s<sup>-1</sup>. Temperature response curves exhibited only minor changes of  $P_N$ ; dark respiration ( $R_D$ ) increased considerably when the temperature was temporarily increased. Furthermore, cuttings responded to an increase in CO<sub>2</sub> concentration from 300 to 1200 ppm by almost doubling  $P_N$ . When cuttings were rooted permanently under PPFDs of 150 μmol m<sup>-2</sup> s<sup>-1</sup> and 80 μmol m<sup>-2</sup> s<sup>-1</sup> (22/20 °C day/night; CO<sub>2</sub> concentration 400 ppm)  $P_N$  and  $R_D$  of cuttings at the higher PPFD were constantly higher than under the lower PPFD. The CO<sub>2</sub> gas exchange of both treatments was relatively constant during ARF. The light response curves of  $P_N$  differed after one week of exposure to the two light intensities. Light adaptation was comparable to that of stock plants measured using a portable infrared gas analyser equipped with a leaf chamber.

The data indicates that petunia cuttings rooted under conditions frequently applied in young plant production in Central Europe show significant carbon assimilation from the first day onwards. The data also reveals that CO<sub>2</sub> gas exchange under such conditions is not affected by ARF, but is subject to prevailing environmental factors such as light intensity, temperature and CO<sub>2</sub> concentration.

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## 1. Introduction

Petunia is one of the most popular bedding and potted flowering plants in the world. Like many other ornamental plant species,

**Abbreviations:** ABA, abscisic acid; ARF, adventitious root formation;  $C_{\text{fixed}}$ , cumulative carbon fixed in photosynthesis;  $C_{\text{gain}}$ , cumulative carbon gain of the CO<sub>2</sub> gas exchange;  $C_{\text{dry}}$ , cumulative carbon gain of the dry matter; CUE, carbon use efficiency; DLI, daily light integral; dpin, days post insertion; LCP, light compensation point of net photosynthesis; LSP, light saturation point of net photosynthesis;  $P_{\text{gross}}$ , gross photosynthesis;  $P_N$ , net photosynthesis; PPFD, photosynthetic photon flux density;  $P_{N\text{max}}$ , maximum net photosynthesis;  $R_D$ , dark respiration.

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petunia (*Petunia × hybrida*) is propagated vegetatively relying on adventitious root formation (ARF) of the cuttings. In order to obtain high-quality young plants for the market, cuttings must have sufficient capacity to form adventitious roots. The stock plants of cuttings are usually cultivated under high light intensities in regions such as Central or South America. Since the main markets for these ornamentals are Central Europe and the USA and since non-rooted cuttings can be densely packed, they are usually transported to rooting stations close to markets in the temperate zones. To be able to supply consumers with fully grown plants in spring and early summer, these cuttings are usually rooted in winter and spring, when solar radiation is low in these temperate zones.

It is generally known that carbohydrates play a crucial role in the later phase of ARF, providing energy and carbon skeletons to the rooting zone to power root development and growth by

supporting cell division, elongation and differentiation into root structures (Haissig, 1984; Stitt and Krapp, 1999). Furthermore, recent studies on ARF in petunia cuttings reveal the involvement of carbohydrates even in the early stages of ARF when a root sink is established, causing an increased sucrose translocation from source leaves to the stem base (Ahkami et al., 2009; Klopotek et al., 2010). ARF may therefore be affected by carbohydrate availability, which is dependent on the cutting's carbohydrate reserves. Carbohydrate availability can be changed by the exogenous application of sugars to the growth media (Li and Leung, 2000; Calamar and De Klerk, 2002) and by synthesis of photoassimilates by the cutting. Consequently, photosynthesis activity may indirectly affect the rooting process (Okoro and Grace, 1976; Haissig, 1984; Tschaplinski and Blake, 1989; Pellicer et al., 2000; Rapaka et al., 2005; Cross et al., 2006).

The photosynthesis of cuttings differs with the plant species. However, photosynthesis is also affected by mineral nutrition, e.g. phosphorus and nitrogen supply (Davis, 1988; Paul and Driscoll, 1997; Harmens et al., 2000; Matloobi et al., 2008) and other environmental factors such as CO<sub>2</sub> concentration and temperature. Light intensity is of particular importance because even minor changes may alter the photosynthesis. In general, it is assumed that applying higher irradiance leads to an increase in photosynthesis, which in turn enhances translocation and improves the supply of sugars to the stem base, promoting ARF (Svenson and Davies, Jr., 1990). However, cutting propagation is usually carried out in greenhouses, meaning that higher irradiance is associated with an increased temperature of the ambient air and plant leaves. Considering that water uptake is limited at the beginning of the rooting process, greater light intensity may initiate water stress and an increase in the respiration of the cuttings, impairing in turn the rooting process (Evans, 1952; Loach, 1977; Loach and Gay, 1979; Aminah et al., 1997).

CO<sub>2</sub> gas exchange has been analysed during the rooting of cuttings of several plant species, but not of *Petunia*. Several authors have demonstrated that, regardless of whether single leaf (Svenson and Davies, Jr., 1990; Svenson et al., 1995) or whole cuttings (Davis and Potter, 1981; Smalley et al., 1991) were measured in leaf cuvettes or measuring chambers, the photosynthesis per leaf area was generally low at the beginning of ARF and increased significantly upon the emergence of the first roots. During these measurements, however, the cuttings or single leaf were removed from their growth environment and the photosynthesis was measured under different conditions to those prevailing in the growth chamber. Since shoot tip cuttings derived from the root system are highly sensitive to environmental changes (Loach, 1977), the ideal measuring system should resemble the rooting environment as closely as possible.

In a recent study, monitoring carbohydrates in petunia cuttings revealed a strong accumulation during rooting in covered (90% light transmission) rooting trays. This was observed on cuttings which had been adapted to relatively high irradiance (mean daily light integral (DLI)=6.5 mol m<sup>-2</sup> d<sup>-1</sup>, mean photosynthetic photon flux density (PPFD)=180 μmol m<sup>-2</sup> s<sup>-1</sup> per 10 h day length) and were rooted under a low PPFD of 100 μmol m<sup>-2</sup> s<sup>-1</sup> (Klopotek et al., 2010). The latter is within the range of light intensity usually applied in European rooting stations in winter (Rapaka et al., 2005). This result stays in contrast with observations on cuttings of pelargonium (Rapaka et al., 2005), suggesting that petunia cuttings may have a high photosynthetic activity under such conditions.

The objective of this study was to evaluate how current CO<sub>2</sub> assimilation contributes to the carbon balance of petunia cuttings in their non-disturbed rooting environment and to study its dependence on the progress of ARF and on environmental key factors. With irradiance, short-term responses and long-term adaptation irradiance were considered. Therefore, we developed a multiple

open chamber system for measuring the CO<sub>2</sub> gas exchange of whole petunia cuttings using covered rooting trays as measuring cuvettes.

## 2. Materials and methods

Three experimental approaches were applied: Experiment A involved the overall carbon assimilation of cuttings at a PPFD of 100 μmol m<sup>-2</sup> s<sup>-1</sup>, as reflected by the short-term responses of CO<sub>2</sub> gas exchange (net photosynthesis ( $P_N$ ) and dark respiration ( $R_D$ )) to PPFD, the temperature and CO<sub>2</sub> concentration and dry matter production during the monitored time. Experiment B focused on the acclimation of  $P_N$  and  $R_D$  of cuttings to two low-range light intensities (PPFD of 80 versus 150 μmol m<sup>-2</sup> s<sup>-1</sup>) during rooting, simultaneously considering the carbon gain of gas exchange as related to the carbon gain in the dry matter of cuttings. Finally, we analysed the light adaptation of young shoots still attached to the stock plant (Experiment C).

### 2.1. Plant material, growth conditions and treatment of cuttings

Seeds of *Petunia × hybrida* cv. 'Mitchell' were sterilised and germinated and stock plants were established in the greenhouse as described by Klopotek et al. (2010). In the last two weeks leading up to the use of the cuttings or stock plants in the experiments, they were subjected to a daily light integral (DLI) of 7.4 mol m<sup>-2</sup> for Experiment A, 6.7 mol m<sup>-2</sup> for Experiment B and 5.3 mol m<sup>-2</sup> for Experiment C, which correspond to average PPFDs for the 10 h of the light phase of 207, 186 and 148 μmol m<sup>-2</sup> s<sup>-1</sup>, respectively.

Cuttings were obtained using shoot tips containing four to five leaves of a similar size, leaving two nodes of the shoot on the plant. Experiments A and B were performed approximately three months after the stock plants had germinated in May and June. Leafy cuttings were excised from stock plants 1 h before the light period started. Sixteen cuttings per tray were planted in perlite Perligran A with a particle size of 0–6 mm and a pH of 7.0 (Knauf Perlite GmbH, Dortmund, Germany) in plastic trays (46 cm × 28 cm × 5 cm, not sub-divided in cells) covered using a plastic hood with a 90% transmission for photosynthetic active radiation. The cuttings were irrigated daily with tap water. The trays covered with plastic hoods were used as cuvettes for the CO<sub>2</sub> gas exchange measurements.

Trays were placed in a growth chamber (Yorck, Mannheim, Germany) for a 14-day rooting period. Unless stated otherwise in the treatment descriptions, the temperature in the cuvettes was 22 °C during the day and 20 °C at night. The PPFDs under the plastic hood, which averaged 100 μmol m<sup>-2</sup> s<sup>-1</sup>, were generated by high-pressure sodium discharge lamps AGRO SON-T 400 W (Philips, The Netherlands) for a 10-h photoperiod. The CO<sub>2</sub> concentration in the chamber was adjusted to achieve a CO<sub>2</sub> concentration of approximately 400 ppm under the plastic hood. The relative humidity of the air in the cuvettes was approximately 85%. These conditions were defined as standard.

### 2.2. Measurement of the CO<sub>2</sub> gas exchange of the cuttings

Continuous measurements of net CO<sub>2</sub> exchange rates of whole petunia cuttings were conducted using an open chamber system (Fig. 1). The trays covered with plastic hoods were arranged in two rows in a growth chamber. Air from inside the growth chamber was pumped into each cuvette at a rate of 190 L h<sup>-1</sup>, controlled by variable area flow meters (Westphal, Ottobrunn, Germany) using a flexible hose. A second pump was used to alternately extract air from a cuvette or from the growth chamber at a rate of 60 L h<sup>-1</sup> controlled by magnet valves (Sirai, Bussero, Italy). This air was pumped into another hose and conducted to an infrared CO<sub>2</sub> sensor (LI-COR LI820, Lincoln, USA). The measurement signal became stable after 1 min. The signal from each cuvette was recorded successively with

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