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A higher sink competitiveness of the rooting zone and invertases are involved in dark stimulation of adventitious root formation in *Petunia hybrida* cuttings

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ABSTRACT

The contribution of carbon assimilation and allocation and of invertases to the stimulation of adventitious root formation in response to a dark pre-exposure of petunia cuttings was investigated, considering the rooting zone (stem base) and the shoot apex as competing sinks. Dark exposure had no effect on photosynthesis and dark respiration during the subsequent light period, but promoted dry matter partitioning to the roots. Under darkness, higher activities of cytosolic and vacuolar invertases were maintained in both tissues when compared to cuttings under light. This was partially associated with higher RNA levels of respective genes. However, activity of cell wall invertases and transcript levels of one cell wall invertase isogene increased specifically in the stem base during the first two days after cutting excision under both light and darkness. During five days after excision, RNA accumulation of four invertase genes indicated preferential expression in the stem base compared to the apex. Darkness shifted the balance of expression of one cytosolic and two vacuolar invertase genes towards the stem base. The results indicate that dark exposure before planting enhances the carbon sink competitiveness of the rooting zone and that expression and activity of invertases contribute to the shift in carbon allocation.

1. Introduction

Petunia hybrida is one of the economically most important ornamental plant species and a great proportion of plants are generated by vegetative propagation. The key developmental process in determining its successful propagation is adventitious root (AR) formation in the stem base of leafy shoot tip cuttings. Stock plants for cutting production of petunia and other plant species are cultivated in tropical regions or low latitude sites. After harvesting the cuttings, they are densely packed and transported to rooting stations in the main business markets of Central Europe and the USA [1,2]. There, cuttings are rooted under low irradiation during winter to provide plants to the consumers in spring and early summer. The transport period is a necessary step within the production chain of petunia young plants and usually takes place in the dark. Additionally, cuttings are intermediately stored usually in the dark at reduced temperatures for collecting cuttings before rooting to cope with the temporarily high demands for young plants of certain cultivars [3]. Low temperature storage slows down plant metabolism and extends shelf life of cuttings [4–6]. However, the storage potential of the plant is dependent on its genotype. *Pelargonium* has been repeatedly described as storage-sensitive genus responding to dark exposure with senescence of cuttings or insufficient AR formation thereafter [7–10]. A good storage tolerance was found for carnation [11–13], chrysanthemum [14] and also for petunia [2]. Dark exposure of cuttings of the petunia cv. 'Mitchell' strongly increased the intensity of subsequent AR formation under light.

AR formation in cuttings is a multistage developmental process, which is controlled by plant hormones particularly auxin [15]

Abbreviations: AR, adventitious root; cw, cell wall; cyt, cytosolic; DM, dry matter; dpe, days post excision; dpin, days post insertion; FW, fresh weight; INV, invertase; P_N, net photosynthesis; PPFD, photosynthetic photon flux density; R_D, dark respiration; vac, vacuolar.

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and also depends on the availability of diverse resources in the rooting zone. There, carbohydrates are particularly important for providing the energy and carbon skeletons to power and feed the cell differentiation, development and growth of new roots [16–18]. In addition, carbohydrates may directly control root development via modulation of gene expression and interaction with plant hormones [13,18–20]. Interestingly, the level of sugars particularly of sucrose and the partitioning between sucrose and starch in cutting leaves seem to provide important bottlenecks for AR formation in the stem base [9,10,14,21]. This stays in accordance with the outstanding role of sucrose as major carbohydrate fraction exported from source tissues and translocated to the diverse utilization and storage sinks in the plant [22,23]. Carbohydrate levels in the cutting tissues are affected by the initial carbohydrate reserves [9,24] and the current carbon assimilation during rooting by photosynthetic activity [10,25–28].

Diminishing the carbon source is only one factor which might limit AR formation. Differences in the strength among the diverse carbohydrate sinks together with the activity of source to sink pathways additionally determine the channelling of assimilates to the different utilization and storage sinks in a plant [22,29]. Invertases are important molecular drivers of sink strength, since they reduce the sucrose pool by converting it into glucose and fructose, which are further channelled into the metabolic pathways, and thereby regulate utilization and storage of organic carbon. The activities of invertases indirectly modulate gene expression via modifying the level of hexoses that regulate cell cycle and cell division programs. In this context, invertases are already proven to be vital for the establishment of "young sinks" such as flowers and fruits [30]. Among the compartment-specific types of vacuolar invertases (INVvac), cytosolic invertases (INVcyt) and cell wall invertases (INVcw), the latter are considered to have an outstanding role in sink activity via modifying phloem unloading particularly in those sink tissues that undergo cell division and elongation [29].

In petunia cuttings, the establishment of the new sink in the rooting zone is an early metabolic key event involved in AR formation at standard conditions under diurnal light [31,32]. During the "sink establishment" phase, increased INVcw activity obviously contributes to an apoplastic unloading of sucrose, while simultaneous depletion of sugars indicates carbohydrate utilization in the rooting zone. Recent studies of the response of cuttings of *P. hybrida* 'Mitchell' to dark exposure revealed a decrease of sugar levels in fully developed source leaves and the stem base during the dark period of seven days, while formation of root meristemoids already started [2]. Interestingly, strong enhancement of AR development during the subsequent light period was associated with higher accumulation of carbohydrates particularly in the stem base during the first three days post insertion (planting) of dark pre-exposed cuttings when compared to cuttings which did not experience dark exposure before planting [2]. The increased carbohydrate levels could be the result of increased photosynthesis caused by possible feed-forward control of carbohydrate depletion during darkness [33–35].

The first objective of this study was to evaluate, whether the enhanced carbohydrate levels and root formation in the stem base of dark pre-exposed cuttings is the outcome of a higher source activity (net carbon assimilation) or of higher sink strength in the rooting zone. This question was addressed by the analysis of CO_2 gas exchange and of dry matter production and allocation between the shoot and root. Based on the results, we followed the hypothesis that organ-specific activation of invertases is involved in dark-stimulated dry matter allocation towards the rooting zone. Therefore, we monitored enzyme activities and RNA accumulation levels in the stem base and in the shoot apex, which constitutes an important utilization sink competing with the rooting zone.

2. Material and methods

2.1. Plant material, growth conditions and treatment of cuttings

Seeds of *P. hybrida* cv. 'Mitchell' were sterilised and germinated and stock plants were established as described by Klopotek et al. [2]. Eighty potted stock plants (fertilised peat substrate: Einheitserde Typ ED-73 with Optifer, Patzer, Sinntal-Jossa, Germany) were placed in the greenhouse and fertilised repeatedly with Hakaphos special (16% N, 8% P_2O_5, 22% K_2O, 3% MgO + micronutrients, COMPO GmbH Münster, Germany) following commercial horticultural practice. Temperature and light in the greenhouse were controlled as described [2]. The experiments were performed about three months after the germination of the stock plants. Leafy cuttings were excised from the stock plants four hours after commencing the light period. Cuttings were obtained by using shoot tips containing four to five leaves (Fig. 1), leaving two nodes of the shoot on the plant [36].

The general experimental set-up is illustrated in Fig. 1. Control cuttings, which were planted and exposed to diurnal light conditions immediately after excision, were compared with cuttings, which first experienced a dark exposure for 7 days and thereafter were planted and exposed to diurnal light conditions. In the results part, the term "dark" is used, when the dark-treated cuttings are under the dark conditions, the term "post-dark" or "dark pre-exposed" is used for the dark-treated cuttings when they have been planted after the dark exposure and are cultivated under same diurnal light conditions as the immediately planted control cuttings. Two time-scales were used (Fig. 1). Days post excision (dpe) refer to the time when cuttings were excised. Days post insertion (dpin) refer to the time when cuttings were inserted (planted) into the rooting substrate perlite and exposed to diurnal light conditions. The presented data involved six experiments (one on photosynthesis, two on dry matter partitioning, two on invertase activity, one on RNA accumulation of invertase genes) focussing on different specific phases of this experimental set-up.

Since the promotive influence of the dark exposure on AR formation was evident with both temperatures of 10 °C and 20 °C but leaf yellowing and drying was observed with 20 °C [2], the dark exposure was conducted at 10 °C. For a seven day dark exposure, cuttings were put in non-perforated bags in a cardboard box that was stored in a dark cabinet at 10 °C.

For rooting, 16–20 cuttings per tray were inserted in perlite Perligran A with a particle size of 0–6 mm and a pH of

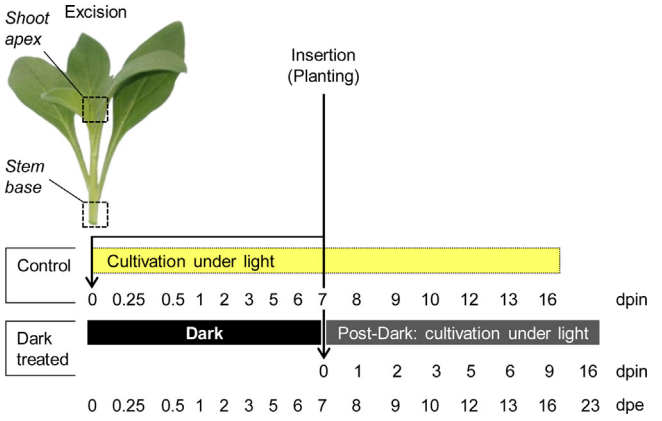

Fig. 1. Schematic presentation of the experimental set-up applied to study the influence of dark treatment of cuttings on photosynthesis, dry matter allocation between shoot and roots and invertase activities and RNA accumulation in shoot apex and stem base. Dpe indicate days post excision, dpin indicate days post insertion (planting). The time points considered depended on the particular focus of the experiment.