Journal of Plant Physiology 167 (2010) 547–554

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.de/jplph

Dark exposure of petunia cuttings strongly improves adventitious root formation and enhances carbohydrate availability during rooting in the light

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ARTICLE INFO

Article history:
Received 3 July 2009
Received in revised form
23 September 2009
Accepted 6 November 2009

Keywords:
Root development
Carbon metabolism
Meristematic cells
Post-harvest
Storage

ABSTRACT

The effect of temporary dark exposure on adventitious root formation (ARF) in *Petunia* × *hybrida* 'Mitchell' cuttings was investigated. Histological and metabolic changes in the cuttings during the dark treatment and subsequent rooting in the light were recorded. Excised cuttings were exposed to the dark for seven days at 10 °C followed by a nine-day rooting period in perlite or were rooted immediately for 16 days in a climate chamber at 22/20 °C (day/night) and a photosynthetic photon flux density (PPFD) of 100 μmol m^{-2} s^{-1}. Dark exposure prior to rooting increased, accelerated and synchronized ARF. The rooting period was reduced from 16 days (non-treated cuttings) to 9 days (treated cuttings). Under optimum conditions, despite the reduced rooting period, dark-exposed cuttings produced a higher number and length of roots than non-treated cuttings. An increase in temperature to 20 °C during the dark treatment or extending the cold dark exposure to 14 days caused a similar enhancement of root development compared to non-treated cuttings. Root meristem formation had already started during the dark treatment and was enhanced during the subsequent rooting period. Levels of soluble sugars (glucose, fructose and sucrose) and starch in leaf and basal stem tissues significantly decreased during the seven days of dark exposure. This depletion was, however, compensated during rooting after 6 and 24 h for soluble sugars in leaves and the basal stem, respectively, whereas the sucrose level in the basal stem was already increased at 6 h. The association of higher carbohydrate levels with improved rooting in previously dark-exposed versus non-treated cuttings indicates that increased post-darkness carbohydrate availability and allocation towards the stem base contribute to ARF under the influence of dark treatment and provide energy for cell growth subject to a rising sink intensity in the base of the cutting.

Introduction

Cultivation of vegetatively propagated petunia (*Petunia* × *hybrida*) plants relies on adventitious root formation (ARF) in leafy stem cuttings, which are usually produced at low latitude production sites. With the success of this species as an ornamental flowering plant, the transport of high-quality cuttings to distant rooting stations, for example in Central Europe, has become increasingly important. This production chain requires intermediate storage of cuttings, usually in darkness, to assure transport and to cope with the number of cuttings produced, which are not all required for rooting at the same time. Lowering temperature during darkness slows down the metabolism and, consequently, extends the shelf life of cuttings (Behrens, 1988).

The storage potential of cuttings is influenced by genotype, conditioning of the stock plant and diverse environmental factors during the post-harvest chain as reflected by the specific responses of ARF. Rooting of cuttings of storage-sensitive plant species like pelargonium seems to be negatively affected by dark pre-treatment (Paton and Schwabe, 1987; Druege et al., 2004). A positive effect on adventitious root growth occurred, however, when chrysanthemum and carnation cuttings were stored in darkness at low temperatures (0.5 or 5 and 4 °C, respectively) before rooting (Druege et al., 2000; Garrido et al., 1996). There are no studies published to date that deal with histological analysis during dark exposure of cuttings.

Root development demands high energy and an adequate supply of carbohydrates (Haissig, 1984; Veierskov, 1988), which serve as energy source and also as structural and storage

Abbreviations: ARF, adventitious root formation; dpe, days post excision; dpin, days post insertion; FW, fresh weight; hpe, hours post excision; hpin, hours post insertion; PPFD, photosynthetic photon flux density; TNC, total non-structural carbohydrates

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0176-1617/$ - see front matter © 2009 Elsevier GmbH. All rights reserved.
doi:10.1016/j.jplph.2009.11.008

compounds and as osmoticum. Carbohydrates also play a role in the control of developmental processes in plants (Gibson, 2005). Sugars are physiological signal molecules that are able to induce or repress various genes affecting plant development and growth (Koch, 1996, Smeekens, 2000; Price et al., 2004). The initial status of carbohydrate levels in leaves and in the stem base is an important factor in root formation (Veierskov et al., 1982; Druege et al., 2004). The availability of soluble sugars for root development is influenced both by reserves at the beginning of ARF and by current photosynthesis (Rapaka et al., 2005). Allocation and fluxes of sugars in the cutting apparently play an important role in ARF, as suggested by the fact that earlier stages of ARF in pelargonium cuttings are limited by carbohydrate availability, especially of sucrose in leaves (Rapaka et al., 2005). An integrated study of molecular physiological events during ARF in *Petunia* provided evidence that early establishment of a carbohydrate sink in the region of root regeneration is probably an important step in this developmental process (Ahkami et al., 2009).

The aim of this study was to use *Petunia* × *hybrida* 'Mitchell' as an established model system to elucidate (i) whether dark pre-exposure of cuttings affects rooting, and (ii) whether soluble and insoluble carbohydrates might contribute to the changed rooting performance. To this end, rooting parameters were determined. Further, we combined histological and biochemical methods and recorded metabolic changes in *Petunia* × *hybrida* 'Mitchell' cuttings during dark exposure and upon transfer to ambient light intensities.

Material and methods

Plant material and growth conditions

Seeds of *Petunia* × *hybrida* cv. 'Mitchell' were sterilized with 70% ethanol, 10% NaOCl (0.1% SDS solution) and rinsed in sterile water three times. Germination took place in Petri-dishes with 1/2 MS medium (Murashige and Skoog, 1962) at 25 °C, 16 h day/8 h night. After 14 days, seedlings were transplanted in peat-based substrate (Einheitserde Typ VM, Patzer, Sinntal-Jossa, Germany) in plastic trays and placed in the greenhouse. After four weeks, the small petunia plants were placed in pots (final volume 1.1 L) containing fertilized peat substrate (Einheitserde Typ ED-73 mit Optifer, Patzer). Plants were watered with tap water of low alkalinity (German degrees of water hardness (°dH)=4) and low electric conductivity (0.23 mS cm^{-1}), which provided continuously low pH values below pH 5.3 ($CaCl_2$). Stock plants were repeatedly fertilized with Hakaphos special (16% N, 8% P_2O_5, 22% K_2O, 3% MgO+micronutrients, COMPO GmbH Münster, Germany) aiming at the following concentrations of nutrients in the substrate: nitrate N: 150 mgL^{-1}, P: 100 mgL^{-1} and K: 300 mgL^{-1}. Plants were shaded when natural irradiance exceeded a photosynthetic photon flux density (PPFD) of 720 µmol m^{-2} s^{-1} (400–700 nm), while day length was restricted to 10 h with a black fabric. Heating/ventilation set points for temperatures were 20/22 °C (day) and 16/18 °C (night). During the development of side shoots providing the cuttings for the experiments (three-week periods before excision), the average temperature and relative humidity in the greenhouse were 20.8 °C, and 59.3%, respectively. The average daily light integral at plant level measured for the same periods was 6.5 mol m^{-2} d^{-1} (400–700 nm). Twenty stock plants were cultured for each replication plot (four plots). Cuttings were obtained using shoot tips containing four to five leaves of similar size, leaving two nodes of the shoot on the plant. Plants remained vegetative due to the short day and repetitive excision of cuttings.

Treatment of cuttings and determination of rooting response

Excision of leafy cuttings was performed 4 h after the beginning of the light period. For the experiments, 10 cuttings per replication plot were used for each treatment (dark exposure; non-treated). Immediately after excision, dark-treated cuttings were put in non-perforated plastic bags in a cardboard box that was stored in a dark cabinet at temperatures specified in Table 1. After dark exposure, cuttings were unpacked, placed in trays and exposed to light conditions in a climate chamber. Non-treated cuttings were exposed to same climate chamber conditions immediately after excision. Trays (46 cm × 28 cm × 5 cm, not sub-divided in cells) contained perlite as rooting substrate and were covered by a plastic hood to maintain humidity (transmission of light (400–700 nm)=90%). In the climate chamber, temperature and relative humidity were set to 22/20 °C (day/night) and 85/60% (day/night), respectively. Light was provided for 10 h per day by fluorescence tubes (Master TLD 58W 830, warm-white, Phillips, The Netherlands). PPFD (400–700 nm) at plant level was adjusted to 100 ± 10 µmol m^{-2} s^{-1} at the start of each experiment using a hand sensor. Exemplary logging of air temperature revealed that mean day and night temperatures at plant level in covered trays were 21.8 and 18.5 °C, respectively.

The different temperatures and durations of dark exposure and periods of rooting in the light are specified in Table 1. To evaluate the response of ARF to the excision of cuttings from the stock plants and to rooting under light in the climate chamber, two time-scales were used. Days and hours post excision (dpe and hpe, respectively) refer to the time when cuttings were excised. Days and hours post insertion (dpin and hpin, respectively) refer

Table 1
Effect of duration and temperature of dark exposure and rooting time post dark exposure on ARF in *Petunia* × *hybrida* 'Mitchell'.

No exp.	Factor tested	Dark treatment duration [d]	Dark treatment temperature [°C]	Rating date		No of roots per cutting	Mean length per root [cm]	Total root length per cutting [cm]
				dpe	dpin			
1	Dark treatment duration	Non-treated	–	15	15	7.8[a]	0.5[a]	5.4[a]
		7 d	9	20	13	25.8[b]	2.1[b]	54.4[b]
		14 d	9	27	13	28.9[b]	2.4[b]	68.7[c]
2	Dark treatment × rooting time	Non-treated	–	16	16	21.1[a]	1.0[a]	23.8[a]
		7 d	10	16	9	28.9[b]	1.5[b]	44.2[b]
3	Dark treatment × temperature	Non-treated	–	16	16	7.4[a]	1.2[a]	8.3[a]
		7d	10	16	9	24.9[b]	0.9[a]	20.2[b]
		7d	20	16	9	25.7[b]	1.3[a]	33.7[b]

Rooting was rated at specified days after excision (dpe), which corresponded with specified days after insertion (dpin) of cuttings into the rooting substrate and exposure to climate chamber conditions (10 h at 100 µmol m^{-2} s^{-1} (400–700 nm) and 22 °C; 14 h darkness at 20 °C). n=4 (each consisting of 10 cuttings) for each experiment. Values with different superscript letters are significantly different within one experiment ($p \leq 0.05$).