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Research Paper

Sugar and starch dynamics in the medium-root-leaf system indicate possibilities to optimize plant tissue culture

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ABSTRACT

Sucrose is commonly provided as a carbohydrate source in a plethora of plant tissue cultures to stimulate *in vitro* plant growth. However, this exogenous supply might compromise photosynthetic performance and hamper the transition from *in vitro* to *in vivo*. To evaluate sucrose addition to *in vitro* media a novel, holistic carbohydrate approach was used in this research taking into account carbohydrate contents in the whole medium-root-leaf system. Therefore *in vitro* plants of *Guzmania* 'Hilda' were initially provided with 15, 37, 73 and 117 µmol sucrose g^{-1} medium. After 4 weeks of plant culture most of the sucrose had been consumed by the plants while significant concentrations of glucose and fructose were measured in the media, indicating invertase activity. Plants growing on higher sucrose enriched media showed higher gains in dry weight and a massive increase of leaf hexose sugars and starch of about 600% in comparison with physiological concentrations. However, this potential advantage was not valorized as no differences in growth were observed during acclimation and on the longer term during 12 months of growth in the greenhouse. On the contrary, the carbohydrate dynamics data indicated that plants originating from high enriched media depend on sugar and starch breakdown during the first weeks after transfer to the greenhouse to enucleate sugar mediated feedback inhibition. Plants originating from low enriched media engage easily in photosynthesis to sustain growth and maintenance. As such, lower enriched media (e.g. 15 µmol g^{-1} medium instead of 25 µmol g^{-1} medium) can effectively be used to grow healthy *in vitro* plants which are able to perform photosynthesis immediately when transferred to the greenhouse, without compromising plant development and growth in the entire production cycle.

1. Introduction

In vitro propagation of plants is common for a range of ornamentals including orchids and bromeliads. These cultures involve different kinds of abiotic stresses. Plants are exposed to low light intensities, high air humidity and low CO_2 concentrations in most culture vessels. These photosynthesis restricting conditions imply the general use of external carbohydrates as energy source in tissue culture media (Cournac et al., 1991; Pospíšilova et al., 1999; Desjardins et al., 2009; Eckstein et al., 2012). In general practice carbon delivery is obtained by sucrose addition to the medium (George 1993; Roh and Choi 2004). As an end product of photosynthesis it can be anticipated that sucrose might trigger feedback inhibition to the photosynthetic plant metabolism and hence reduce overall growth (Hdider and Desjardins 1994; Sheen 1994). However, most *in vitro* grown plants show stimulated growth with increasing sucrose content of the medium. An increase in dry weight was noticed for example in coconut (Fuentes et al., 2005), *Alocasia amazonica* (Jo et al., 2009) and *Phalaenopsis* (Yoon et al., 2009). In addition to above ground biomass, root formation and development were also affected as more and longer roots developed in *Alocasia amazonica* and *Phalaenopsis* cultured on high sucrose concentrations (Jo et al., 2009; Yoon et al., 2009). In contradiction, Cui et al. (2000) found a decrease in shoot growth for *Rehmania glutinosa in vitro* plants exposed to 30 g L^{-1} sucrose in comparison to those exposed to 0 g L^{-1}. In a more detailed study Roh and Choi (2004) suggested an optimal sucrose concentration of 40 g L^{-1} for growth of tobacco *in vitro* as smaller shoots and roots were observed with either lower sucrose levels or with 50 g L^{-1}. It is obvious that whilst sucrose is generally applied in tissue culture of plants, its effects on plant development, photosynthetic performance and growth under these conditions seem to be variable and species dependent.

Sucrose is a major transport sugar in most plants. After formation or uptake by *in vitro* plants sucrose is transported to sink tissues where it is used as energy source. Source activities (e.g. photosynthesis, export) are upregulated under low sugar concentrations whilst sink processes (e.g. growth, storage) are upregulated when carbon sources are

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abundantly present (Rolland et al., 2006). Following invertase activity, sucrose is split into glucose and fructose (Straus 1962). This hydrolysis can take place both inside and outside the plant (in the *in vitro* medium). The amounts of hydrolyzed sucrose and its reaction rate is species dependent (George 1993). Once hexoses are present in the medium (by adding it as the preferable carbon source or by invertase action on sucrose), these sugars can also be used by the *in vitro* plants (Wyse 1979; George 1993).

In different studies, the content of sucrose in culture media has been related to the sugar and starch contents in the cultivated plantlets. Roots and leaves of *Rehmania glutinosa* contained more soluble sugars and starch when more sucrose was available in the growth medium (Cui et al., 2000). Also for tobacco, addition of sucrose resulted in an increase of soluble sugars and starch in the leaves (Tichá et al., 1998). It is hypothesized that these higher levels of sugars and starch might favor acclimation to *in vivo* by providing energy to these plants (Van Huylenbroeck et al., 1998). However, considerable losses are still experienced during acclimation in a range of cultures (Van Huylenbroeck et al., 2000; Carvalho et al., 2005).

In this study the influences of exogenous sucrose added to the *in vitro* medium of *Guzmania* 'Hilda' plants will be discussed by depicting the complete carbon flow from the medium (enriched with different amounts of sucrose) into the plants. A novel picture emerges by determining and quantifying the amounts of hexose sugars (i.e. glucose and fructose), sucrose and starch in both medium and plants (roots and leaves). Plant growth and development will be evaluated not only during *in vitro* but also during acclimation and on a longer term *in vivo* in the greenhouse to account for potential long-term effects on plant metabolism from *in vitro* sucrose supply.

2. Materials and methods

2.1. Plant material

Guzmania 'Hilda' is an ornamental hybrid belonging to the Bromeliaceae family. *In vitro* plants of *Guzmania* 'Hilda' were grown in tissue culture for 4 weeks of multiplication and 8 weeks of shoot elongation. Sampling of established phase 2 *in vitro* plants was performed during the subsequent rooting phase. The standard sucrose level in commercial production (25 g L^{-1} = 73 µmol g^{-1} medium), as well as lower (5 and 12.5 g L^{-1} = 15 and 37 µmol g^{-1} medium) and higher (40 g L^{-1} = 117 µmol g^{-1} medium) concentrations were added to the medium (1/2 MS, Murashige and Skoog 1962) at the start of the rooting phase (time = 0). One culture recipient (polystyrene, volume = 500 mL) contained 30 *in vitro* plants. Culture vessels were kept at 23 °C, a 10 h photoperiod and a light intensity of 90 µmol m^{-2} s^{-1} (PAR light from fluorescent tubes, a mix of 50/50 Osram FH 28W/830HE warm white and Osram FH 28W/840HE cool white). After 8 weeks of growth on rooting medium the *in vitro* plants were transferred to the greenhouse (Venlo type) where they were grown under a 16 h photoperiod (daylight supplemented with Son-T lamps, 30 µmol m^{-2} s^{-1}) as earlier described by Croonenborghs et al. (2009). The first month, acclimation occurred while plantlets were kept under a plastic lid to keep a high relative humidity. After 1 month, the lid was removed and 70% relative humidity and 21 °C were achieved during the day. During night, the relative humidity was 80% and the temperature was 19.5 °C.

2.2. Sugar and starch analyses

Sampling for the analyses of sugars and starch was performed by freezing plant material and medium at different time points in liquid nitrogen followed by storage at −20 °C. The plant material was then lyophilized and a powder of dry plant material was produced.

Contents of soluble sugars (glucose, fructose and sucrose) and starch (only measured in leaf samples) were determined enzymatically (Enzytec, Scil Diagnostics GmbH, Viernheim, Germany) and analyzed by measuring the presence of produced NADPH at 340 nm using a spectrophotometer (UV-1800, Shimadzu Scientific Instruments, Colombia, US) according to Ceusters et al. (2008) (n = 3).

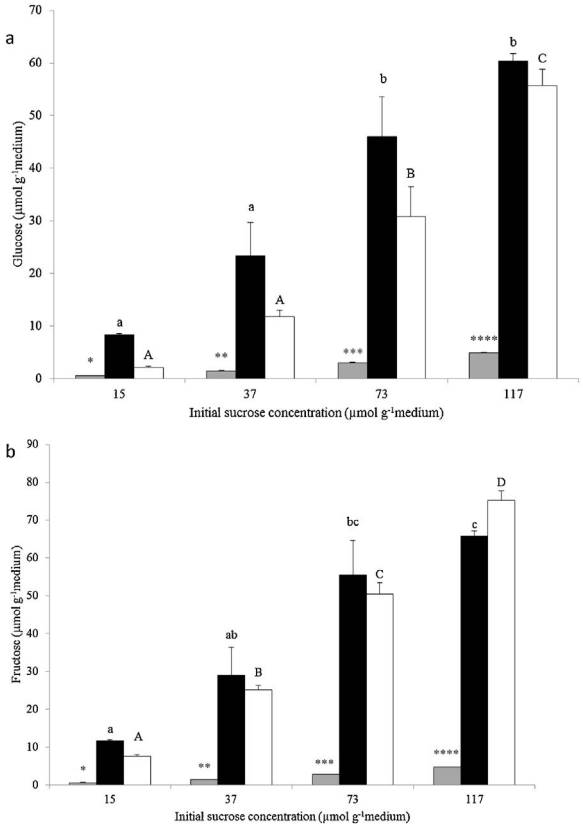

Fig. 1. Glucose (a) and fructose (b) contents (µmol g^{-1} medium) in media with *in vitro* plants of *Guzmania* 'Hilda' with different initial sucrose concentrations at the start (grey), after 4 (black) and after 8 weeks (white) of growth (n = 3). SE is shown and the same symbols or letters mean no significant difference (asterisk: week 0, small letter: week 4, capital letter: week 8) ($P \leq 0.05$).

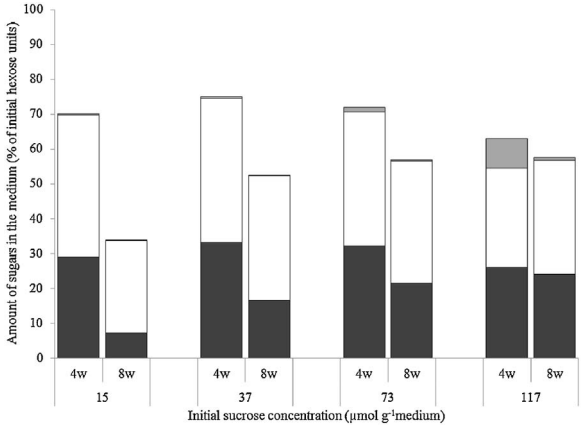

Fig. 2. Percentage of sucrose (grey), fructose (white) and glucose (black) to the quantity of initial sucrose levels (100%) after 4 and 8 weeks of growth of *Guzmania* 'Hilda' on tissue culture media with different initial sucrose concentrations (n = 3).

2.3. Plant growth characteristics

For the *in vitro* plants, fresh weight was determined per plant (leaves and roots) (n = 30). After drying the *in vitro* plants at 70 °C for 48 h, dry weight was determined (n = 10). To evaluate growth of *Guzmania* 'Hilda' plants after transfer to the greenhouse, a non-destructive