EFFECTS OF MEDIUM SUGAR ON GROWTH AND CARBOHYDRATE STATUS OF SWEETPOTATO AND TOMATO PLANTLETS IN VITRO

Sandra B. Wilson[1], Chieri Kubota[2] and Toyoki Kozai[2]
[1]Department of Environmental Horticulture, Indian River Research and Education Center, University of Florida, 2199 South Rock Road, Fort Pierce, FL 34945, USA.
E-mail: sbwilson@gnv.ifas.ufl.edu
[2]Department of Bioproduction Science, Chiba University, Matsudo, Chiba 271, Japan.

Abstract. Carbohydrate status of tomato (*Lycopersicon esculentum* Mill., 'HanaQueen') and sweetpotato (*Ipomoea batatas* (L.) Lam., 'Beniazuma') plantlets was investigated in plantlets cultured for 17 d in vitro photoautotrophically (without sucrose in the medium) or photomixotrophically (with 30 g/L sucrose in the medium) under enriched CO_2 conditions (1500 $\mu mol \cdot mol^{-1}$) and 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux (PPF). After 17 d of enriched CO_2 conditions, there was no significant difference in leaf, stem or root fresh weight among photoautotrophic and photomixotrophic tomato and sweetpotato plantlets, with the exception that the stem fresh weight of photomixotrophic tomato plantlets was higher than that of photoautotrophic plantlets. The addition of sucrose to the medium increased the stem, root and total dry weight of tomato plantlets and increased the root dry weight of sweetpotato plantlets. Tomato plantlets had higher soluble sugar levels (sucrose, glucose, and fructose) than did sweetpotato plantlets, regardless of medium composition. Photomixotrophic plantlets had higher soluble sugar levels that did photoautotrophic plantlets, regardless of plant species. Starch concentrations of photomixotrophic tomato plantlets were higher than that of photomixotrophic sweetpotato plantlets. However, there was no difference in starch concentration of photoautotrophic sweetpotato and tomato plantlets. Regardless of plant species or media composition, carbohydrate status was maintained under enriched CO_2 conditions.

Key index words. *Ipomoea batatas*, *Lycopersicon esculentum*, micropropagation, photoautotrophic, photomixotrophic.

1. Introduction

Plantlets are conventionally cultured photomixotrophically (with sugar and atmospheric CO_2 as their carbon sources) in vitro. Sugar in culture medium has been considered the main carbon source for the growth of cells, buds, shoots, and even plantlets. Providing sucrose in the medium has proven beneficial to maintain plant quality and photosynthetic ability during low temperature storage of in vitro broccoli (Wilson et al., 1998a; Wilson et al., 1998b) and hosta (Wilson et al., 2000) plantlets. Capellades et al. (1991) cultured *Rosa multiflora* L. plantlets with four different initial levels of sucrose, and found that plantlets receiving 10 g/L and 30 g/L sucrose in the culture medium had higher net photosynthetic rates and lower starch concentration in leaves than that with 50 g/L sucrose. It was demonstrated that exogenous supply of sugars increases starch and sucrose reserves in micropropagated plants and that appropriate higher levels of carbohydrates can favor plantlet survival upon transfer to ex vitro conditions, improve acclimatization and speed up physiological adaptations (Capellades et al., 1991; Van Huylenbroeck and Debergh, 1996; Wilson et al., 1998b).

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However, the absolute requirement of exogenous sugars in tissue culture has been contested. Sugar in the medium has been shown to inhibit photosynthesis and research has revealed that the low net photosynthetic rates of chlorophyllous shoots or plantlets in vitro are largely due to the low CO_2 concentration in the vessel during the photoperiod (Kozai et al., 1997). Kozai et al. (1997) report CO_2 enrichment and supplemental lighting to significantly reduce the period of acclimatization in the greenhouse, and improve the size and quality of in vitro plantlets.

Although sugar is considered as a carbon source for in vitro cultured plantlets, only a few reports document sugar uptake and metabolism of in vitro plantlets. As mentioned by Desjardins et al. (1995), there are extensive studies on sugar uptake using cell suspension cultures as model systems, but it can not be assumed that responses observed in cultured cells will be the same with tissue cultured plantlets. Quantitative understanding of carbohydrate status is useful for predicting plantlet growth and acclimatization to greenhouse conditions. The objectives of this study were to investigate the influence of initial sucrose concentration of the medium on growth and carbohydrate status of in vitro plantlets grown in enriched CO_2 conditions. Tomato and sweetpotato plantlets were selected as model systems for this study, since both plants can be micropropagated either photoautotrophically or photomixotrophically.

2. Materials and Methods

2.1 Plant material and culture conditions

Single-node stem cuttings each with a leaf were excised from tomato and sweetpotato plantlets and cultured photoautotrophically and photomixotrophically under conditions shown in Table 1.

Table 1 Experimental culture conditions for tomato and sweetpotato plantlets. Plantlets were cultured photoautotrophically (no sucrose in the medium) or photomixotrophically (30 g/L sucrose in the medium) under otherwise identical culture conditions.

Plant species:
Tomato (*Lycopersicon esculentum* Mill., 'HanaQueen')
Sweetpotato (*Impomoea batatas* (L.) Lam., 'Beniazuma')
Culture period: 17 d
Explant: Single node cuttings each with a leaf
Mean fresh weight per explant: tomato (225 ± 20 mg), sweetpotato (120 ± 20 mg)
Vessel: polycarbonate (volume: 480 mL)
Medium volume: 100 mL/vessel
Basal composition: Murashige and Skoog (1962)
Sugar: 0 or 30 g sucrose/ L
Substrate: Sorbarod cellulose plugs (Baumgartner Papiers SA, Switzerland)
pH: 5.8
No. of air exchanges: 1.6 (days 1-5) and 3.05 (days 6-17) h^{-1}
Culture room conditions:
Air temperature: day (26.8 ± 0.2 °C), night (23.5 ± 0.2 °C)
Relative humidity: 80%
CO_2 concentration: 1500 ± 10 $\mu mol \cdot mol^{-1}$
Photosynthetic photon flux: 100 ± 10 (days 1-5); 150 ± 15 (days 6-17) $\mu mol \cdot m^{-2} \cdot s^{-1}$.
Photoperiod: 16 $h \cdot d^{-1}$
Light source: cool white fluorescent bulbs

2.2 CO_2 concentration and gas exchange rate

Three holes (10 mm in diameter) in opposite sides of the vessel lids were covered with 0.5 μm membrane filter disks (Milli-Seal, Millipore K.K., Tokyo) to provide air exchange. At the start of the experiment, two of the air diffusive filters of the vessel were covered with plastic tape to minimize the number of air exchanges. On day 5, the remaining two filters were uncovered to increase the number of air exchanges. CO_2 concentrations inside the vessels were monitored after days 0, 5, and 17. The number of air exchanges per hour was determined by the method described by Kozai et al. (1986). The CO_2 concentrations inside and outside the vessel were determined during the photoperiod by analyzing gas samples using a gas chromatograph (GC-12A, Shimadzu Co., Kyoto) equipped with a flame ionization detector.

2.3 Plant carbohydrate analysis

At day 0 and 17, leaves, stems, and roots were frozen in liquid N_2 and freeze-dried for dry weight measurements. To obtain sufficient tissue for carbohydrate analysis, two plantlets were pooled to generate a sample. The procedures for soluble sugar extraction were modified as previously described (Boersig and Negm, 1985; Miller and Langhans, 1989). Leaves, stems, and roots were separately ground, and approximately 50 mg was loaded into glass Pasteur pipettes with glass wool plugs (1 cm) and extracted 3 times with 1.5 mL of 12 methanol : 5 chloroform: 3 water (by volume, MCW). 100 μL sorbitol (10 mg/mL) was added as an internal standard. Distilled water (3.5 mL) was added to samples and aqueous phase was removed and applied to polyethylene columns containing 3 mL 1 methanol : 1 water (v:v, MW) and cation and anion resin (1 mL Amberlite IRA-45 layered with 1 mL Dowex 50-W, Sigma-Aldrich Co.,St. Louis, MO). Soluble carbohydrates were eluted with 1 mL MW twice and evaporated to dryness using a rotary flash evaporator. The dry residue was resuspended in 1 mL HPLC-grade water and filtered through a 0.45 um membrane prior to HPLC injection. Sucrose, glucose, and fructose were separated and detected using an HPLC with a refractive index detector (Hitachi Ltd., Tokyo, Japan) and a Gelpack GL-C611 column (Hitachi Chemical Co., Tokyo, Japan) maintained at 70 °C. Quantification was determined using a D-2000 Chromato-Integrator (Hitachi Ltd., Tokyo, Japan) and a regression equation describing the sucrose, glucose, and fructose calibration lines.

The procedures for starch determination were modified as described (Haissig and Dickson, 1979; Miller and Langhans, 1989). The tissue residue left in the pasteur pipets following soluble sugar extraction was dried overnight at 60 °C, suspended in 4 mL Na-acetate buffer (100mM, pH 4.5) and placed in a boiling water bath for 20 min. After cooling to room temperature, 1.0 mL amyloglucosidase solution (from *Aspergillus niger*, Sigma-Aldrich Co., St. Louis, MO) (50 units/assay in 0.1 M pH 4.2 Na-acetate buffer) was added to each test tube. Samples were incubated for 48 hr at 55 °C with occasional agitation. Glucose determination via a glucose oxidase and peroxidase enzymatic method was completed on a 100 μL sample. Absorbance was determined at 450 nm on a spectrophotometer and starch content calculated based on the regression equation describing the glucose calibration line (0.0 to 0.5 μmol).

2.4 Experimental design and statistical analysis

All vessels were arranged in a completely randomized design. Each vessel containing four plantlets was considered a replication and there were three replications

per treatment. Data were analyzed by ANOVA and mean separation evaluated using LSD at P=0.05.

3. Results and Discussion

3.1 Plant growth

After 17 d, there was no significant difference in fresh weight among photoautotrophic and photomixotrophic tomato and sweetpotato plantlets, with the exception that the stem fresh weight of photomixotrophic tomato plantlets was higher than that of photoautotrophic plantlets (Table 2). Kozai and Iwanami (1988) also observed enhanced growth of carnation plantlets under a CO_2 concentration level of 1000-1500 $\mu mol \cdot mol^{-1}$ and a PPFD level of 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$, regardless of the addition of sugar in the medium. Photomixotrophic tomato and sweetpotato plantlets generally had higher stem, root, and total dry weight than did photoautotrophic plantlets (Table 2). Similarly, Kubota et al. (1998) showed that final dry weight of in vitro tomato and sweetpotato plantlets increased as initial sucrose concentration increased from 0 to 7.5 $g \cdot L^{-1}$. In their experiments, final dry weight continued to increase as sucrose increased to 30 $g \cdot L^{-1}$ for tomato plantlets but remained unchanged for sweetpotato plantlets (Kubota et al., 1998). Deng and Donnelly (1993) showed that both CO_2 and sucrose affect in vitro plantlet growth of *Rubus idaeus* L. (red raspberry) independently. In their experiments, in vitro CO_2 enrichment significantly increased root count, root length, and total plantlet fresh weight compared with those of plantlets grown under ambient CO_2, plantlet height, and percent dry weight were similar among plantlets grown under ambient or enriched CO_2 concentrations (Deng and Donnelly, 1993).

Table 2 Effects of photoautotrophic (PA) and photomixotrophic (PM) conditions on fresh and dry weight of sweetpotato and tomato plantlets after 17 days at 1500 $\mu mol \cdot mol^{-1}$ CO_2.

Treatment	Fresh weight (g)				Dry weight (g)			
	Leaf[z]	Stem	Root	Total	Leaf	Stem	Root	Total
Sweetpotato PA	.429	.077	.188	.693	.038	.006	.012	.056
Sweetpotato PM	.365	.086	.191	.641	.040	.009	.016	.065
Tomato PA	.516	.279	.164	.959	.053	.019	.011	.082
Tomato PM	.509	.386	.184	1.08	.063	.042	.015	.120
ANOVA[z]								
Species	**	**	NS	**	**	**	NS	**
Sucrose	NS	**	NS	NS	NS	**	**	**
Sp * suc	NS	**	NS	*	NS	**	NS	*

[z]ANOVA = analysis of variance.
NS, *, ** Nonsignificant, significant at $P < 0.05$ or 0.01, respectively.

3.2 Carbohydrate analysis

Tomato plantlets had higher sucrose, hexose, and total soluble sugar concentrations than did sweetpotato plantlets, regardless of medium composition. However, total sugar uptake from the media based on dry weight was not significantly different between species (data not presented). Photomixotrophic plantlets had higher sucrose, hexose, and total sugar concentrations than did photoautotrophic plantlets, regardless of plant species. Sucrose concentrations were generally higher than hexose

concentrations in stem tissue but lower than hexose concentrations in leaf or root tissue indicating sucrose cleavage and the mobilization of its hexoses. Cournac et al. (1991) showed that the soluble sugar concentrations of *Solanum tuberosum* L. (potato) plantlets cultured photomixotrophically in aerated vessels were higher than those cultured photoautotrophically either with or without CO_2 enrichment. Starch concentrations of photomixotrophic tomato plantlets were higher than that of photomixotrophic sweetpotato plantlets. However, there was no difference in starch concentrations of photoautotrophic sweetpotato plantlets verses photoautotrophic tomato plantlets. Photomixotrophic plantlets had higher starch concentrations than photoautotrophic plantlets. This is similar to work of Capellades et al. (1991) who showed that the leaf starch concentration increased when the plantlet was cultured on medium with an elevated sucrose concentration. Sugar in the medium has been reported to reduce the rubisco activity in plants (Desjardins et al, 1995) thereby lowering the net photosynthetic rate (Capellades et al., 1991). Piqueras et al. (1998) reported that micropropagated *Calathea louisae* Gagnep plantlets had higher starch contents in roots and stems compared with leaves, while sucrose concentration was highest in stems, followed by leaves and roots. Providing CO_2 enrichment for micropropagated plantlets resulted in highest total soluble sugars in the leaf and root tissue of tomato and in the stem and root tissue of sweetpotato. In vitro tomato and sweetpotato plantlets grown in enriched CO_2 conditions without sucrose had sufficient carbohydrate reserves after 17 d in culture.

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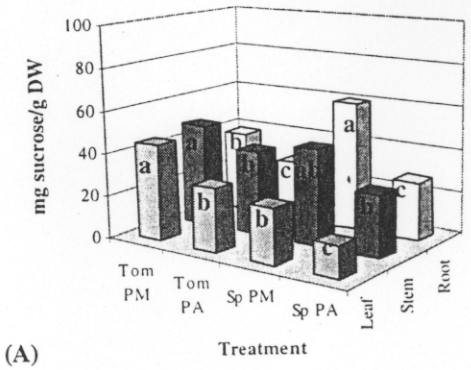
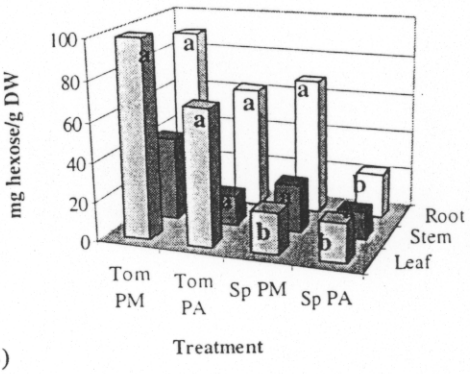


(A)



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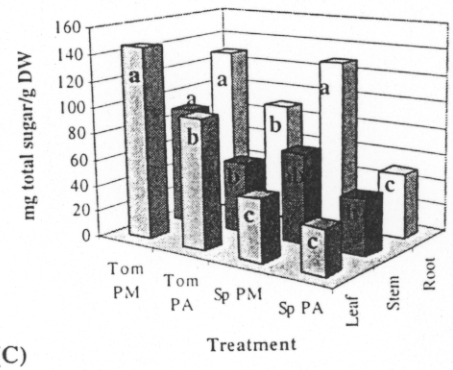

(C)

Fig. 1 Sucrose (A), hexose (B), and total sugar (C) concentrations in leaf, stem and root tissue of tomato (Tom) and sweetpotato (Sp) after 17 d of photoautotrophic (PA) or photomixotrophic (PM) culture.

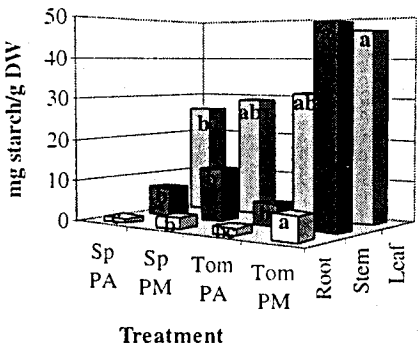

Fig. 2 Starch concentrations in leaf, stem, and root tissue of sweetpotato (Sp) and tomoato (Tom) plantlets grown photoautotrophically (PA) or photomixotrophically (PM).

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