In Situ Estimation of Carbon Balance of In Vitro Sweetpotato and Tomato Plantlets Cultured with Varying Initial Sucrose Concentrations in the Medium

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ABSTRACT. The effects of initial sucrose (suc) concentrations in the medium (S0) on the carbon balance and growth of sweetpotato [Ipomoea batatas (L.) Lam. `Beniazuma'] and tomato [Lycopersicon esculentum Mill. `HanaQueen'] plantlets were studied under controlled environmental conditions. Plantlets were cultured with 0, 7.5, 15, or 30 g·L–1 of S0 under high photosynthetic photon flux (160 to 200 µmol·m–2·s–1) and CO2 enriched (1400 to 2050 µmol·mol–1) conditions. Net photosynthetic rate per leaf area (Pl) decreased and dry weight per plantlet (Wd) increased with increasing S0, but did not differ significantly between S0 of 7.5 to 30 g·L–1 for sweetpotato or 15 to 30 g·L–1 for tomato. Carbon influxes and effluxes of the plantlets by metabolism of medium suc and/or photosynthesis, and respiration were estimated based on measurements of in situ and steady state CO2 exchange rates and sugar uptake during culture. At S0 from 7.5 to 30 g·L–1, photosynthesis was responsible for 82% to 92% and 60% to 67% of carbohydrate assimilation for sweetpotato and tomato, respectively. Estimated carbon balances of plantlets based on the estimated and actual increases of moles of carbon in plant tissue demonstrated that in situ estimation of carbon balance was reasonably accurate for sweetpotato at S0 of 0 to 15 g·L–1 and for tomato at S0 of 0 g·L–1 and that the actual contribution of photosynthesis for tomato at high S0 might be lower than the values estimated in the present experiment. Results showed that initial suc concentration affected the relative contribution of photosynthesis on their carbon balances and that the responses were species specific. The failure of validation at S0 in a range specific to each species suggested the need for further study on carbon metabolism of in vitro plantlets cultured with sugar in the medium.

In vitro plant culture environments are recognizably different from greenhouse or field environments. The uniqueness includes the presence of sugar in the medium and use of the relatively airtight vessels, which results in the accumulation of ethylene and water vapor, and the accumulation or depletion of CO2. Measurement of CO2 concentration inside vessels containing chlorophyllous plantlets showed that CO2 concentrations inside the vessel dropped below ambient levels and often fell as low as the CO2 compensation point during the light period (Desjardins et al., 1988; Fournioux and Bessis, 1986; Fujiwara et al., 1987; Infante et al., 1989; Kozai and Sekimoto, 1988). Therefore, regardless of their photosynthetic ability, chlorophyllous plantlets grown in vitro are required to grow photomixotrophically; supported by relatively large amounts of available sugar in the medium and limited CO2. Such a physiological phenomenon of having two carbon sources (photomixotrophy) is not the norm for ex vitro plants, except for parasitic plants, such as Striga hermonthica (Del.) Benth. (Pageau et al., 1998). Consequently, the in vitro environment serves as a unique model for studies focusing on photosynthesis and carbon metabolism. A quantitative understanding of the relative contribution of each carbon source gives important information on the carbon metabolism of in vitro plants.

For more practical reasons, a quantitative understanding of sugar uptake and photosynthesis is necessary to enhance plantlet growth with a minimal input of energy and other resources, thereby maximizing the biomass production efficiency. Reducing suc concentration in the medium and increasing photosynthetic photon flux (PPF) and CO2 concentration in the headspace have reportedly enhanced net photosynthetic rates and growth of plantlets (Kozai, 1991). However, increasing the medium suc concentration may increase dry matter accumulation of the plantlets. When growth promotion is attempted while minimizing production costs, a decision must be made whether one should promote plantlet photosynthesis by controlling light and CO2 levels, or enhancing carbon uptake from the medium by increasing the sugar supply, or both.

In vitro photosynthesis has been measured many ways including an in situ measurement method based on CO2 balance in the vessel and the use of open flow or closed measurement systems, as reviewed by Desjardins et al. (1995). The in situ measurement method of net photosynthetic rates (Fujiwara et al., 1987; Fujiwara and Kozai, 1995) is preferred over open flow or closed measurement systems, to minimize the possible disturbance of the in vitro environment. For photomixotrophically cultured plantlets, net photosynthetic rates give a limited assessment of plantlet carbon balance because plantlets use sugar in the medium as carbon source in addition to CO2. Moreover, net photosynthetic rates measured as CO2 exchange rates include dark respiration rates which dissipate sugar absorbed from the medium (Fujiwara et al., 1995). To optimize the environmental conditions for photomixotrophic plantlets, it is necessary to determine their carbon balance with each...
carbon flux (influx and efflux) under various environments.

The carbon balance of photomixotrophic and photoautotrophic plantlets has been studied for rose, *Rosa multiflora* L. (De Riek et al., 1991); potato, *Solanum tuberosum* L. (Fujiiwara et al., 1995; Wolf et al., 1998); and gardenia, *Gardenia jasminoides* Ellis. (Serret et al., 1996, 1997) using several different methods. De Riek et al. (1991) quantified components of plantlet carbon flow (net carbon fixed by photosynthesis, carbon released by respiration, and medium carbohydrates accumulated in dry weight and respired as CO2) using 14C quantified components of plantlet carbon flow (net carbon fixed by photosynthesis and respiration, resulting in a low estimate of degree of photoautotrophy (Serret et al., 1996). Furthermore, most sugar absorbed from the medium was reportedly released as CO2, resulting in small amounts of carbon originating from medium sugar accumulating in the plantlets as indicated in the study using 13C-suc and 14CO2. Use of carbon isotopes can be straightforward for determining carbon influx and efflux separately, and the proportion of tissue carbon originating from different sources. However, the analysis requires specially designed vessels or chambers and use of radioisotopes, limiting practical application of the method.

Serret et al. (1996, 1997) used a stable carbon isotope (13C) and estimated the percentage of carbon in leaflet tissue originating from photosynthesis. A similar method using 13C to evaluate the “degree of photoautotrophy” in terms of percent tissue carbon derived from photosynthesis has also been tested (Wolf et al., 1998). Use of 13C is suitable for in situ evaluation of carbon balance without altering culture conditions. However, percentages of carbon originating from photosynthesis and medium sugar over the total carbon in the plantlet (residual after respiratory carbon loss) may not provide enough information for understanding the contribution of photosynthesis in photomixotrophic plantlet growth. Because the culture vessel represents a relatively closed system, carbon from the metabolism of medium suc in plantlets through respiration may be fixed by photosynthesis, resulting in a low estimate of degree of plantlet photoautotrophy (Serret et al., 1996). Furthermore, most sugar absorbed from the medium was reportedly released as CO2, resulting in small amounts of carbon originating from medium sugar accumulating in the plantlets as indicated in the study using 13C-suc (Borkowska and Kubik, 1990; De Riek et al., 1991).

To understand photomixotrophic growth of plantlets under different culture conditions, a quantitative approach for determining carbon influx by photosynthesis and sugar uptake is necessary. Fujiiwara et al. (1995) developed a culture system where plantlet CO2 exchange rates were continuously measured by employing forced ventilation in the vessel. This method required a number of assumptions to estimate the gross photosynthetic rate from CO2 exchange rates during light and dark periods, but alternatively, allowed the estimation of carbon influx from photosynthesis. Unfortunately, forced ventilation disturbs the culture environment, resulting in misleading interpretation of results. To estimate carbon exchange in culture systems using natural ventilation, in situ measurement of CO2 exchange rates is preferred over methods which use forced ventilation. The objectives of our research were to determine the effects of initial suc concentration (Su) on in situ plantlet carbon balance estimated by in situ and steady state CO2 exchange rates and medium sugar concentration.

**Materials and Methods**

**CULTURE CONDITIONS.** Sweetpotato (*Ipomoea batatas*, ‘Beniazuma’) and tomato (*Lycopersicon esculentum*, ‘HanaQueen’) were selected as model plants because both species have been micropropagated using photoautotrophic conditions (Afreen-Zobayed et al., 1999; Kubota et al., 2001). Culture conditions selected were specific for each species. Sweetpotato single node cuttings with a leaf (FW: 80 ± 15 mg) and tomato terminal shoots (FW: 80 ± 15 mg) were cultured for 20 and 15 d, respectively. Cylindrical polycarbonate vessels (volume: 480 mL) and lids (Nicca Chemical Co. Ltd., Fukui, Japan) were modified by drilling two holes (10 mm in diameter) in addition to the original hole (8 mm in diameter) for each lid and covering the holes each with a gas permeable membrane disk (pore size 0.5 mm; Milliseal, Millipore Japan). The number of air exchanges of the vessel (Kozai et al., 1986) was increased accordingly on days 6 and 11 by removing adhesive plastic tape that covered the gas permeable membrane disks, resulting in 1.3 (days 0 to 5), 1.9 (days 6 to 10), and 4.7 (days 11 to 20) air exchanges/h for sweetpotato; 1.3 (days 0 to 5) and 4.7 (days 6 to 15) air exchanges/h for tomato. Suc was added to the Murashige and Skoog (1962) medium at 0.75, 15, or 30 g·L–1 before adjusting the pH (5.7 for sweetpotato; 5.9 for tomato). Vessels containing the liquid medium (100 mL per vessel) and cellulose plugs (20 plugs per vessel; Sorbarod, Baumgartner Papiers SA, Switzerland) were autoclaved for 20 min at 120°C.

After transferring explants, vessels were placed under fluorescent tubes (FPL27EX-N, Matsushita Electric Co., Osaka, Japan) mounted horizontally above the shelf inside a growth chamber (AEL-3280, Advantec Co., Tokyo). The PPF was adjusted to 100 (days 0 to 10) and 200 (days 11 to 20) µmol·m–2·s–1 for sweetpotato; 100 (days 0 to 5) and 160 (days 6 to 15) µmol·m–2·s–1 for tomato under a 16 h d–1 light period. Air temperatures inside the vessel during the light/dark period were 28.0/27.0°C for sweetpotato and 24.0/23.0°C for tomato, respectively, and were monitored by a 0.1-mm copper-constantan thermocouple inserted into a vessel placed in the center of the shelf. Relative humidity in the culture room ranged from 70% to 80%. The CO2 concentration in the culture room was enriched to 1400, 1800, and 2000 µmol·mol–1 on days 0 to 5, 6 to 10, and 11 to 20, respectively, for sweetpotato; 2050 µmol·mol–1 on days 0 to 15 for tomato.

**GROWTH MEASUREMENTS.** Initial fresh weight was measured for each explant and initial dry weight of the explant (W0) was estimated using the percent dry matter over the fresh weight obtained from 10 explants sampled separately. Dry weight was measured after drying fresh samples at 80°C for more than 48 h. Plantlet fresh and dry weights and leaf areas were recorded 15 d (tomato) and 20 d (sweetpotato) after the start of the experiment. Leaf area was measured using image analysis software (LIA32, Nagoya University) on black and white images that were photocopied when harvested. The change in dry weight was calculated for each plantlet by subtracting W0 from the final dry weight per plantlet (Wf), and converted into mole carbon per plantlet (Dc) with an assumption that 90% of dry matter was equivalent to carbohydrate [(CH2O)n] (Murayama et al., 1984).

**SUGAR CONCENTRATION OF THE MEDIUM.** After autoclaving the medium, and harvesting plantlets, 10 mL of medium was sampled from each vessel and filtered through a 0.22 µm (pore size) membrane. Concentrations of suc, glucose (gluc), and fructose (fruc) in the sampled media were measured using a HPLC with a refractive index detector (L6000, Hitachi Ltd., Tokyo) and regression equations describing the suc, gluc, and fruc calibration lines. Prior to sampling the medium, each vessel was weighed. Medium volume was calculated from specific mass of the medium (g·L–1) and the mass of the medium obtained by subtracting mass of plantlets, dried plugs, and empty vessel from the total mass of the vessel.

The absorbed mass of sugar per plantlet was calculated from sugar concentrations and medium volume, and was converted to absorbed mole carbon through sugar uptake from the medium (Uc, mol C/plantlet):
Carbon dioxide exchange rate during the light and dark periods every 5 d when the light and dark periods were based on typical diurnal changes in CO2 concentration inside vessels containing photosynthetically active plantlets. Therefore, validation of this method was made by comparing increases in moles of carbon per plantlet estimated from the carbon balance and those derived from the actual dry weight increase of the plantlets. Carbon balance of in vitro plantlets was expressed as follows:

\[ U_d + U_p - L_d - L_p - D_p = 0 \]  

where, \( U_p \) is carbon absorbed during photosynthesis (mol C/plantlet); \( L_d \) and \( L_p \) are carbon released during dark respiration and photosynthesis (mol C/plantlet), respectively; and \( D_p \) is the carbon increase in plantlet tissue (mol C/plantlet). The percentage of \( U_d \) over the sum of \( U_d \) and \( U_p \) was calculated and expressed as percent.

Fig. 1. Dry weight and net photosynthetic rate per leaf area during the light period of sweetpotato on day 20 (A) and of tomato on day 15 (B), as affected by initial sucrose concentration (0, 7.5, 15, or 30 g·L\(^{-1}\)). \( L^* \), \( L^{**} \), \( Q^* \), \( Q^{**} \) = nonsignificant or significant linear (L) or quadratic (Q) response at \( P \leq 0.05 \) or 0.01, respectively.
contribution of photosynthesis [%P; %P = \frac{U_P}{U_S + U_P}]. The \( D_b \), estimated by solving Eq. [2], was compared with the increase of moles of carbon in dry weight (\( D_d \)).

### Statistical Analysis

There were five and six vessels for each treatment in experiments using tomato and sweetpotato plantlets, respectively. Vessels containing four plantlets were considered replicates. Experiments for sweetpotato and tomato were conducted separately each with a completely randomized experimental design. All data within each experiment were subjected to analysis of variance (ANOVA), and effects of initial suc concentration were analyzed by partitioning into orthogonal multiple contrasts using the Statistical Analysis System (Version 6.12, SAS Institute).

### Results and Discussion

#### Plantlet Growth and Net Photosynthetic Rate

Total (\( W_d \)) and root dry weight of sweetpotato and tomato plantlets increased whereas net photosynthetic rate per leaf area (\( P_l \)) decreased when suc was added to the medium (Fig. 1). The dry weight and net photosynthetic rate of the plantlets cultured without sugar in the medium (\( S_0 = 0 \)) may be further enhanced at a higher CO2 concentration and PPF more favorable to photosynthesis.

For sweetpotato plantlets, \( W_d \) increased and \( P_l \) decreased with addition of suc to the medium, but both stabilized at \( S_0 \) greater than 7.5 g·L\(^{-1} \) (Fig. 1A). For tomato plantlets, \( W_d \) and \( P_l \) stabilized around 15 to 30 g·L\(^{-1} \) of \( S_0 \). The increase of \( W_d \) was associated with the decrease of \( P_l \), probably because the plantlets had greater relative carbon influx from sugar in the medium at higher \( S_0 \). Decreased photosynthetic activities due to the sugar added to the medium have been reported with many species (Cristea et al., 1999; Kozai, 1991; Pospisilova et al., 1992). Langford and Wainwright (1987) demonstrated that decreasing the medium suc concentration (10, 20 or 40 g·L\(^{-1} \)) increased the net photosynthetic rate of in vitro grown shoots for two rose cultivars (Rosa hybrid). Net photosynthetic rate per leaf area of tomato plantlets was about 60% lower with 30 g·L\(^{-1} \) than with no suc when examined under similar culture conditions as in the present experiment (Kubota et al., 2001).

The biochemical aspect of depression of photosynthesis by sugar in the medium has been extensively studied. Cappellades et al.
Calathea luisae (1991) showed that rose plantlets grown on a high suc concentration (50 g·L⁻¹) medium had high accumulation of starch in the leaves resulting in reduced photosynthesis. Accumulation of carbohydrate in leaves has a strong correlation with inhibition of CO₂ assimilation, although the exact mechanism is not known (Foyer and Galtier, 1996). Wilson et al. (2000) demonstrated potential differences in carbohydrate status between tomato and sweetpotato plantlets cultured under similar culture conditions. They reported that leaves of tomato plantlets cultured with 30 g·L⁻¹ suc had significantly higher soluble sugar (suc, gluc, and fru) and starch concentrations than those without sugar in the medium, but that leaves of sweetpotato with and without sugar in the medium did not show significant differences in soluble sugar and starch concentrations. Desjardins et al. (1995) suggested that the most probable cause of reduced photosynthesis with sugar in the medium was a series of biochemical reactions leading to the down-regulation or feedback inhibition of ribulose bisphosphate carboxylase (rubisco). In the present experiment, reduction of Pₚ observed for sweetpotato plantlets may have been caused by enzymatic activities mediated by sugar absorbed from the medium rather than carbohydrate accumulation in the leaves, assuming that carbohydrate did not accumulate in the sweetpotato leaves with sugar in the medium as found by Wilson et al. (2000). For tomato, both increased carbohydrate levels and limited rubisco activities remain as possible reasons for a reduction of net photosynthetic rate. Further biochemical analyses will be needed to determine the actual limiting factor of photosynthesis at high S₀ for these species.

Root growth increased with increasing S₀ (P linear ≤ 0.01; P quadratic ≤ 0.05) (Fig. 1). Shoot to root dry weight ratio was 2.8, 2.9, 2.3, and 1.8 for sweetpotato; and 3.1, 2.6, 2.3, and 2.0 for tomato at S₀ of 0, 7.5, 15, and 30 g·L⁻¹, respectively, indicating that more carbohydrate was allocated to roots with increasing S₀ in both species. Accumulation of soluble sugar and starch in roots was reported for sweetpotato and tomato plantlets cultured with 30 g·L⁻¹ suc in the medium as compared with no suc (Wilson et al., 2000). High total soluble sugars were observed in leaf and root tissue of tomato and in root tissue of sweetpotato when cultured with 30 g·L⁻¹ suc (Wilson et al., 2000). Piqueras et al. (1998) reported that Calathea luisae Gagnep. plantlets had higher starch concentration in roots and stems than in leaves, while suc concentration was highest in the stems, followed by leaves and roots. Although root dry weight of sweetpotato and tomato similarly responded to S₀, carbon partitioning and thereby soluble sugar and starch concentrations may have been different between species.

Sugar concentration and medium volume. During autoclaving, a portion of the original suc present was hydrolyzed to gluc and fruc in all media containing sugar. Specific sugar concentrations after autoclaving were 6.5, 14.4, and 29.5 g·L⁻¹ for suc; 0.7, 1.0, and 1.4 g·L⁻¹ for gluc; and 0.7, 1.1, and 1.4 g·L⁻¹ for fruc at S₀ of 7.5, 15 and 30 g·L⁻¹, respectively. The increase in total sugar (suc, gluc, and fruc) concentrations after autoclaving was 5% to 10%. This indicates that, in addition to hydrolysis of suc, a small portion of water may have evaporated from the vessel during autoclaving or condensed on the inner wall of the vessel, resulting in an increase in the total sugar concentration.

After day 20 (sweetpotato) and 15 (tomato), suc still comprised the majority of the sugar in the medium and it was 4.8, 9.2, and 20.0 g·L⁻¹ for sweetpotato; and 3.1, 7.8, and 17.3 g·L⁻¹ for tomato at S₀ of 7.5, 15 and 30 g·L⁻¹, respectively (Fig. 2). No suc was detected in the medium without suc. All sugar concentrations at the end of culture increased with increasing S₀ (P linear ≤ 0.01). Medium volume at the end of culture was not affected by S₀, and was 15% to 20% less in sweetpotato and 13% to 16% less in tomato than the initial volume (data not shown). Based on the suc, gluc, and fruc concentrations and residual medium volume, the total mass of sugar left in the medium was 77%, 87%, and 99% (0.58, 1.31, and 2.96 g/vessel) of the initial values (0.75, 1.5, and 3.0 g/vessel) for sweetpotato; and 64%, 82%, and 85% (0.48, 1.23, and 2.54 g/vessel) for tomato at S₀ of 7.5, 15, and 30 g·L⁻¹, respectively. Although the culture period for tomato was shorter than that for sweetpotato, more sugar remained in the medium for sweetpotato than for tomato. The low consumption of sugar (most sugar remained unconsumed in the medium) was probably due to CO₂ enrichment and high PPF that promoted photosynthesis by the cultured plantlets. Under conditions with lower PPF and more limited CO₂ (without CO₂ enrichment and/or lower ventilation of the vessel), the plantlets may rely more on sugar in the medium as the major carbon source, and the sugar consumption would be higher than those in the present experiment. This hypothesis had been confirmed for tomato in a separately conducted experiment (Ezawa, 1997), where tomato plantlets cultured under low PPF (100 µmol·m⁻²·s⁻¹) in vessels with a low number of air exchanges (1.6 h⁻¹). Under these conditions, the plantlets absorbed 32% of initial sugar when cultured at 30 g·L⁻¹ of S₀, while they absorbed 15% of initial sugar at the same S₀.

Fig. 3. Simulated changes in CO₂ concentration (Cₑ) inside a vessel containing four tomato plantlets without sugar in the medium (S₀ = 0). The Cₑ during light and dark periods was assumed to decrease/increase linearly and reach steady state conditions 1 and 6 h, respectively, after onset of the period. CO₂ concentration outside the vessel was 2050 mmol·mol⁻¹ throughout the culture period.

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under a high number of air exchanges (4.7 h⁻¹) and high PPF (160 µmol·m⁻²·s⁻¹).

**Steady state CO₂ concentration inside the vessel (C_in,s) and estimation of changes in C_in during culture.** In both plant species, C_in,s measured during the light period decreased with time, except for sweetpotato during days 5 to 10 (Table 1). Increased C_in,s during the light period in sweetpotato for days 5 to 10 was due to an increase in C_in,s (from 1400 µmol·mol⁻¹ on day 5 to 1800 µmol·mol⁻¹ on day 6). Although differences among S₀ were relatively small, C_in,s during the light period increased with increasing S₀ (P linear ≤ 0.01), except for tomato on days 10 and 15 in which no significant influence of S₀ was observed. The C_in,s attained a range of 250 to 390 µmol·mol⁻¹ in sweetpotato and 450 to 890 µmol·mol⁻¹ in tomato by the end of culture. The C_in,s measured during the dark period increased with time in sweetpotato. The S₀ did not affect C_in,s during the dark period except for day 15. In tomato, C_in,s during the dark period increased with time regardless of S₀ except for 0 g·L⁻¹, and with increasing S₀ (P linear ≤ 0.01).

The time course of changes in C_in was simulated for each vessel based on the measured C_in,s by our assumptions (Fig. 3). The C_in during the dark period was simulated as reaching steady state conditions 6 h after initiation of the dark period, although changes in C_in during the dark period did not affect the estimation of cumulative CO₂ exchange rates since the Rₜ was assumed to be constant. Simulated results were considered representative of the typical changes in C_in of the vessels containing plantlets under natural ventilation conditions.

**Plantlet carbon balances.** The S₀ affected Uᵢ, Uᵢₛ, %P, and Lₛ, but did not affect Lₛ for sweetpotato (Table 2). The %P decreased and attained 77% to 92% when suc was added to the medium. For tomato plantlets, S₀ affected Uᵢ, Uᵢₛ, %P, Lₛ, and Lₛ (P quadratic ≤ 0.01). The %P decreased with S₀ and attained 60% to 67% for S₀ of 7.5 to 30 g·L⁻¹. When the plantlets were cultured with sugar in the medium, the range of Uᵢ was relatively higher for tomato (2.4 to 4.5 mmol C per plantlet) than for sweetpotato (0.6 to 1.6 mmol C per plantlet), while Wᵢₛ was relatively higher for sweetpotato (133 to 136 mg per plantlet) than for tomato (67 to 99 mg per plantlet). This observation corresponded to the higher %P for sweetpotato (77% to 92%) than for tomato (60% to 67%).

Mole fractions of Uᵢ to the carbon added as sugar in the medium (6.5, 13, and 26 mmol C per plantlet were added at 7.5, 15, and 30 g·L⁻¹ of S₀, respectively) can be considered as indicating the sugar utilization efficiency, and were 0.25, 0.14, and 0.02 mol·mol⁻¹ for sweetpotato; and 0.37, 0.20, and 0.17 mol·mol⁻¹ for tomato at 7.5, 15, and 30 g·L⁻¹ of S₀, respectively, decreasing with increasing S₀. In R. multiflora, 50% to 75% of tissue carbon was derived from sugar uptake from the medium; however, 68% to 84% of sugar taken up from the medium was used for respiration (De Riek et al., 1991). Borkowska and Kubik (1990), using ¹⁴C-suc, showed that sour cherry (Prunus cerasus L.) plantlets absorbed 23% of sugar added to the medium but only 5% of the original activity of the medium remained in dry weight, indicating that most of the carbon absorbed as sugar from the medium had been respired. In the present experiment, the ratios of Lₛ to Uᵢ increased with increasing S₀ (0.44, 0.54, 0.52, and 0.56 for sweetpotato; 0.56, 0.60, 0.66, and 0.78 for tomato at S₀ of 0, 7.5, 15, and 30 g·L⁻¹, respectively), indicating increased Rᵢ with increasing S₀. High dark respiration rates have been reported for many species when cultured with sugar in the medium (Cristea et al., 1999; Galzy and Compan, 1992; Kubota et al., 2001). If the sugar taken up from the medium merely enhances the dark respiration rate and is mostly released as CO₂ to the atmosphere, addition of sugar in the medium reduces the efficiencies of energy and sugar incorporated to dry matter in plantlets, especially chlorophyllous plantlets that have already gained photosynthetic ability.

The higher %P in sweetpotato than in tomato suggests that controlled environments (e.g., CO₂ enrichment and increased PPF) can more effectively enhance the dry weight increase of sweetpotato plantlets, but that increasing sugar concentration in addition to CO₂ enrichment and increased PPF may be necessary to effectively increase dry weight of tomato plantlets. De Riek et al. (1991) used ¹⁴C-labeled suc and ¹⁴CO₂ to show that 75% of the dry weight carbon originated from suc (25% was from photosynthesis) for rose plant-
Fig. 4. Increase of moles of carbon per plantlet calculated from the balance equation, \( D_d = D_p + D_p(1 - \alpha) - L - U \), and from dry weight increase, \( D_p = U + L - L_d - L_s \). Increasing PPF from 50 to 300 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \) and 1.6 air exchanges/h in the vessel showed that \( D_d \) increased this percentage from 16\% to 56\% for the gardenia leaflets (Serret et al., 1997). Increasing PPF from 50 to 300 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \) increased this percentage from 16\% to 56\%. For sweetpotato plantlets, increased dry matter when cultured in tightly sealed vessels (Serret et al., 1997). Lower sugar concentrations (Serret et al., 1997; Wolf et al., 1998) and osmotic potentials (Wolf et al., 1998) increased percent tissue carbon derived from photosynthesis of photomixotrophic plantlets. The percentage of tissue carbon derived from photosynthesis of potato plantlets was about 40\% when cultured at 30 g·L\(^{-1}\) suc, while it was only 10\% at 80 g·L\(^{-1}\) suc (Wolf et al., 1998).

Effects of physical environmental conditions on percent carbon derived from photosynthesis over the total carbon in dry weight have been well studied in gardenia. Gardenia plantlets at shoot multiplication stage were shown to have 36\% photosynthates in leaflet dry matter when cultured in tightly sealed vessels, but 93\% when cultured in loosely sealed vessels (Serret et al., 1997). Increasing PPF from 50 to 300 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \) increased this percentage from 16\% to 56\% for the gardenia leaflets (Serret et al., 1997). Our preliminary data for potato plantlets (Ezawa, 1997) cultured under 100 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \) and 1.6 air exchanges/h in the vessel showed that contribution of photosynthesis (%P) was 24\% when cultured at 30 g·L\(^{-1}\) suc, while it was 60\% at the same suc level under 160 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \) and 4.7 air exchanges/h. Under conventional culture conditions (lower PPF and less ventilation), plantlets are generally dependent on sugar in the medium mainly due to limited availability of CO\(_2\) in the head space and thereby the %P is expectedly smaller. Fujiwara et al. (1995) showed that the contribution from photosynthesis to dry weight increase, expressed as mass of carbohydrate on a basis of plantlet dry weight increase, was three to four times greater than the contribution from sugar taken up from the medium for potato plantlets cultured at 15 g·L\(^{-1}\) initial suc concentration under 100 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \) PPF and 1000 \( \mu \text{mol} \cdot \text{mol}^{-1} \) CO\(_2\) concentration.

This result agrees with those obtained by us for sweetpotato at 15 g·L\(^{-1}\) of \( S_0 \) (the ratio of \( U_i \) to \( U_p \) was 3.3). In the present experiment, the %P can be further increased for both species by increasing CO\(_2\) concentration and PPF inside the vessel.

Validation of the increase of moles of carbon per plantlet showed that the estimation using the carbon balance equation was reasonably accurate for plantlets cultured without sugar in the medium \((S_0 = 0)\), regardless of species. This conclusion indicates that the assumptions made for simulating \( C_o \), and estimating cumulative photosynthesis and respiration were acceptable for no sugar in the medium. For sweetpotato plantlets, increased moles of carbon per plantlet, calculated according to the carbon balance equation \((D_p)\), generally agreed with the increased moles of carbon calculated from the dry weight increase \((D_d)\) except at the highest \( S_0 \) of 30 g·L\(^{-1}\) (Fig. 4) where the \( D_d \) were underestimated. For tomato plantlets, \( D_d \) agreed with \( D_d \) only at no sugar \((S_0 = 0)\), and was overestimated when cultured with sugar in the medium. The disagreement of \( D_d \) and \( D_d \) for tomato may be due to overestimation of photosynthetic carbon uptake \((U_i)\) as indicated by Fujiwara et al. (1995). Dark respiration rate for plantlets cultured with sugar in the medium includes CO\(_2\) through dissimilation of sugar in the medium. Therefore the present estimate of \( U_i \) based on the CO\(_2\) exchange rate measured during light and dark periods may be an overestimate. For tomato, the differences between \( D_d \) and \( D_d \) were 1.7, 1.0, and 2.0 mmol C per plantlet at 7.5, 15, and 30 g·L\(^{-1}\) of \( S_0 \), which accounted for 40\% to 70\% of \( U_i \). This finding may indicate that a significant portion of sugar absorbed from the medium was released as CO\(_2\), as reported for other species under conventional culture conditions (Borkowska and Kubik, 1990; De Riek et al., 1991). If the 40\% to 70\% of \( U_i \) were respired as CO\(_2\), from 50 to 300 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \) increased this percentage from 16\% to 56\% for the gardenia leaflets (Serret et al., 1997). Our preliminary data for potato plantlets (Ezawa, 1997) cultured under 100 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \) and 1.6 air exchanges/h in the vessel showed that contribution of photosynthesis (%P) was 24\% when cultured at 30 g·L\(^{-1}\) suc, while it was 60\% at the same suc level under 160 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \) and 4.7 air exchanges/h. Under conventional culture conditions (lower PPF and less ventilation), plantlets are generally dependent on sugar in the medium mainly due to limited availability of CO\(_2\) in the head space and thereby the %P is expectedly smaller. Fujiwara et al. (1995) showed that the contribution from photosynthesis to dry weight increase, expressed as mass of carbohydrate on a basis of plantlet dry weight increase, was three to four times greater than the contribution from sugar taken up from the medium for potato plantlets cultured at 15 g·L\(^{-1}\) initial suc concentration under 100 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \) PPF and 1000 \( \mu \text{mol} \cdot \text{mol}^{-1} \) CO\(_2\) concentration.
photosynthesis (Foyer and Galtier, 1996). The high $S_o$ could enhance the carbohydrate concentration of the plantlets and thereby alter the diurnal changes in CO$_2$ exchange rates.

Nonphotosynthetic carbon fixation by phosphoenolpyruvate carboxylase (PEPC) has been reported for in vitro plantlets (Desjardins et al., 1995). However, PEPC activity was not determined in the present experiment. Desjardins et al. (1995) found high PEPC activity when sugar was added to the medium for early growth stages of strawberry plantlets. A nonnegligible amount of PEPC activity when sugar was added to the medium for early mined in the present experiment. Desjardins et al. (1995). However, PEPC activity was not deter-

The carbon balance of in vitro plantlets was estimated in situ with reasonable accuracy when grown with an initial medium suc concentration of 0 to 15 g L$^{-1}$ for sweetpotato and with no suc for tomato. Tomato plantlets were more dependent on sugar in the medium than sweetpotato. For both sweetpotato and tomato, the dry weight increased, but the net photosynthetic rate during the light period and the efficiency of sugar use from the medium decreased with increasing initial suc concentrations. Such analysis of carbon balance and use of resources for tissue culture is crucial for a better understanding of carbon metabolism in vitro and for selection and improvement of methods and systems for micropropagation.

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