Light quality and CO_2 induced changes in saccharide content of strawberry (*Fragaria x ananassa*) 'Red Joy' plants *in vitro*

John H. Miranda[1] and Richard R. Williams[2]

[1] School of Life Science, Queensland University of Technology, Gardens point, 2 George St. Brisbane Qld 4001, www.qut.edu.au, Email john_miranda@hotmail.com

[2] School of Agronomy and Horticulture, University of Queensland, Gatton Campus, Lawes, Qld.4343, www.uq.edu.au, Email richard.williams@mailbox.uq.edu.au

Abstract

Blue and yellow light increased the level of sucrose in plants developed *in vitro* when compared to white light. CO_2 enrichment and airflow into the culture container increased sucrose content compared to closed system. Light qualities or CO_2 levels did not alter fructose or glucose concentration. Light quality effect on sugar concentration was shadowed by high concentrations of CO_2.

Media summary

Strawberry plants developed *in vitro* under blue and yellow light produced more sugar than those developed under white light. Carbon dioxide and airflow into the culture container enhanced sugar levels in the plant.

Key words

Light, CO_2, sucrose, fructose, glucose, strawberry, *in vitro*

Introduction

Until recently it was considered that the main function of sucrose in the plant was as a substrate for growth and development of sink points, and in some cases storage of carbohydrate (fruits, tubers and cane sugar). Recent evidence shows that expression of many different plant genes, such as those for RubisCO small and large sub units and polysaccharide biosynthesis, have regulatory mechanisms mediated by sugar (Koch 1996).

The expression of genes that are regulated by sugar concentration are generally grouped as famine and feast genes. Famine gene expression is enhanced by sugar depletion (eg. sucrose synthesis) and feast gene expression is enhanced by sugar abundance (eg. starch synthesis).

Depletion of sugars activates gene expression and increases photosynthetic rate. When output exceeds the use and/or export capacity the subsequent accumulation of sugars in the leaf triggers repression of photosynthesis genes and ultimately photosynthesis. At the same time genes required for synthesis of carbon metabolites for future use are activated (Stitt 1991; Krapp *et al.* 1993; Van Oosten and Besford 1994 and Smeekens and Rook 1997).

The relative type and size of the sugar pools varies dramatically during tissue development, between different plant species, and within the same species subject to different environmental conditions (Quick and Schaffer 1996). Changes in CO_2 concentration can alter the level of sugar pools, for example under CO_2 enriched condition, sucrose concentration generally increases (60 to 160%), soluble proteins and RubisCO decrease and photosynthesis is inhibited (Drake *et al.* 1997).

Light quality and CO_2 had significant effect on photomorphogenic development of *in vitro* strawberry 'Red Joy' (Miranda *et al*. 2002). However, the independent effects of light quality and interactive effects of light

quality with CO_2 on sucrose accumulation are not known. Also, nothing is known about the possible role of *in vivo* sucrose levels in morphogenic expression either *in vitro* or *ex vitro* developed plants.

The objective of the study was to quantify changes in sucrose concentration due to light quality and the interaction of light quality with CO_2 on *in vitro* development of strawberry 'Red Joy' plantlets.

Methods

Plant culture

–

Strawberry 'Red Joy' plantlets of uniform size, ≈ 1.5-2 cm height, ≈ 0.105 g weight, with 2-3 leaves, were cultured in 250 mL polycarbonate bottles with 30 mL of half strength of De Fossard's solid media plus 2 μML^{-1} of benzene amino purine (BAP). The mean light intensity within the experimental area was 45 ? 5.5 $\mu mol.m^{-2}.s^{-1}$, with a constant temperature of 26?C ? 2.5. A 16 h photoperiod and 8 h dark was provided during the culture period.

Media composition, culture containers and growth conditions

Half strength De Fossard's multiplication medium was used. The pH of the media was adjusted to 5.6 after mixing all ingredients and before adding agar and autoclaving. The 2.2 cm deep transparent polypropylene screw lids were fitted with one or two rubber septa (Shimadzu, Japan) to facilitate CO_2 enrichment and air flow.

CO_2 enrichment and air flow system

The *in vitro* plantlets were enriched with CO_2 (996 ? 5ppm) or air, both gases supplied from compressed cylinders (G sized 15.0 MPa). A gas flow system developed at UQG campus (Miranda, 2002) was used to enrich the tissue culture containers with sterile gases. These cylinders were connected via upstream (0-30000 KPa) and down stream (0-1500 KPa) regulators. The down stream was connected to a flow meter, which was marked from 0-15 litres with 30 equal divisions (set for 200 KPa). The outlet of the flow meter was connected to a solenoid time dosing control system. Using this system one can control the gas flow to the tissue culture bottles 16 times/day for any specified length of time. The down stream gas flow through the solenoid valve was directed to a manifold which divided the flow into 10 equal streams which were then directed into the tissue culture bottles (experimental units) through 2 µm filters (Millipore).

The CO_2 flow rate and duration of flow for enrichment was standardised based on CO_2 utilisation (photosynthesis), rate of diffusion through the lid, solubility in the medium and rate of plantlet respiration. The enrichment occurred for 15 minutes every half an hour commencing at 11:45 am.

Experimental design

The four light qualities; white, blue, yellow and red, of known wavelength spectra were used in combination with three CO_2 levels; CO_2 enriched (1000 ppm), air flow, and closed systems, laid out as a 4 x 3 factorial with 5 replications.

Analysis of fructose, glucose and sucrose

Whole plantlet samples were subjected to HPLC analysis of fructose, glucose, and sucrose using the extraction method modified from Lopez *et al*. (1994) with 15 minute double extraction used to extract soluble sugars instead of a single 30 minute extraction.

Sample preparation and analysis

After five weeks *in vitro* culture under treatment conditions, strawberry plants were deflasked at the end of the dark period. Whole plant samples were freeze dried, ground in liquid nitrogen for double extraction. Standard solutions of fructose, glucose and sucrose of 0.1% and 0.3% strength were prepared. From this, 2µL of each standard were injected into the HPLC-RID 10 (Shimadzu Japan) set at 30?C using acetonitral and water (75:25) as mobile phase with a flow rate of 1.2 mL min^{-1}. The individual sugar concentrations in the original sample were calculated using the sample dry weight and total extract volume and expressed as g/g dry weight.

Results

Sucrose accumulation

Blue and yellow light increased the level of sucrose, but red had no significant effect (Table 1). The concentration of sucrose also increased with the supply of CO_2, from the closed system to CO_2 enrichment.

Table 1. Effect of light quality and CO_2 levels on *in vivo* sucrose concentration of strawberry plants developed *in vitro.*

	Mean sucrose content (?g/g dry wt)				
CO_2 regimes *	Light quality levels *				
	White	Blue	Yellow	Red	Average *
CO_2	39.2 ap	50.6 ap	47.1 ap	28.6 ap	41.4 m
Air	14.2 aq	36.7 bp	60.6 cq	23.8 ap	33.8 m
Closed	13.1 aq	23.6 aq	22.9 ar	30.3 ap	22.5 n
Average *	22.1 i	37.0 j	43.5 jk	27.5 i	32.5

* = significant, ns = not significant, P = 0.05. Pooled standard error = 7.6. Letters a b c d for comparison of means within rows. Letters i j k l for comparison of means within average row. Letters p q r for comparison of means within columns. Letters m n o for comparison of means within average columns. Means with the same letter are not significant.

Under all light treatments except red, CO_2 enrichment increased the sucrose level. The response to the air treatment varied among the light qualities, from no effect under white or red, to a doubling under blue and trebling under yellow.

Fructose and Glucose

Neither light quality nor CO_2 had any independent effect on fructose and glucose accumulation.

Discussion

The high sucrose content in plants cultured under yellow or blue light, compared with white light, can be attributed to the light quality. Assuming the uptake of sucrose directly from the media was negligible (De Riek *et al.* 1991), the high sucrose content of the plants may be an indicator of either a high photosynthetic rate under these light qualities and/or the rate of utilisation of sucrose was lower. The increase in mean sucrose concentration over CO_2 regimes under blue and yellow light is largely due to accumulation in the open systems with a reduced effect in the closed system.

As the mean sucrose content over CO_2 regimes increased under yellow or blue light, glucose content tended to decrease and fructose increased marginally, compared with white. This indicated that as more sucrose accumulated more glucose was used in plants developed under yellow and blue than under white. It was interesting to note the similarities ($P>0.05$) in the response of sucrose to light quality in CO_2 enrichment and closed systems. High levels of CO_2 in these systems may have overshadowed the light quality effect. CO_2 enrichment provided a continuous CO_2 supply, and the control also had periods of high CO_2 concentration at the end of each dark period, whereas in the air system CO_2 accumulation was negligible. Thus, the lack of response to light quality under CO_2 enrichment and in closed systems may be attributed to the periods of high CO_2 levels.

In general chlorophyll has the capacity to utilise red wavelengths for CO_2 assimilation more effectively than other wavelengths. However the low and similar level of sucrose across CO_2 regimes indicate a low level of sucrose synthase activity or conversion of sugar to starch at a much faster rate than under other light qualities.

The significant increase in the effect of the open system over different light qualities compared to the control, was a clear indication that continuous CO_2 availability during photoperiod increased sucrose content *in vitro*. However the low level of sucrose under white light in the air system may be due to the high intensity mercury peak in the white light, resulting in photoinhibition (data not shown here) and this needs to be investigated further.

The CO_2 regime did not effect glucose and fructose content in the way sucrose did, probably because the quantity of fructose and glucose synthesised *in vivo* was pooled together with that absorbed directly from the media. It is known that fructose and glucose are taken up from the medium (De Reik *et al.* 1991).

References

De Riek J, Van Cleemput O and Debergh P (1991). Carbon metabolism of micropropagated Rosa multiflora L. In vitro Cell Developmental Biology 27, 57-63.

Drake BG, Gonzzalez-Mele, MA and Long SP (1997). More efficient plants: a consequence of rising atmospheric CO_2? Annual Review of Plant Physiology and Plant Molecular Biology 48, 609-639.

Koch KE (1996). Carbohydrate-modulated gene expression in plants. Annual Review of Plant Physiology and Plant Molecular Biology 47, 509-540.

Koch KE, Ying Z, Wu Y and Avigne WT (2000). Multiple paths of sugar-sensing and a sugar/oxygen overlap for genes of sucrose and ethanol metabolism. Journal of Experimental Botany 51, 417-427.

Krapp A, Hofman B, Schafer C and Stitt M (1993). Regulation of the expression of rbcS and other photosynthetic genes by carbohydrates: a mechanism for the 'sink regulation' of photosynthesis? The Plant Journal 3, 817-828.

Lopez HJ, Gonzaez CME, Vazquez BML Vazquez O and Simal L (1994). HPLC determination of sugars and starch in green beans. Journal of Food Science 59, 1084 -1049.

Miranda JH (2002). Control of plant development by light CO_2 and oligosaccharins *in vitro* with emphasis to strawberry 'Red Joy' PhD Thesis 2002 University of Queensland.

Miranda JH and Williams R (2004) Developmental effects of light quality and CO_2 on strawberry plants *in vitro*. Submitted for peer review.

Quick PW and Schaffer AA (1996). Sucrose metabolism in source and sinks. In 'Photoassimilate Distribution in Plants and Crops: Source Sink Relationships. (Ed. Zamski E and Schaffer A A), New York, Dekker.

Smeekens S and Rook F (1997). Sugar sensing and sugar mediated signal transduction in plants. Plant Physiology 115, 7-13.

Stitt M (1991). Rising CO_2 levels and their potential significance for carbon flow in photosynthetic cells. Plant Cell Environment 14, 741-762.

Van Oosten JJ and Besford RT (1994). Sugar feeding mimics effect of acclimation to high CO_2: rapid down regulation of RubisCO small subunit transcripts, but not of the large subunit transcripts. Journal of Plant Physiology 143, 306-312.