Assessment of the carbon dissimilation methodology in the *in vitro* growth of the 'Paulsen 1103' grapevine

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ABSTRACT

A simple and non-destructive carbon dissimilation method to assess plant cell growth was tested for its *in vitro* suitability for grape vines. *In vitro* culture plants can either use atmospheric CO_2 or the sugars present in the culture medium as carbon source. Carbon dissimilation predicts that the sugar metabolism of the culture medium results in a net weight loss of the culture flask contents and may thus be used to follow culture growth. The 'Paulsen 1103' grapevine rootstock was introduced and multiplied *in vitro* by the auxiliary shoot methodology. Tubes with and without plants were weighed daily throughout an experimental growth period of 60 days. Results from the *in vitro* plants showed that the variety studied presented dissimilation and assimilation activity over the culture period and therefore presented *in vitro* fotomixotrophy. There were three distinct growth phases: an initial phase, during the first 10 days of culture, where there was intense weight loss by dissimilation; a second stationary phase, which lasted approximately 10 days, and a final phase of 40 days, where an important weight gain was observed (37.68 mg). Thus carbon use and micropropagated plant growth could be assessed quickly and efficiently.

KEY WORDS: *Vitis,* rootstock, i*n vitro* culture, photoautotrophy.

INTRODUCTION

The contribution of photosynthesis to total carbon metabolism in *in vitro* plants has been greatly studied. Tissue culture systems have used exogenous sugars as an energy source. Thus atmospheric CO_2 is available to the plant , but it can equally use the sugar present in the culture medium (Galzy, 1990; Galzy and Compan, 1992). This normally results in the state called photomixotrophy (Capelades et al., 1991; Desjardins, 1995; Silva et al., 1996).

Carbon dissimilation analysis considers that the sugar consumed by the culture medium is partially converted to biomass and the other part is used to supply energy to the plant metabolic processes. The sugars are dissimilated by the plants under O_2 consumption to produce CO_2 and H_2O. In this process, an equimolar quantity of O_2 is exchanged with CO_2, resulting in a net weight loss in the culture flask. This methodology was developed by Schripsema et al. (1990) to assess the growth of *in vitro* heterotrophic cell cultures.

This study was carried out to assess the application of the carbon dissimilation method for differentiated tissues and to estimate the growth of *in vitro* plants of the 'Paulsen 1103' grapevine rootstock variety in order to evaluate these non-destructive methodologies as useful and effective tools in genetic improvement using biotechnology techniques.

MATERIAL AND METHODS

The experiments were carried out in the Plant Morphogenesis and Biochemical Laboratory (LMBV) and the Genetics and Development Physiology Laboratory (LGFD) from the Crop Science Department (FIT) at the Federal University of Santa Catarina (UFSC) Center for Agrarian Sciences (CCA).

The 'Paulsen 1103' grapevine rootstock (*Vitis rupestris* x *Vitis berlandieri*) was multiplied *in vitro* using the auxiliary shoots methodology described by Galzy (1969). The stock plants of this variety were kept in a greenhouse at the Crop Science Department/ CCA/UFSC.

The explants, approximately 2 cm long, were obtained from the fifth auxiliary shoot from *in vitro* plants aged 60 days in culture and were inoculated in test tubes containing 15 ml DSD1 culture medium (Silva and Doazan, 1995) with the addition of 20.0 g L^{-1} sucrose

and 7.0 g L^{-1} agar-agar. Pyrex (22 x 220 mm) test tubes were used closed with transparent plastic caps and wrapped in three layers of plastic film to prevent gas exchange with the external atmosphere. Later, the cultures were transferred to a growth room at 25°C, 16 hour photoperiod, 40-45 $mmol.m^{-2}.s^{-1}$ of photosynthetically active radiation and 60-70% relative humidity.

The carbon dissimilation analysis was carried out with ten test tubes containing explants and five control tubes (without plants) that were weighed daily from the moment of inoculation to 60 days *in vitro* growth (experimental period). The set of test tube weight data were assessed by the arithmetic mean and the weight loss by carbon dissimilation from the culture medium was estimated according to methodology proposed by Schripsema et al. (1990).

RESULTS AND DISCUSSION

Figure 1 shows the results obtained from the daily weighing of the tubes with plants during the 60 days *in vitro* culture of the 'Paulsen 1103' variety.

The tubes with *in vitro* plants gradually lost weight during the experimental period (466.05±35.49 mg). These results may have been caused by two different processes: weight loss because of water loss from the culture medium or weight loss because of dissimilation activity of the sugars in the culture medium or the two processes concomitantly.

Tubes without plants (controls) were also weighed daily to assess the effect of water loss on the total weight loss of the cultures (Figure 2).

The behavior for weight loss in the tubes with and without plants was similar, indicating that much of the weight variation shown by the cultures was due to water loss from the culture tubes.

Considering that the water loss suffered by the tubes without plants was similar to that of the tubes with plants, since the experimental conditions were the same, the evaporation rate of the controls was subtracted from that of the tubes with plants. Thus a weight loss curve by dissimilation from the tubes with plants was obtained (Figure 3).

The weight loss curve by dissimilation of the tubes with plants showed great variation during the period of culture. This type of behavior observed in the *in vitro* 'Paulsen 1103' grapevine rootstock shows, according to the method, intervals of heterotrophic and autotrophic carbon use that characterizes photomixotrophic nutrition.

Initially the values were positive, indicating that the plants consumed and metabolized the carbon sources added to the culture medium, thus losing weight by dissimilation. In this period, therefore, the plants presented heterotrophic nutrition.

This result is confirmed by analysis of the sugars in the culture medium performed by Moreira (2000) where it was observed that the 'Paulsen 1103' variety consumed the sugars added to the culture medium especially in the first 15 days of culture.

After a period of stability, the curve values fell and became negative, suggesting a weight gain in the

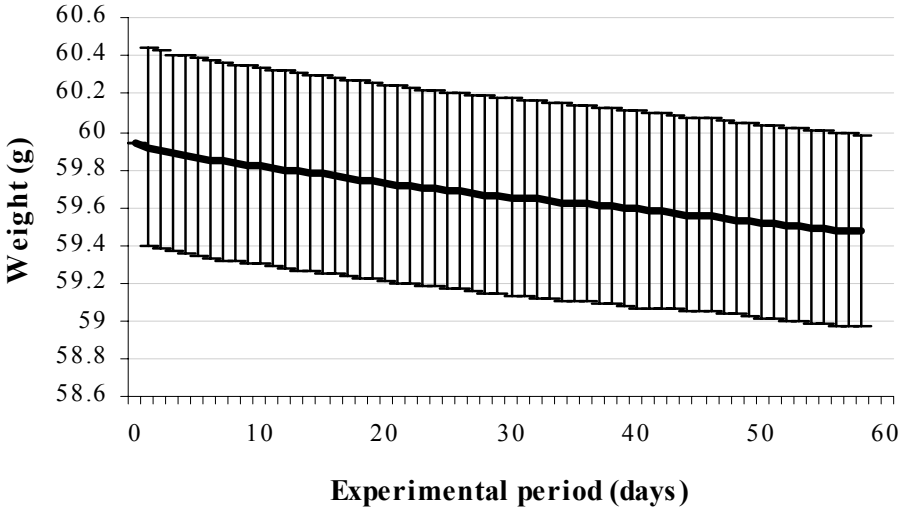

Figure 1. Mean weight evolution of the tubes with *in vitro* plants of the 'Paulsen 1103' grapevine rootstock. UFSC, Florianópolis, SC, 2000.

tubes with plants compared to the tubes without plants (Schripsema et al., 1990). That is, instead of carbon dissimilation in the culture medium, there was an atmospheric carbon assimilation by the plants, that is, photosynthesis, and this external source of carbon was converted to biomass or stored in the form of starch or sucrose.

According to Moreira (2000) the Rubisco and chlorophyll analysis and the stomata assessment of this same variety of *in vitro* grapevine rootstock showed similar results to those found in plants maintained in greenhouse suggesting the presence of a normal photosynthesis apparatus in these *in vitro* plants.

Photosynthesis in plants cultivated *in vitro* was also observed in other grape vine species and varieties such as *Vitis vinifera* and *Vitis rupestris* (Galzy and Compan, 1992) and Gravesac (Silva et al., 1995, 1996) and for other plant species, such as potato (Cournac et al., 1991), raspberry (Deng and Donnelly,

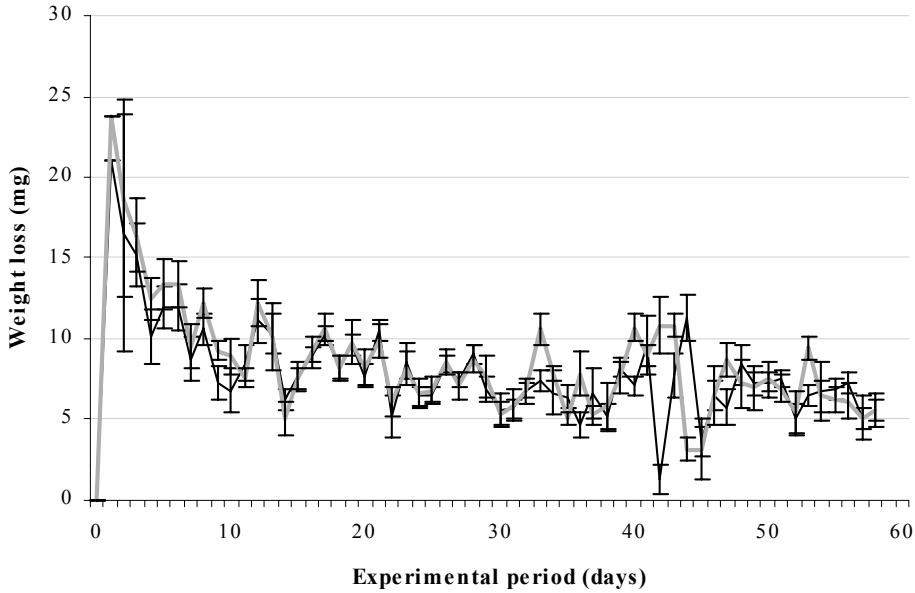

Figure 2. Mean variation in daily weight loss of tubes with *in vitro* 'Paulsen 1103' grapevine rootstock plants and tubes without *in vitro* plants. UFSC, Florianópolis, SC, 2000.

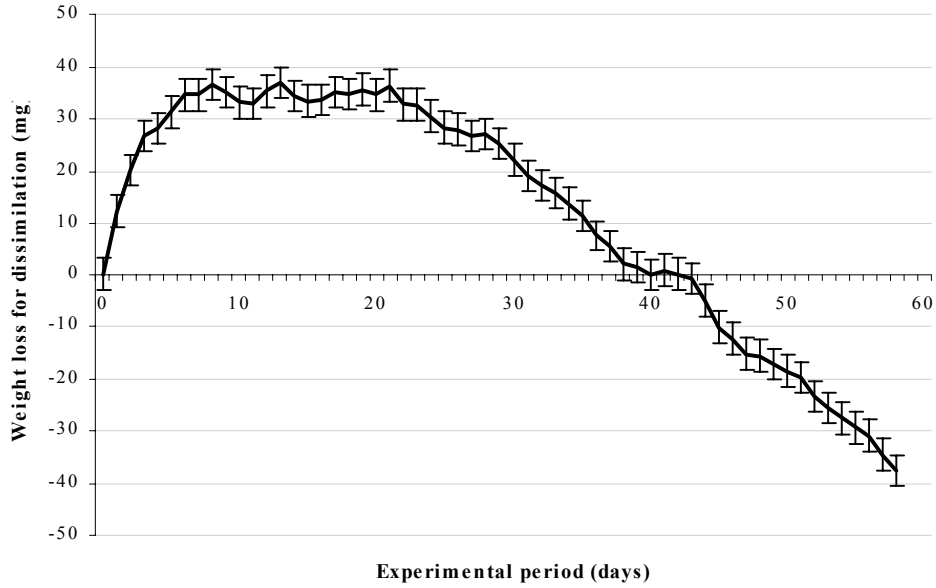

Figure 3. Mean weight loss by dissimilation from the tubes with plants of the *in vitro* 'Paulsen 1103' grapevine rootstock. UFSC, Florianópolis, SC, 2000.

1993), *Spathiphyllum* (Huylenbroeck and Debergh, 1996) and tobacco (Ticha et al., 1998).

Different results, however, were detected in cell suspension cultures of *Tabernaemontana divariacata* (Schripsema et al., 1990) and *Mandevilla velutina* (Maraschin, 1998) where the cultures showed positive weight loss by dissimilation, characterizing a totally *in vitro* heterotrophic nutrition.

Regarding to the loss weight assessed by dissimilation curve, three further phases could be identified by assessing and following the weight of the tubes with plants during the period of culture. An intense weight loss by dissimilation was detected (36.48±2.71 mg) during the first ten days of culture. It is believed that the explants used the exogenous sugars from the culture medium in this period to supply the intense metabolism required by the root and leaf re-differentiation process as, in this stage, the photosynthesis apparatus is not yet formed.

There was practically no variation in the weight of the cultures in the period between 10 and 20 days of culture. In this stage, the plants formed roots and leaves and began to grow, requiring energy. The use of exogenous carbon sources to the culture medium and the beginning of autotrophic activity met this demand. Thus there was a balance between the assimilation and dissimilation activities that resulted in little weight variation in the cultures.

There was a clear reduction in the dissimilation activity after the 21st day and the *in vitro* plants began to gain weight compared to the tubes without plants. This weight gain was related to photosynthesis, as the plants in this age already presented completely differentiated leaves, with formed photosynthesis apparatus (Moreira, 2000).

At the end of the culture period (60 days) there was a 37.69±3.52 mg weight gain in the flasks with plants compared to those without plants. This value expresses the total biomass production of the plants and was equivalent to the values found for this same variety *in vitro* by Moreira (2000).

CONCLUSION

The carbon dissimilation analysis was shown to be efficacious in analyzing carbon metabolism allowing estimates of the biomass production of *in vitro* vine plants to be made in a non-destructive methodology.

The assessment of the 'Paulsen 1103' grapevine rootstock showed photomixotrophic nutrition during the *in vitro* culture, with heterotrophy at the start and autotrophy at the end of the culture.

RESUMO

Avaliação da metodologia de dissimilação de carbono no crescimento in vitro do porta-enxerto de videira 'Paulsen 1103'

Um método de dissimilação de carbono, simples e não-destrutivo, de avaliação do crescimento de células vegetais foi testado quanto à sua aplicabilidade em videira *in vitro*. As plantas de cultura *in vitro* podem utilizar como fonte de carbono tanto o CO_2 atmosférico quanto os açúcares presentes no meio de cultura. A dissimilação de carbono prevê que a metabolização de açúcares do meio resulta numa perda líquida de peso dos conteúdos dos frascos de cultura e pode, dessa forma, ser usado para acompanhar o crescimento das culturas. A variedade de porta-enxerto de videira 'Paulsen 1103' foi introduzida e multiplicada *in vitro* através da metodologia de gemas axilares. Tubos com e sem plantas foram pesados diariamente ao longo de um período experimental de 60 dias de crescimento. O acompanhamento das plantas *in vitro* demonstrou que a variedade estudada apresentou atividade de dissimilação e assimilação ao longo do período de cultura, apresentando, portanto, fotomixotrofia *in vitro*. Os resultados demonstraram três fases distintas de crescimento: uma fase inicial, durante os 10 primeiros dias de cultura, onde ocorreu intensa perda de peso por dissimilação; uma segunda fase estacionária, que durou aproximadamente 10 dias, e uma fase final de 40 dias, onde observou-se importante incremento de peso (37.68 mg). Dessa forma, foi possível avaliar de forma rápida e eficiente a utilização de carbono e o crescimento de plantas micropropagadas.

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