RESEARCH ARTICLE

Chlorophyll *a* fluorescence of sweet potato plants cultivated *in vitro* and during *ex vitro* acclimatization

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ABSTRACT

Sweet potato (*Ipomoea batatas* L.) plants were cultivated *in vitro* in Murashige and Skoog (MS) medium with 20 and 40 g L^{-1} of sucrose under two different photon flux densities (21 and 60 μmol m^{-2} s^{-1}). Leaves developed *in vitro* mostly showed high variable to maximum fluorescence ratio (F_V/F_M) , indicating good development of photosynthetic apparatus. This ratio decreased during leaf aging, especially in the plants cultivated *in vitro* on medium with higher sucrose concentration and higher photon flux. Leaves developed *ex vitro* showed high F_V/F_M ratio during whole experiment. The effective photochemical efficiency (F_V'/F_M') was maximum at 15th day after emergence of leaves. Photosynthetic potential rate was higher in leaves developed *in vitro* than in leaves originated *ex vitro*.

Key words: culture medium, photon flux density, photosystem, sucrose.

Abbreviations: BA – 6 - benzylaminopurine; F_V/F_M - maximum photochemical efficiency; F_V'/F_M' - effective photochemical efficiency; MS - Murashige and Skoog; PPF - photosynthetic photon flux.

INTRODUCTION

During *in vitro* culture, plantlets usually grow under nutritional and physical conditions which characterize a mixotrophic and/or heterotrophic growth, depending on external sugar source (Le et al., 2001). In general, leaves developed during *in vitro* culture show reduced capacity to synthesize organic compounds. Also, the photosynthetic apparatus of plants growing *in vitro* is not completely active (Fuentes et al., 2005). According Amâncio et al., (1999), variations of the photosynthetic photon flux (PPF) during *in vitro* culture should influence both leaf and chloroplast development. In addition, *in vitro* conditions should determine alterations of morphologic, anatomic and physiological characteristics of the plantlets, doing the *ex vitro* establishment of the plantlets a hardly mission (Semorádová et al., 2002). When plantlets cultivated *in vitro* are transferred to *ex vitro* conditions, the development of new leaves assume primordial importance to development and establishment of plantlets (Viña et al., 1999).

The chlorophyll *a* fluorescence is an efficient tool used in photosynthetic investigations as fast and non-destructive method suitable to indicate the photosynthetic response of plants to stress (Saquet and Streift, 2002; Barbagallo et al., 2003), providing an indication of photosynthetic performance of plants. Moreover, preliminary studies showed that the

sucrose concentration in the culture media and photon flux density, at which plants are subjected *in vitro*, alter the F_V/F_M leaves ratio (Cassana et al., 2008). This study aimed to evaluate the photosynthetic capacity of sweet potato plantlets cultivated in *vitro* under different sucrose concentrations and photosynthetic photon flux using the chlorophyll *a* fluorescence and oxygen evolution rate measurements.

MATERIAL AND METHODS

Nodal segments (1 cm length) from sweet potato (*Ipomoea batatas* L. cv. ILS19) meristem cultures were inoculated in MS (Murashige and Skoog, 1962) basal medium supplemented with 0.5 mg L^{-1} of 6 - benzylaminopurine (BA) and two sucrose concentrations (20 and 40 g L^{-1}). In order to compare the photosynthetic capacity of plantlets in *in vitro* and *ex vitro* conditions, measurements also were performed after the acclimatization of plantlets in greenhouse conditions. Therefore, after inoculation, the plantlets were placed in plant growth chamber for 45 days, with 16 h photoperiod, 25/23 ± 1 ºC air temperature (during light and dark period, respectively) and 21 and 60 μmol m^{-2} s^{-1} PPF provided by fluorescent lamps.

The maximum and effective photochemical efficiency (F_V/F_M and F_V'/F_M', respectively) were measured using a portable fluorometer FMS 2 (Hansatech, UK). The first measurement was made at last day of *in vitro* culture (day zero). Afterwards, the plantlets were transplanted and acclimatized in greenhouse under 23-28ºC air temperature and 80% relative humidity. In these (*in vitro*) leaves, the chlorophyll fluorescence determinations were repeated weekly until 29 d. On the other hand, for new leaves emerged during the acclimatization (*ex vitro*), which appeared after 45 days in greenhouse, the assay was repeated weekly the same determinations until the 29th day.

The photosynthetic potential rate (μmol O_2 m^{-2} s^{-1}) was estimated at day zero (*in vitro* leaves) and day one (*ex vitro* leaves) using the LeafLab 2 (Hansatech, UK). In this experiment, we had two growth conditions (combinations between sucrose and PPF) and leaves originated *in vitro* and *ex vitro*. Four replications per treatment were carried out. Each replication consisted of one vessel containing five plantlets and one plant, during *in vitro* and *ex vitro* culture, respectively.

All experiments were repeated three times and the statistical analysis was performed using the Statistical Analysis System for Microcomputers – SANEST (Zonta & Machado, 1987) and the means compared by Tukey test ($p < 0.05$).

RESULTS AND DISCUSSION

The maximum photochemical efficiency (F_V/F_M) values were similar in the different treatments in leaves originated *in vitro* at day zero (Table 1), however, the lowest value of F_V/F_M (0.773) was obtained by combination of 21 μmol m^{-2} s^{-1} PPF and 20 g L^{-1} sucrose. Reduced values of this ratio have been related to damages on photosynthetic apparatus (Guo et al., 2006). During the acclimatization, the leaves originated *in vitro* showed different behaviors pattern along the time, depending on pretreatments to which the plantlets were submitted during the *in vitro* culture (Table 1). Values for the F_V/F_M ratio reduced significantly in leaves of plantlets exposed to 60 μmol m^{-2} s^{-1} PPF and 40 g L^{-1} sucrose during 15 days after the transfer of plantlets to greenhouse and after 29 days of acclimatization, the F_V/F_M value reached 0.6.

The effective photochemical efficiency in light adapted state (F_V'/F_M') can be used to estimate the photosystem II (PSII) operational efficiency (Baker and Rosenqvist, 2004). In the present study, F_V'/F_M' values obtained at day zero ranged from 0.707 to 0.762, in the different pretreatments to which the plantlets were submitted, but such variation was not statistically significant (Table 1). However, the F_V'/F_M' ratio decreased with the acclimatization of plantlets, mainly in the leaves pretreated with 40 g L^{-1} sucrose, a decrease independent on the PPF. These results show that leaves of sweet potato plants cultured *in vitro*, although been persistent as well as been increased in size due mainly of cellular elongation (Fabbri et al., 1986), they could be classified such as competent species group, endowed with low photosynthetic capacity after acclimatization. This feature can be related to physiological stress caused by the environmental changes imposed to the plant during its transfer from *in vitro* to *ex vitro* conditions, especially in conditions of low sucrose concentration during *in vitro* culture.

Table 1. Maximum photochemical efficiency (F_V / F_M) and effective photochemical efficiency (F_V' / F_M') in sweet potato leaves originated in vitro. Different small letters indicate significant difference in the columns (means of sucrose; $p<0.05$). Different capital letters indicate significant differences in the rows (means of days; $p<0.05$). Different Greek letters indicate significant differences in the columns (means of PPF; $p<0.05$). Day zero (0) was considered when the leaf showed 10 mm of diameter and measured immediately before acclimatization.

	PPF (μmol m^{-2} s^{-1})	sucrose (g L^{-1})	days after acclimatization				
			0	8	15	22	29
F_V/F_M	21	20	0.773Aaβ	0.830Aa$\propto$	0.779Aa$\propto$	0.782Aa$\propto$	0.687Ba$\propto$
		40	0.822Aa$\propto$	0.786ABa$\propto$	0.771Aba$\propto$	0.746BCa$\propto$	0.681Ca$\propto$
	60	20	0.827Aa$\propto$	0.855Aa$\propto$	0.807ABa$\propto$	0.750Ba$\propto$	0.668Ca$\propto$
		40	0.823Aa$\propto$	0.799Ab$\propto$	0.666Bbβ	0.650Bbβ	0.603Bbβ
F_V'/F_M'		20	0.707 Aa	0.593 Aa	0.425 Ba	0.289 Ca	0.207 Ca
		40	0.762 Aa	0.458 Bb	0.160 Cb	0.169 C$_B$	0.148 C$_A$

The maximum photochemical efficiency (F_V/F_M) of *ex vitro* leaves just varied with time (Table 2). At first day of evaluation, when the leaves were still very young, the maximum photochemical efficiency ranged from 0.808 to 0.837 with the lowest value obtained in leaves previously cultivated in 60 μmol m^{-2} s^{-1} PPF and 40 g L^{-1} sucrose. However, during the growth and development of the leaves, it was observed an increased F_V/F_M ratio in all treatments, and after 29 days the F_V/F_M values were very high, varying from 0.829 to 0.852. According Lichtenthaler et al., (2005), such a high values characterize non-stressed leaves, indicating that sweet potato plants were highly adapted to the greenhouse environmental condition.

The F_V'/F_M' of new leaves originated under greenhouse conditions (Table 2) was low at first day of evaluation, but it reached a maximum value (0.817) at the 15th day regardless the treatments applied *in vitro* culture. After this period, the effective photochemical efficiency decreased, but it remained higher than that values observed at first day of evaluation. The F_V'/F_M' ration indicates the capacity of absorption of excitation energy by leaves, and it is usually lower in young leaves, increasing with age and decreasing thereafter as a consequence of leaf senescence.

Table 2. Maximum photochemical efficiency (F_V/F_M) and effective photochemical efficiency (F_V'/F_M') in sweet potato leaves originated *ex vitro* (after acclimatization in greenhouse). Different small letters indicate significant differences in the columns (means of sucrose; $p<0.05$). Different capital letters indicate significant differences in the rows (means of leaf age; $p<0.05$). Different Greek letters indicate significant differences in the columns (means of PPF; $p<0.05$). Day one (1) was considered when the leaf showed 10 mm of diameter.

	PPF (μmol m^{-2} s^{-1})	sucrose (g L^{-1})	Leaf age (days)				
			1	8	15	22	29
F_V/F_M	21	20	0.831	0.875	0.883	0.882	0.829
		40	0.834	0.872	0.885	0.875	0.844
	60	20	0.837	0.875	0.883	0.878	0.852
		40	0.808	0.871	0.881	0.883	0.846
		Mean	0.827 C	0.873 A	0.883 A	0.879 A	0.842 B
F_V'/F_M'	21	20	0.432 Ca$\propto$	0.741 ABa$\propto$	0.815 Aa$\propto$	0.643 Ba$\propto$	0.594 Baβ
		40	0.440 Ca$\propto$	0.723 ABa$\propto$	0.816 Aa$\propto$	0.614 Ba$\propto$	0.679 ABa$\propto$
	60	20	0.476 Ca$\propto$	0.641 Ba$\propto$	0.815 Aa$\propto$	0.584 BCa$\propto$	0.706 ABa$\propto$
		40	0.521 Ba$\propto$	0.606 Baβ	0.817 Aa$\propto$	0.611 Ba$\propto$	0.490 Bbβ

When the photosynthetic potential rate measured in leaves at the end of *in vitro* cultivation was compared with the rate found in leaves originated after acclimatization (*ex vitro*), it was observed that only the condition factor in which the leaves were generated was significant (Table 3). Leaves generated by tissue culture, regardless the treatment to which the plants were subjected, presented average photosynthetic potential rate significantly higher (29.60 μmol O_2 m^{-2} s^{-1}) than the one shown by *ex vitro* originated leaves (22.21 μmol O_2 m^{-2} s^{-1}). Le et al., (2001) studied the effects of exogenous sucrose (0 and 3%) on the oxygen evolution rate of tomato plants (*Lycopersicon esculetum* Mill.) cultured *in vitro* under low and high PPF (50 and 500 μmol m^{-2} s^{-1}) and verified that the exogenous sucrose had a positive effect on oxygen evolution process (from 11.22 to 15.75 μmol O_2 m^{-2} s^{-1}, after 20 days of *in vitro* cultivation with low PPF and low carbon dioxide concentration).

Table 3. Oxygen evolution rate (μmol O_2 m^{-2} s^{-1}) of sweet potato plant *in vitro* originated leaves immediately before being transferred to acclimatization and of leaves originated during acclimatization (*ex vitro*). Different capital letters indicate significant differences in the rows and compare condition in which the leaves were generated ($p < 0.05$).

Photon flux density (μmol m^{-2} s^{-1})	Sucrose (g L^{-1})	Photosynthetic potential rate (μmol O_2 m^{-2} s^{-1})	
		In vitro	*Ex vitro*
21	20	27.08	21.65
	40	31.03	21.26
60	20	30.46	22.18
	40	29.84	23.75
Mean		29.60 A	22.21 B

The data from the present study taken together with those of the literature cited make it possible to conclude that: (1) the leaves growth in *in vitro* and *ex vitro* conditions showed similar maximum photochemical efficiency and (2) the conditions of *in vitro* culture can influence the chlorophyll fluorescence parameters.

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