http://dx.doi.org/10.22161/ijeab/3.2.33 ISSN: 2456-1878

Effects of Photomixotrophic Conditions on Plants of *Eucalyptus Urograndis* Propagated in Temporary Immersion Bioreactors

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***Abstract*—** *Eucalyptus is one of the crop, which has been investigated with commercial purposes in the world. There are more than 500 species, being the Eucalyptus urograndis one of the most important, because of its intensive use in the production of wood pulp to make papers. The multiplication by means of temporary immersion bioreactors (TIB) is among the present techniques to obtain higher productions of the pulp to satisfy the demands of the market. The effects of the photomixotrophic crop were studies during the elongation of the propagation of shoots in the TIB in order to increase the quality of them. This process consisted of the use of 30 g L^{-1} combined with two concentration of CO_2 (350 and 1200 µmol mol) and two flows of photosynthetic photon flux (PPF= 80 and 250 µmol $m^{-2}s^{-1}$). The higher percentage of suitable plants were found with the treatment of high PPF (250 µmol m^{-2} s^{-1}) and 1 200 µmol mol of CO_2. The photosynthetic capacity of propagated shoots was of 64% of the adult plants. Also was observed that photomixotrophic conditions reduced the stressed environment that is imposed by the growing in vitro. The catabolic activity in the enzymes of the metabolism of carbon was also reduced, increasing the activity of the Sucrose Phosphate Synthase.*

Keywords*— *Eucalyptus, micropropagation, photomixotrophism, vitroplants.

I. INTRODUCTION

According to predictions, the world population will increase annually in more than 80 million of people and it is estimated that in 2050 it will be about 7 000 millions of inhabitants, because of this, it is believed that the use of wood for building and for the paper industry will increase constantly. Whereas the global demand of wood products increase, the wood resources available in the world are decreasing dizzily due to the lack of knowledge and the inappropriate use of them. It has permitted the destruction and the degradation of the forests in the tropics [1].

The *Eucalyptus* specie is one of the most investigated with commercial purposes in the world. This species is characterized by its easy management in tree nursery, its fast growth, its erectile shape and the properties of its wood, which is used for numerous applications as: the production of wood pulp for making papers, charcoal and also for construction. *Eucalyptus* is another specie of great commercial in forest because of its extensive use in the production wood pulp for making paper [2]

The vegetative propagation of some species of *Eucalyptus* by means of rooting presents some limitations as the change of the rooting ability among and the gradual decrease of the potentiality of rooting associated with the ontogenetic aging of trees. A method that is exploited in several clonal propagation programs. It has also been promoted the use of the micropropagation as a method to produce clones very fast [3, 4, 5].

The techniques of tissues culture haves been used for propagating the clones with superior characteristics. More than 50 *Eucalyptus* species haves been propagated using the techniques of tissue culture, most of them using semi-solid means [6]. Micropropagation of *Eucalyptus* clones is more expensive than the techniques of macropropagation using the shoot rooting. The operations that increase cost correspond to the manual subcultivation because of the repeated labor, which are carried outs to obtain high rate of multiplications and the rooting of plantlets. These limitations can be solved with automatization of some of the phases of the organogenesis and the somatic embryogenesis to reduce labor and the cost of production [7, 8]. Starting from the first semi-automatized system of temporal immersion (e.g. RITA) some investigations about the use of this techniques in the proliferation of some crops have been carried [9, 10, 11, 12].

http://dx.doi.org/10.22161/ijeab/3.2.33 *ISSN: 2456-1878*

The acclimatization *in vitro* is the term used when carrying out photomixotrofism and autotrofism works in plants cultivated under *in vitro* conditions [13]. These works haven't been studied in depth in the cultivation of *Eucalyptus urograndis* when temporal immersion bioreactors are utilized. That's why the objective of this investigation is to establish the photomixotrofic conditions which increase the quality of *Eucalyptus urograndis* explants propagated in TIB for the acclimatization of plants under *ex vitro* conditions.

II. MATERIAL AND METHODS

2.1 General procedures:

All the experiments carried out during this investigation were fulfilled in the specialized areas of plant cell and tissue culture laboratory in Bioplant Center of Ciego de Avila University.

In the research was used vitroplants clone of shoots that come from elite trees of *Eucalyptus urograndis (E. grandis Hill ex Maiden x E. urophylla)* which were brought from the Biotechnology Center of the Universidad Católica de Oriente, Medellín, Colombia. The donating plants, from which the explants were taken to be inserted *in vitro* conditions, were kept in greenhouses under 80% relative humidity (RH), 25.5 °C temperature and photosynthetic photon flux (PPF) of 350 µmol $m^{-2}s^{-1}$ as average under natural photoperiods during eight months. The plants were planting with a combinations (1:1, v/v) of zeolite + filter cake (derived from sugarcane bagasse) as substrate. The top of these plants were pruned in several moments. They were applied foliar fertilizer (Bayfolan Forte® produced by Bayer CropScience) each week at a rate of 2.0 ml L^{-1} in order that each plant takes 100 ml of final solution.

2.2 Establishment and micropropagation of *E. urograndis in vitro*:

The methodology proposed by Grattapaglia *et al*. [14] was the one used but with some modifications. The shoots obtained after the pruning were utilized as source of explants for the establishment phase *in vitro*. The sections taken as explants were segments of stalk from one to two centimeters of length, from 1.5 to 3.0 mm of diameter and with one or two preformed shoots after being washed with commercial detergent and rinsed several times with much fresh water, under asepsis conditions they were submerged in bichloride of mercury on 0.05% during ten minutes.

After this; the shoots were washed three times with purified sterile water. The explants were planted in test tubes in liquid medium with zeolite previously sterilized as support.

As basic means; mineral salts and MS vitamins [15], 3.0% of sucrose and 0.1 mg L^{-1} of BA were utilized. pH solution was adjusted to 5.8 with 1 N NaOH and after this it was sterilized during 20 minutes, under 121 °C and a pressure of 1.2 kg cm^2. The test tubes were placed under a photoperiod of 16 hour-light with photosynthetic photon fluxes (PPF) about µmol $m^{-2}s^{-1}$, measured by means of a sensor of photon fluxes installed in a universal cuvette matched to a portable system of measuring photosynthesis (CIRAS-2) of PP system.

After 30 days, the shoots were subcultivated and multiplied in 250 ml flask in a semisolid MS medium enriched with BA (0.5 mg L^{-1}), L-glutamine (500 mg L^{-1}), MS vitamins, saccharose (3.0%) and solidified with agar (6.5 g L^{-1}) in all the cases; a volume of 25 ml was used. Five subcultivation were carried out, each one with a frequency of three weeks.

2.3 Establishment of the methodology for the propagation in the temporary immersion bioreactors (TIB):

A design of a temporal immersion bioreactor, modified by the Bioplant Center of Ciego de Avila University, Cuba [9], was used. As recipients flasks of Nalgene Company with a capacity of one liter, which were interconnected with couples using silicone hoses, in a flask the medium of liquid cultivation was placed and in the other the *Eucalyptus* explants, additionally each one was connected to an air entrance system coming from a compressor, which was turned on by an automatic programmer to control the frequency and the length of immersion, the light and the gas fluxes. All the entrance or outgoing of air fluxes was sterilized by hydrophobic filters of 0.2 µm, in such a way that each recipient was handled independently without risk of contamination.

The number of explants (13), the time (3 minutes) and the frequency of immersion (every 12 hours) were used during the experiments. The average volume corresponded to 750 ml and 13 explants, each with five shoots, were contaminated. The environment for cultivation consisted in the mineral salt of MS from which the nitrate of ammonium was reduced to 1 237.5 mg L^{-1} also the organic (ingredients) which included: thiamine-HCI (0.4 mg L^{-1}), mio-inositol (100 mg L^{-1}), pyridoxine HCI (0.5 mg L^{-1}), nicotinic acid (0.5 mg L^{-1}), glycine (2.0 mg L^{-1}), L- glutamine (500 mg L^{-1}), saccharose (30 g L^{-1}), polyvinyl polypirrolidona (PVPP) in concentration of 250 mg L^{-1} was used as a preventive agent of oxidation, and it was also supplemented with BA (0.5 mg L^{-1}). The medium was sterilized by autoclave (40 minutes, 121 °C), previously the pH was adjusted to 5.8 with Na OH. The cultivations were incubated to 24±1 °C with a photoperiod of 16 hours with artificial light helped supported by fluorescent lamps (PHILIPS TL 40 W/54) which irradiated an intensity of 80 µmol m^{-2} s^{-1}.

http://dx.doi.org/10.22161/ijeab/3.2.33 *ISSN: 2456-1878*

2.4 Determination of photomixotrophic effects in elongation phase of *E. Urograndis* shoots propagated in temporary immersion bioreactors:

The experiment consisted of a bifactorial under a design completely random with three repetitions for treatments, where the factors corresponded to different levels of light intensity and of CO_2 in the phase of elongation in TIB. The photomixotrophic conditions of *E. Urograndis* are shown on table 1.

Table.1: Photomixotrophic conditions in elongation phase of Eucalyptus Urograndis shoots propagated in temporary immersion bioreactors (TIB).

CO_2 Concentration (µmol mol CO_2)	**Light intensity** (µmol m^{-2} s^{-1})
350	80 250
1200	80 250

A photoperiod of 16 hours light was used during the development of this experiment, the photosynthetic photons fluxes (µmol $m^{-2}s^{-1}$) for the photosynthetic activity were measured with a sensor to measure photosynthesis (CIRAS-2), a portable PP system.

2.5 Photosynthetic, stomatic conductance and transpiration activity of *E. urograndis* plants during photomixotrophic conditions in TIB:

Net photosynthesis (A, µmol (CO_2) $m^{-2}s^{-1}$), stomatic conductance (mmol (H_2O) $m^{-2}s^{-1}$) and transpiration (µmol (H_2O) $m^{-2}s^{-1}$) was measured at the ambient relative humidity and air temperature. The analyzer and cuvette were automatically calibrated before every measurement. Light was fixed at 600 µmol $m^{-2}s^{-1}$ for the determination of the A/Ci curve, while the CO_2 was fixed at 600 µmol mol^{-1}, these values were obtained from saturation curve of light and CO_2 in *Eucalyptus* plants.

Measurement were always made on the youngest fully expanded leaves and between 9:00 am and 10:00 am. Five plants were measured with 10 repetitions each one for a total of 50 values.

2.6 Measurements of enzyme activity in *E. urogran*dis plants during photomixotrophic conditions in TIB:

Enzyme extraction and assays. Leaf samples were taken before 10:00 am and immediately frozen in liquid N_2 and stored at -80 °C until use. A 250 mg frozen tissue was ground to a very fine powder with a mortar and pestle in liquid N_2. The sucrose phosphate synthase (SPS) enzyme (EC 2.4.1.14) activity was determined to pH 7.5 with 50 µL from the unsalted extract. The mixing was incubated for 20 minutes in 30 ^{0}C. The reaction stopped with the addition of 70 µL (5.35 mol L^{-1}) of KOH. For the white the reaction stopped with the addition of 70 µL (5.35 mol L^{-1}) of KOH in 0 minutes [16]. The formation of sucrose was determined by means of the antrone method [17].

The activity of invertase (EC 3.2.1.26) was determined in a mixing of reaction (reaction mixing) of 500 µL from the total volume that contains enzymes free of salt and 50 mmol L^{-1} of saccharose, a tampon of acetate of sodium to pH 4.5 was used. The reaction started with the addition of enzyme, previously incubated during 20 minutes to 30 ^{0}C. The formation of hexosa was determined enzymatically [18].

For the case of the phosphoenolpyruvate carboxylase enzymes (PEPC; EC 4.1.1.31) and pyruvate kinase (PK; EC 2.7.1.40) were extracted in 1 ml of tampon 50 mM Hepes-KOH containing 12 mmol L^{-1} Mg Cl_2, 1 mmol L^{-1} EGTA, 1 mmol L^{-1} EDTA, 1 mmol L^{-1} DTT, 10% glycerol, 2 mmol L^{-1} benzamidine, 2 mmol L^{-1} amino-n-caproic acid to pH 7.4 [19]. The catalysis of reaction was joined to the reacting of dehydrogenated malate to 25 ^{0}C for the decreasing of NADH used to 340nm in a spectrophotometer Pharmacia. The PQ activity was joined to the dehydrogenated lactate to 25 ^{0}C by monitoring the utilization of NADH to 340 nm.

2.6 Statistical analysis:

At each sampling date, six plantlets were randomly selected to measure photosynthesis and transpiration. For enzymatic determination, randomized plantlets were used. Three extraction in each evaluation moments and three repetitions for each extraction were performed. Twenty plantlets were used for morphological measuring. Analysis of variance was conducted using SPSS Program. Duncan's multiple range tests were used for mean separation at the $p<0.05$ level.

III. RESULTS AND DISCUSSION

3.1 Effects of photomixotrophic conditions on growing variables of *E. urograndis* plants during photomixotrophic conditions in TIB.

Light intensity showed the best results with the treatment in which the plants were submitted to a greater light intensity (250 µmol m^{-2} s^{-1}), this shows statistical differences in all variables evaluated, with the exception in the dry mass. In the leaf number the plants developed five news leaves, which is in correspondence with the fresh dough found. Meanwhile CO_2 brought about significant differences just on the fresh mass variable,

http://dx.doi.org/10.22161/ijeab/3.2.33 *ISSN: 2456-1878*

where the higher values were got in plants submitted to treatments with high concentration of CO_2 (Table 2).

Table.2: Effects of photomixotrophic conditions on growing variables of E. urograndis plants during photomixotrophic conditions in TIB.

Factors		Length (cm)	Leaf number	Mass (g)	
				fresh	Dry
I - Light	80	2.14 b	11.17 b	0.13 b	0.0189
(µmol m^{-2} s^{-1})	250	2.86 a	17.02 a	0.21 a	0.0184
	Sign.	*	*	*	ns
	SE	0.13	1.22	0.023	0.0018
II - CO_2	350	2.42	13.56	0.12 b	0.0186
(µmol mol)	1 200	2.58	15.14	0.22 a	0.0188
	Sign.	ns	ns	*	ns
	SE	0.13	1.22	0.023	0.0018
Interacction	80 + 350	2.34 bc	11.24 b	0.14 b	0.0197
I - II	80 + 1 200	1.93 c	12.16 b	0.13 b	0.0182
	250 + 350	2.49 b	19.04 a	0.11 b	0.0174
	250 + 1 200	3.23 a	14.96 ab	0.32 a	0.0194
	Sign.	*	*	*	ns
	SE	0.18	1.72	0.033	0.0025

Measures with different letters differ statistically (one-way ANOVA, Tuckey, $p<0.05$). Each data represents the mean for n=30.

Effects of the interactions of the two factors showed that plants which were submitted to a higher light intensity and enriched environment with CO_2, highest values are obtained in the studied variables of plant quality, with the exception the dry mass and the number of leafs, which shows significant differences among the other interactions. We should point out that treatments submitted to the same conditions were the ones which obtained the greater percentage of optimal size plants, motivated too for the BIT ability to aerate plant tissue and provide contact between entire explants and the liquid medium.

The shoot length, the number of new nodal segments for explant and the multiplication coefficient were significantly higher in *Eucalyptus tereticornis* under photomixotrophic conditions than in photoautotrophic condition [20]. High significant differences too were found in fresh mass and net photosynthetic rate in *E. camaldulensis* plants when they were submitted to forced ventilation [21].

Growth of plantlets *in vitro* is often greater under photomixotrophic conditions than under heterotrophic conditions, provided that the *in vitro* environment is properly controlled for promoting photosynthesis. In this case and in some others, it is speculated about the metabolic routes favored under the conditions established for cultivation, this information is too important for better interpretation of the results.

Some variables related to the photosynthetic activity, measures during the photomixotrophic treatments when finishing the phase of elongation of the *E. Urograndis* shoots propagated in temporary immersion bioreactors should be evaluated in these experiments.

3.2 Stomatic conductance, transpiration and photosynthetic activity of *E. urograndis* plants during photomixotrophic conditions in TIB:

E. urograndis plants showed a high stomatal conductance measured in terms of water flow. As the stomatal conductance is equivalent to the permeability and inverse to the resistance of water fluxes, it is inferred that transpiration in these leafs had to be proportional, however this reasoning is not always materialized (Table 3).

Table.3: Values of transpiration, stomatal conductance and net photosynthesis in E. urograndis shoots propagated under photomixotrophic conditions in TIB.

Factors	Transpiration (mmol (H_2O) $m^{-2}s^{-1}$)	Stomatic Conductance (mmol (H_2O) $m^{-2}s^{-1}$)	Photosynthesis (µmol (CO_2) $m^{-2}s^{-1}$)

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I - Light	80	6.34	4067.32 a	4.34 a
(µmol $m^{-2}s^{-1}$)	250	6.37	2633.33 b	0.00 b
	Sign.	ns	*	*
	SE	0.043	115,40	0.20
II - CO_2	350	6.78 a	4261.63 a	3.26 a
(µmol mol)	1 200	5.90 b	2439.02 b	1.12 b
	Sign.	*	*	*
	SE	0.043	115,40	0.20
Interaction	80+350	6.51 b	4960.63 a	6.52 a
I - II	80+1 200	6.17 c	3174.00 b	2.25 b
	250+350	7.05 a	3562.63 b	0.00 c
	250+1 200	5.63 d	1704.03 c	0.00 c
	Sign.	*	*	*
	SE	0.06	163,20	0.28

Means with different letters differ statistically (one–way ANOVA, Tuckey, $p<0.05$). Each data represents the means for n=30.

When analyzing the effect of light, the first variation of this postulate is found. The plants submitted to a higher PPF reduce significantly the stomatal conductance but it doesn't modify the transpiration. As it is known a general behavior of replying of the stoma to the increasing of light intensity, turns out in a greater number of open stomas and in the increasing of transpiration.

In the case of concentration of CO_2, the behavior of the *E. Urograndis* shoots cultivated in TIB was similar to the most general tendency that plants flow. When air was enriched with CO_2, plants reduced their stomatal conductance associated to a reduction in the quantity of open stomas, and the transpiration decreased significantly. It is known that the effects of CO_2 are very powerful over the opening of stomas, the increase of its concentration in occlusive cell cause a partial closure of stomas and it can inhibit the assimilation of CO_2, just partially compensated when the photosynthetic activity increases.

In the integration of the effects of both factors, the concentration of factors, the concentration of CO_2 in the environment where the shoots were cultivated, had greater influences when comparing each level of light with the two levels of CO_2 the stomatal conductance was reduced and the transpiration too. However in the comparison of each concentration of CO_2 with the two levels of PPF evaluated, it is reiterated that the increase of light reduced the stomatal conductance, but transpiration of plants cultivated with 350 µmol (CO_2) mol of air increased.

The regulation of the opening of stomas is a complex process which does not depend only on the factors previously analyzed. Others as temperature, relative humidity, cytosolic concentration of calcium, hormones, and enzymes which indicates the related metabolic routes, they also exert important influences and development. Some of these measurements should be considered in future investigations.

The effects of CO_2 over stomatal opening is adjusted in function of the demand of photosynthesis in plants. The measurement of this relative indicator demonstrated that in low levels of CO_2 and light the greater quantity of fixed CO_2 are registered.

The addition of sugar in the environment of cultivation showed a negative effect in the growing and photosynthesis in plants *in vitro* [22]. Besides the saccharose provoked the stimulations of the growth and the photosynthesis activity of tobacco plants *in vitro* [23] also in beet plants [24] and in potato plants [25]. These results indicate that the photosynthesis capacity depend mainly on the species and the environmental conditions and of cultivation that plants are submitted.

The integral analysis of the results showed on table 4 are not enough to justify the effects of treatments about the quality of plants cultivated in TIB, because the behavior of the physiological indicators is insufficient when they depend on some other factors, among them the metabolic routes.

3.23 Activity of enzymes of the carbon metabolism in the *E. urograndis* shoots propagated under photomixotrophic conditions in TIB.

In order to specify the results of the treatments under photomixotrophic conditions, in table 4 is shown the activity of important enzymes involved in the metabolism of carbon, measured in the *E. urograndis* shoots once the cultivation *in vitro* phase is finished in the TIB.

The enzymes of the catabolic routes of the metabolism of carbon were much more active than the metabolic means.

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The activity of the acid invertase and pyruvate kinase surpass several times the saccharose phosphate synthase and the phosphoenolpiruvate carboxylase (table 4).

Table.4: Activity of enzymes of the carbon metabolism, sucrose phosphate synthase (SPS) acid invertase (AI), pyruvate kinase (PK) and phosphoenolpyruvate carboxylase (PEPC) in E. urograndis shoots propagated under photomixotrophic conditions in TIB.

Factors		SPS (µmol g^{-1} FM h^{-1})	AI (µmol g^{-1} FM h^{-1})	PK (µmol g^{-1} FM h^{-1})	PEPC (µmol g^{-1} FM h^{-1})
I - Light	80	7.04	24.34 a	51.16 b	-
(µmol m^{-2} s^{-1})	250	5.95	15.54 b	223.48 a	-
	Sign.	ns	*	*	
	SE	0.77	0.84	24.38	
II - CO_2	350	5.72	10.43 b	112.90	-
(µmol mol)	1 200	7.27	29.46 a	161.74	-
	Sign.	ns	*	ns	
	SE	0.77	0.84	24.38	
Interaction	80+350	8.22 a	0.17 d	31.63 c	10.18
I - II	80+1 200	5.86 ab	48.52 a	70.68 bc	0.00
	250+350	3.21 b	20.69 b	194.16 ab	0.00
	250+1 200	8.68 a	10.40 c	252.81 a	0.00
	Sign.	*	*	*	
	SE	1.09	1.18	34.48	

Means with different letters are statistical different (one-way ANOVA, test TUCKEY, $p<0.05$). Each data represents the means for n=9.

The PK registered the greatest activity in all the treatments with significant differences marked by the light factor. As it was previously analyzed (table 3) the increasing of light reduced the stomatal conductance, apparently the resistance of the flow of water increased and although it could be one thousand superiors to the entrance of CO_2, the closing of stomas should have reduced the capture of it. Nevertheless, the entrance of oxygen, necessary for the oxidized process, specifically the respiratory ones. The relevant activity of PK is an evidence that these were the favored process.

Anyhow the activity of AI is also big, it is supposed that they degraded the saccharose from the environment of cultivation (in all cases to 30 g L^{-1}) and the resulting hexoses were degraded in the glycolytic means which marks the catalytic action of PK. Probably the high concentrations of saccharose limited the photosynthetic fixation of CO_2 as it has been shown by [26, 27], because they were sufficient to guarantee the metabolic demands which improved the quality of plants propagated under a greater flux of photosynthetic photons (PPF).

Comparing the enzyme behavior of plants propagated in the treatments with greater intensity of light, which were the ones that obtained better plants, differences between IA and SFS were observed. The treatment with best results in the production of competent plants, those which were elongated with greater levels of light and CO_2 in the air, registered lower activity of AI and higher activity of SPS. This shows that absorbed fewer saccharose from the means of cultivation and that synthesized more, favored by higher concentration of CO_2 in the atmosphere.

Activities of SPS and AI were increased much more in tomato plants cultivated in 3% of saccharose than without saccharose when they were submitted under PPF and lower concentration of CO_2, while it was observed a different behavior in the activity of SPS, when its increase was higher in conditions of high PPF and high concentration of CO_2 and in an environmental mean of cultivation without saccharose [28].

With this analysis, the interpretation of the best results in quality of plants, which were obtained under conditions of photomixotrophic cultivation, is completed. To the intense metabolic activity measured in those shoots (table 4) it is added the best management of the hydraulic related to transpiration (table 3). In *E. urograndis* plants propagated with high PPF and concentration of CO_2, these factors provoke the least stomatal conductance, the lowest loss of water and they obtained the highest values of length and fresh mass. The ones which are elongated with high PPF but with the lowest concentration of CO_2, duplicate the stomatal conductance and they registered the

highest loss of water, which showed the significant reduction of length and fresh mass.

In both treatments with higher light intensity, the null values of PEPC are corresponded with the increased activity of PK, because the two enzymes compete for the phosfoenolpiruvate and the data show that the breathing consumption prevailed because of the PK. Just with the control treatment, with the lowest levels of PPF and with CO_2, PEPC activity was detected in the *E. urograndis* shoots, the lowest activities of PK were also measured in them.

Finally, the increase of concentration of CO_2 with the lowest PPF resulted the worst combination to stimulate the elongation of the *E. urograndis* shoots in the TIB, because the percentage of plants non competent was increased to more than 20%. They were distinguished for the higher activity of the acid invertase in the catabolic process already analyzed, which together to the action of PQ, resulted determinant over the anabolic activity represented by SFS.

The interpretation of these results is complex, as it is the action of each enzyme evaluated. They go from the optimal levels enzymatic complexes to other particularities as the regulation of SPS by the CO_2 and the temperature [29] mediating other functions as the Rubisco activity and the nitrogenous metabolism, as the important relation between PEPC and the malic enzymes, the presence of isoforms in both, the regulation by light and temperature [30] as the integrated behavior for Rubisco, SPS, SS, AIC, PEPC associated to a fixation of CO_2, hormones and photoperiod [31]

Although some of the factor previously quoted were not included in this study, the results show the effects of the growth regulators in the multiplication and the photomixotrophic treatments in the elongation of *E. urograndis* shoots in TIB. The combination of high levels of PPF and of CO_2 in the air favored the quality of the propagated plants, in biochemical level approximations were obtained to reasons that justify these answers, based on the metabolism of carbon.

Nevertheless the treatments applied also influenced in the concentration of phytohormons and in its regulation [32] which are integrated to the system of signals that should have existed in this species in reply to the action of the regulators of growing, concentration of CO_2 in the air and levels of PPF, which include changes in the metabolism [33].

IV. CONCLUSION

In conclusion, the results of the present study indicated that during the photomixotrophic cultivation the light improved the quality of *E. urograndis* shoots, and that the saccharose limited the photosynthetic assimilation of CO_2 while the high PPF and concentration of CO_2 provoked less stomatal conductance, lower-loss of water and higher values of length and fresh mass. Nevertheless the catabolic activity was intense, but the combination of high levels of light and CO_2 reduced the activity of AI and increased the activity of SPS.

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