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EFFECT OF LEVEL OF PFD ON PHOTOSYNTHETIC PARAMETERS AND PRODUCTION OF STEVIOL GLYCOSIDES BY HYDROPONIC CULTURE FROM STEVIA REBAUDIANA BERTONI

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ABSTRACT

Stevia rebaudiana produces steviol glycosides (GSt) 300 times sweeter higher than sugar cane. The problem is that the global demand is increasing and the agricultural production is insufficient. Is reported that Hydroponic cultivation and high irradiance increase the yield of *S. rebaudiana* and the production of steviol glycosides. This is due to the management and control of nutrition and the effect of light on the photosynthetic system, respectively. Therefore, the objective was to evaluate the effect on three levels of PFD (440.8, 383.9 and 273.2 μmol $m^{-2}s$) efficiency of photosystem PSII, and production of glycosides of steviol in *S. rebaudiana* var. Morita II at 40 and 90 days, using a NFT hydroponic culture (Nutrient Film Technique). The highest photosynthetic rate was recorded at 440.8 μmol $m^{-2}s$ with 15.4 and 17.72 ppm CO_2 min^{-1} g at 40 and 90 of culture days respectively and the higher content of steviol glycosides, with this same PFD, the lowest fluorescence emission occurred in PSII, while with low luminosity, the absorbed energy was harnessed more efficiently by the $PSII_{RC}$. The photosynthesis and production of steviol glycosides in *S. rebaudiana* were affected by level of PFD and culture time in a hydroponic system.

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Keywords: NFT hydroponic system, *Stevia rebaudiana*, Irradiance, OJIP test, Chlorophyll (Chl) a fluorescence, CO2 assimilation.

1. INTRODUCCTION

The South American Species *S. rebaudiana* (Bertoni) is the only one of genus that has economic importance, because low-calorie sweeteners are extracted from its leaves.The steviol glycosides: stevioside, rebaudioside (A to F), steviolbioside, and isosteviol, are responsible for the plant's sweet taste (Momtazi-Borojeni *et al*., 2017). Notably, stevioside, rebaudioside A and rebaudioside C are the major metabolites and these compounds are on average 250-300 times sweeter than sucrose, because of that, have commercial value all over the world as a sugar substitute in foods, beverages and medicines (Lemus-Mondaca *et al*., 2012; Abbas *et al*., 2017).

The global demand for both Stevia leaves and pure steviol glycosides is constantly increasing and is expected to be higher in the future, as metabolic disorders such as type II diabetes and obesity prevail every day. However, there are certain problems to cover the growing global demand, since the agricultural production methods for this crop are still inefficient and insufficient. (Tavarini *et al*., 2018).

Among the investigations reported to increase the yields of leaf and steviol glycosides, stand out the nutrition and photosynthetic activity. The effect of culture systems and nutrition on the performance of *Stevia rebaudiana* has been evaluated; it was shown that the biomass yield is higher when the cultivation is done in floating hydroponics compared to that grown in soil, both with different nitrogen levels (Bolonhezi *et al.*, 2010). It is worth mentioning that among the most outstanding advantages of hydroponics is the management of nutrition and the constant availability of nutrients, enabling optimal development of the plant, consequently high yields of biomass and metabolites of interest, as well as crops free of heavy metals. and pathogenic organisms. So far, the hydroponic cultivation of *S. rebaudiana* by Nutrient Film Technique (NFT), which is one of the most efficient, has not been reported. On the other hand, it is known that the light energy absorbed by the leaves is used mainly for photosynthesis and that when the photosynthetic system becomes saturated, the excess energy is dissipated in the form of heat and fluorescence. The increase or decrease of any of these affects the remaining two, therefore, the measurement of fluorescence is used to know the photochemical efficiency of the photosystem II (PSII). A study carried out on *S. rebaudiana* var. Morita II showed that the net assimilation rate and the harvest index were more efficient under BIOESPACE conditions with low light intensities, than in those grown under direct sunlight with high light intensities (Jarma-Orozco *et al*., 2020). Measurements of the chlorophyll fluorescence is used to examine the photochemical efficiency of plants a wide range of environmental conditions. The quantum yield of non-cyclic electron transport is directly proportional to the efficiency of excitation from the reaction centers

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of Photosystem II (PSII), can be determined variable fluorescence and maximum fluorescence (Fv/Fm) (Rodriguez *et al*., 2014). This parameter, has been utilized to measure the photosynthetic efficiency en*S. Rebaudiana* Bertoni bajo tratamientos de potasio y fotoperíodo (Kakhki *et al*., 2019). Based on the fact that in independent studies it has been shown that floating hydroponic cultivation and light intensity increase the yield of *S. rebaudiana* Bertoni and the production of steviol glycosides, it was assumed that the combination of both could have a synergistic effect. Therefore, the objective was to evaluate the effect on three levels of PFD (440.8, 383.9 and 273.2 μmol $m^{-2}s$) efficiency of photosystem PSII, and production of glycosides of steviol in *S. rebaudiana* var. Morita II at 40 and 90 days, using a NFT hydroponic culture (Nutrient Film Technique).

2. MATERIALS AND METHODS

2.1. STUDY AREA

The study was carried out at the Ecological Hydroponic Laboratory of the Center for the Development of Biotic Products of the National Polytechnic Institute, located in the San Isidro neighborhood, in the municipality of Yautepec, Morelos, Mexico. The geographic coordinates of the experimental area are 36°54'15" N and 30°38'30" E [30]. The climate is warm sub-humid with rains in summer.

2.2. BIOLOGICAL MATERIAL

The 20-day-old seedlings were transplanted on hydroponic baskets 4.0 cm in diameter higher, 3.5 cm de diameter lower and 5.0 cm high, which contained a mixture of the substrates: perlite, vermiculite and peat-moss in a 1:1:1 ratio. A 2cm wide by 15cm long strip of magitel cloth was used as a conductor of the nutrient solution. Subsequently, the baskets containing the seedlings were placed in a system of NFT (Nutrient Film Technique) hydroponic culture that was built with two PVC hydraulic tubes of 7.62 cm in diameter and 199 cm long, with a distance between within-row spacing was 30 cm. In each tube, 10 holes (3.8 cm in diameter) were made in which the baskets with the seedlings were placed (20 seedlings in total). The separation between each of them was 16 cm.

The seedlings, once placed in the hydroponic module, were irrigated with 50.0% Steiner nutrient solution (Steiner, 1961). The recirculation of it was carried out through a plastic container with a capacity of 60 L and a pump with a power of 20 W reaching a head of up to 1.95 m and a flow of 1000 L h^{-1}. The pH was maintained in an interval of 5.5 to 6.5 and with an electrical conductivity of 1.5 dS m^{-1}. Stevia hydroponic cultivation was developed under an average temperature of 33.0 °C and a relative humidity of 60%.

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2.3. LIGHT TREATMENTS

Inside the greenhouse, three zones of 4 m^2 each were implemented. In the first, two meshes were superimposed: 65 and 80.0% shade, in the second a mesh of 80.0% shade was placed, and the third zone was left without a shade mesh. Luminosity was measured as photon flux density (PFD) (µmol/m^2s) with a Sper Scientific 840022 luxmeter and a pyranometer (Pyranometer App). The PFD values were taken every third day at 10:00 am, 12:00 pm, and 2:00 pm, during the 90 days of treatment, with a total of 135 readings. The average values obtained for each zone are presented in Table 1. At each level of PFD was placed a module for NFT culture with 20 cavities in which they were placed at random 20 seedlings.

Table1: Light treatments expressed as Photon Flux Density (PFD)

Zone	Treatments light	Photon flux densityPFD (µmol / m^2s)
1	1. High	440.8 ± 161.2
2	2. Medium	383.9 ± 175.3
3	3. Low	273.2 ± 101.4

2.4. BIOMASS YIELD

Biomass yield was determined in 10 randomly selected *S. rebaudiana* plants in units of grams per dry weight (g/dw) during the 40 and 90 days of culture at each level of PFD. An average of the ten values was obtained and means were compared with Tukey's test. The weight of only the leaves contained from the fourth node to the tip of the stem was measured. Due to this, the plants were pruned at the fourth node and once the leaves were separated from the stem, they were placed in plastic trays which were placed in a dark drying chamber at 25 °C, 60.0% relative humidity (HR). And air recirculation for 15 x 45 min, for 96 h until the samples reached a constant weight. After pruning, the plants were maintained until reaching 90 days of treatment to obtain biomass yield using the same procedure.

2.5. PHOTOSYNTHETIC EFFICIENCY

Photosynthetic efficiency was determined at 40 and 90 days of treatment through stomatal conductance, CO2 assimilation rate, and energy use through fluorescence emitted by chlorophyll a.

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2.6. CO_2 ASSIMILATION

The CO_2 assimilation rate (ppm) was measured in a closed system, with an EGM-4 PP System infrared CO_2 gas analyzer (IRGA). For this, a transparent glass jar with an airtight lid was adapted; in which two 5 mm holes were made, to introduce two houses that were connected to the equipment. The measurement was made from 12:00 to 13:00 h in two randomly selected plants per treatment, with a height of 15 to 18 cm (according to the size of the bottle). For each determination, the entire plant was introduced into the flask, which was hermetically sealed. CO_2 assimilation was measured for 10 minutes, using the pp systems transfer version 1.0 software. With the data obtained, kinetics of CO_2 assimilation was elaborated.

2.7. CHLOROPHYLL A FLUORESCENCE AND QUANTUM EFFICIENCY

The fluorescence emitted by chlorophyll a and the quantum efficiency were evaluated from 12:00 to 13:00 h with a Fluor Pen fluorometer (FP 100), Photon Systems Instruments, with detachable leaf tweezers. The tweezer was placed on a fully mature leaf at the fifth node from the apex. The tweezer was closed to keep the leaf in complete darkness for 5 min, at the end of the time the equipment was placed and fixed in the tweezer, the reading was taken and it was opened. Data were transferred to Fluor Pen version 1.0 software and exported to Microsoft Excel 2010 software. Recording was performed on five randomly selected plants for each treatment.

2.8. QUANTIFICATION OF STEVIOL GLYCOSIDES

Steviol glycosides (stevioside and rebaudioside A) were counted in milligrams per dry weight (mg/dw) of leaf. The methodology of Aranda-González, (2015) was applied, for which a High-Resolution Liquid Chromatograph (HPLC) (PerkinElmer) was used with a C18 column (Thermo Scientific, 250 µm x 4.6 mm, 5 µm) and a UV detector at 210nm. The mobile phase was acetonitrile-water (35:65; v/v), an isocratic method was used. *S. rebaudiana* extracts were obtained from a mixture of dry leaves from 10 plants per treatment, pulverized in a mortar and mixed with hot water (80 °C) in a 1:5 ratio. The mixture was left in an ultrasound bath (Parmer Model CV334) for 30 min and then for a rest time of 48 h, to subsequently filter with a 0.45 µm SPE membrane. 20 µL were taken to inject into the HPLC; Similarly, an aliquot (20 µL) of the standards (stevioside and rebaudioside A, Sigma Aldrich) was injected separately, previously dissolved in 200 µL of water and filtered on a 0.45 µm SPE membrane to obtain the calibration curve in a concentration of 1 mg mL^{-1} in a range of 0.5 to 10 µg mL^{-1}. The identification of the metabolites was made according to the retention time of the calibration curve of the standards. The quantification was done in triplicate by means of the area under the curve that each reference standard presented.

2.9. STATISTIC ANALYSIS

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The data of the variables were analyzed with the Minitab 18 program, through a statistical analysis of variance of one factor (ANOVA) for each variable, applying the Tukey test ($P \leq 0.05$) in order to obtain significant differences.

3. RESULTS AND DISCUSSION

3.1. BIOMASS YIELDING (DW, G)

Results showed that the level of ligth as well as hydroponic culture time provide significant effect on the plant biomass. Higher light intensity had significantly higher dry leaf yields than the lower PFD at 40 and 90 days of culture. Biomass yielding was higher at 90 days of culture than 40 days and there were no significant differences among levels of luminosityin dry leaf yield at 40 days. At 90 days of culture significant differences were found for the highest and the lowest light intensities (4.00 ±1.58 and 1.42±1.35, respectively) (Table 2). Production of plant biomass is related to the ability of photosynthesis and leaf area; lower intensity conditions will cause a reduction in growth and yield, this is due to decreasing photosynthesis (Rachmawati and Asiyah, 2017). Jarma *et al.*, (2010) showed the accumulation of biomass depends on the photosynthetically active radiation that receive the plant. *Stevia* variety *Morita* I and II responded positively by increasing the light intensity in biomass production (Jarma *et al.*, 2010 and Yoneda *et al.* 2017). In 2020, Jarma *et al.*, reported that photosynthesis can reach a point of light saturation close to 1200 µmol photosynthetically active radiation (PAR) m^{-2} s^{-1}. When comparing the development of plants grown with BIOESP technology with that obtained from plants grown in direct light (DR) the harvest index (HI) showed greater efficiency in plants grown under BIOESP compared to those grown in the DR (HI: 0.62 vs. 0.54 % respectively). The ability to accumulate biomass in dry matter in the main harvested organs determines the crop yield (Barrientos *et al.*, 2015).

Table 2: Effect of PFD (µmol $m^{-2}s$) on biomass yielding (dw, g) *in S. rebaudiana var. Morita II* grown 40 and 90 days of treatments under NFT hydroponic culture.

Time (days)	Growth variables	PFD (µmol $m^{-2}s$)		
		440.8	**383.9**	**273.2**
40	Biomass yielding (dw, g)	1.32 ± 0.605 [a]	1.11 ± 0.378 [a]	1.09 ± 0.450 [a]
90	Biomass yielding (dw, g)	4.0 ± 1.58[a]	3.52 ± 1.70[a]	1.42 ± 1.35[a]

Difference letters indicate significant difference (P<0.05), Tukey test

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3.2. ASSIMILATION OF CO_2

Fig. 1 shown the assimilation of CO_2 as net photosynthetic rate (A) of *S. Rebaudiana* at three levels of PFD during 40 and 90 days of culture by NFT hydroponic system. At 40 and 90 days of hydroponic culture, the assimilation of CO_2, decreased when level of light was reduced. No significant difference ($P<0.05$) was found at 40 days of culture, while at 90 days, significant difference ($P<0.05$) were found. The increase of (A) in relation to the intensity of light is caused by the decrease in the concentration of Ci (intercellular CO_2), as the light increases (Xiong *et al*., 2018), thus lower irradiance in *S. Rebaudiana* was with the lower (A), then Ci was higher and it decreased at higher irradiance (440.8 µmol $m^{-2}s$) it generated a greater concentration gradient and therefore a greater CO_2 assimilation. The assimilation of CO_2 in *S. rebaudiana* was limited at PFD of 273.2 µmol $m^{-2}s$; according to light plays an important role in the activation of rubisco (ribulose-1, 5-bisphosphate carboxylase/oxygenase), when the light intensity decreased, the carboxylation activity decreased, thus $CO_2$is not transformed and the concentration of Ci is increased, then the rate of CO_2 assimilation was reduced.

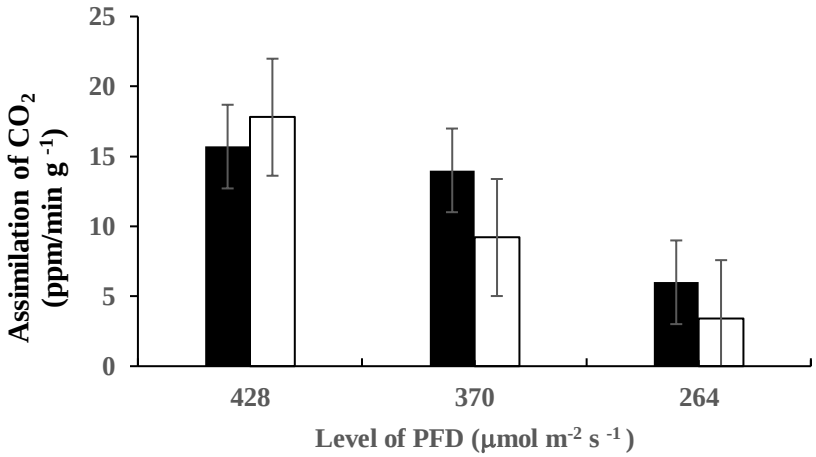

Fig. 1: Net photosynthesis at three levels of PFD by NFT hydroponic culture system of *S. rebaudiana* at 40 (■) and 90 (□) days.

3.3. THE OJIP TRANSIENT ANALYSIS

The curves of Chl a fluorescence during the fast part of the transient (OJIP), measured at different levels of PFD after 40 days of treatment is shown in Fig. 2, where the typical curve OJIP were observed. When plotted on a logarithmic time scale, the kinetics of fluorescence rise exhibited similar profiles, with distinct O-J-I-P steps in all cases (Fig. 3). Significant difference at the three levels of PFD were found for the fluorescence emitted by leaves of *S. rebaudiana*. After 40 days of hydroponic culture, on the photochemical phase (O to J) and thermal phases (J–

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I and I–P parts), PFD of 383.9 μmol $m^{-2}s^{-1}$ showed the higher level of fluorescence emission, this event depends strongly on the intensity of the exciting light while on thermal phases (J–I and I–P parts), the PFD at 273.2 μmol $m^{-2}s^{-1}$ increased the energy dissipation in the form of fluorescenceand their curve was similar to the fluorescence emitted with 383.9 μmol $m^{-2}s^{-1}$, while the emission of fluorescence at higher PFD (440.8 μmol $m^{-2}s^{-1}$) was the lower curve. The greatest fluorescence dissipation observed from phases I-P corresponding to the partial reduction of cofactor QA and QB in electronic transport. On the OJIP transient, O-J parts depends strongly on the intensity of the exciting light, while J-P parts is temperature sensitive (Stirbet and Govindjee, 2011). After 90 days of hydroponic culture, the higher emission of fluorescence was found with 383.9 μmol $m^{-2}s^{-1}$ on the OJIP transient, and the lower PFD reduced the emission of fluorescence and the higher light intensity exhibited the lowest energy dissipation. Then the greater fluorescence emission observed at 383.9 μmol m^{-2} s^{-1} of irradiance from stage J to stage P, the reduction of electron acceptors (QA, QB and PQ) which generated a partial closure of the $PSII_{RC}$, was evidenced.

The JIP test, showed in this study that there was energy transferred between the PSII units from the formation of excited chlorophylls (Chls) related with the exciting light intensity as PFD, that resulted in changes in the emission of florescence for Chl a. When the exciting light intensity is high and saturating and the peak (P, also called FM or Fmax) was reached, the higher PFD (440.8 μmol $m^{-2}s^{-1}$ of irradiance) as control without solar protection, the fluorescence intensity decreases in Chl*a* due the $PSII_{RC}$ were open and then a constant flux of electrons is produced by successive reduction of the electron acceptors, it is consistent that $PSII_{RC}$ is a regulator of Chl*a* fluorescence (Stirbet and Govindjee, 2011; Stirbet *et al*, 2020). It can be summarized that at 40 days of culture the highest fluorescence emission was recorded with intermediate PFD and the lowest fluorescence emission at higher irradiance, in all the stages of photosystem II. Stages O and J, higher and lower PFD with the same level of fluorescence. In stages I and P the fluorescence with the lowest irradiance increased, and the fluorescence with the highest irradiance tended to decrease in the final stage (P) of photosystem II, therefore the flow of electrons tended to increase, therefore, there was greater assimilation of CO_2 while this flux decreased at lower irradiance, which means that the PII reaction centers were closed, therefore the assimilation of CO_2 was lower.

The conversion of light into a stable chemical form is originated from the formation of excited chlorophylls (Chl a) by the absorption of photons by antenna molecules, and it is necessary to make molecules of the next stage of photosynthesis: the energy storage molecule ATP and the reduced electron carrier NADPH. We conclude that *S. rebaudiana var. Morita* II has different levels of susceptibly to the level of PFD subjected and time of culture, thus their photochemical process can be altered.

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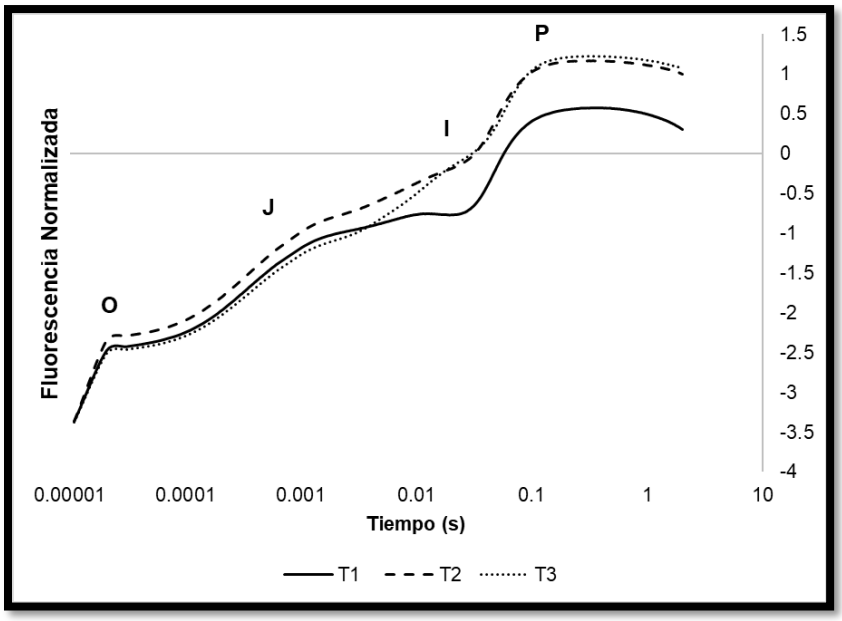

Fig. 2: Fluorescence emission kinetics based on the stages of electronic transport of photosystem II on the three levels of PFD (440.8, 383.9 and 273.2 μmol $m^{-2}s^{-1}$) at 40 days of culture of *S. rebaudiana* under NFT hydroponic system. All curves were recorded with the Handy PEA fluorometer of Hansatech Instruments, Ltd. (UK). Fluorescence data are plotted against linear time scales.

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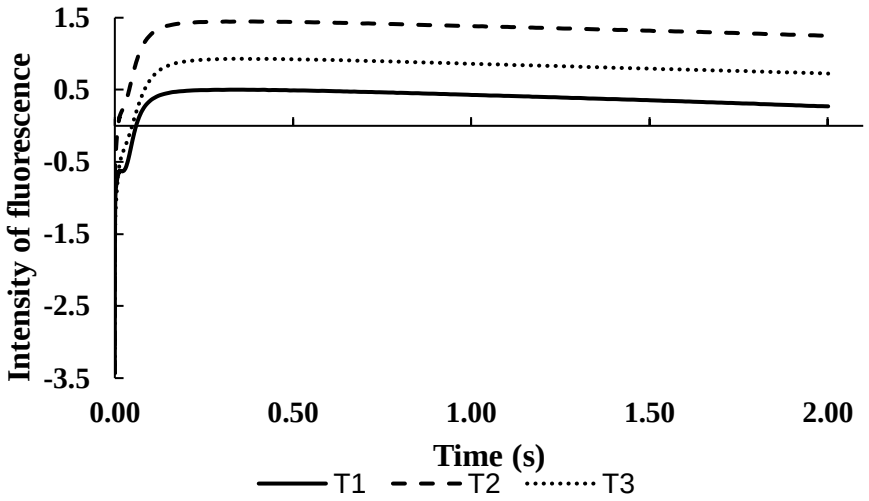
a)



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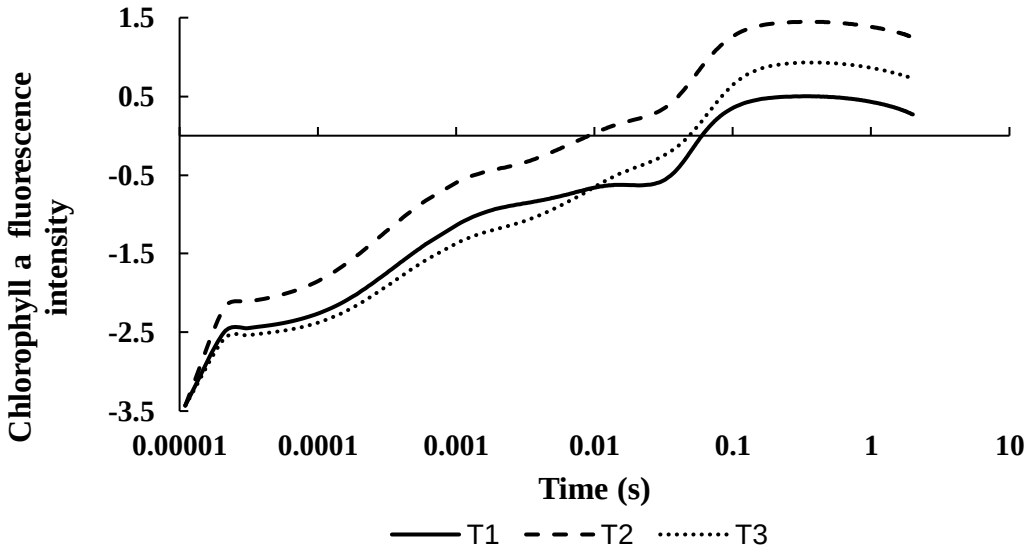

Fig. 3: Kinetics of chlorophyll *a* fluorescence induction transients recorded at three levels of PFD of a *S. rebaudiana* leaf at 90 days of culture by NFT hydroponic system. Top graph: on a linear time scale; Bottom graph: on a logarithmic time scale. For definition of OJIP symbols, see text. O-, J-, I-, and P-levels are marked on the upper most curve. Fluorescence is given in arbitrary units. a) Logarithmic time scale. b) Linear time scale.

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3.4. MAXIMUM PHOTOSYNTHETIC EFFICIENCY (FV/FM)

The maximum photosynthetic efficiencies of *S. rebaudina* at three levels of irradiance after 40 and 90 days of culture by NFT hydroponic system are shown in Table 3. No significant difference ($P < 0.05$) was found between 40 and 90 days of culture or levels of irradiance. Fv/Fm values after 40 and 90 days of culture were maintained in a range of 0.75-0.80 and 0.74-0.79, respectively, indicating greater photosynthetic efficiency in the use of energy and being kept in a range close to that reported by Snider *et al*. (2018) for Fv/Fm, who mentions that the higher plants without stress, maintain Fv/Fm values in a range of 0.78-0.84. In general, in this study, to reduce the luminositythe maximum photosynthetic efficiency increased, and increasing the days of culture, it was reduced. However, in all treatments the PSII kept working in a similar way with better photosynthetic efficiency at lower PFD (273.2 μmol $m^{-2}s^{-1}$) which showed the higher values of Fv/Fm. Fv/Fm. Values of 0.5-0.6 were reported for *S. rebaudiana*, growing in the open field, those values which indicated a stress in the plant and a photo inhibition of the PSII, because the plant was absorbing more energy of the necessary for the assimilation of CO_2 (Libik-Konieczny *et al*. 2018). Photo inhibition is irreversible chemical conversions (Papageorgiou and Govindjee, 2011) and depends on the amount of light intensity and duration of this that the plant is exposed, the level of saturation is specific to each plant and depends on the species or variety, physiological state and nutrition. González-Salvatierra *et al*. (2013) mention that the decrease in Fv/Fm in conditions of high light intensity may be due to the dissipation of energy.PFD of 440.8 to 273.2μmol m^{-2} s^{-1}no showed photoinhibition of PSII. The tolerance of a species to changes in luminosity entails two important features that are the plasticity of a genotype and the ability to acclimatize a phenotype (Valladares *et al.*, 2004). It can establish the great plasticity that *Stevia* has to adapt to different environmental conditions, especially the *Morita II variety*, due to its cultivation that it is highly distributed worldwide. Acosta-Motos *et., al* (2019), obtained Fv/Fm values between 0.74 and 0.79 in Stevia rebaudiana Bertoni plants during the acclimatization process at 2 and 14 days respectively. Hajihashemi et al., (2018) found Fv/Fm values between 0.40 and 0.70 in nine cultivars of S. rebaudiana grown at a temperature of 5ºC, while at 25 ºC the values in the nine cultivars were very close to 0.8.

In this study, a decreased of Fv/Fm was observed in plants at 90 days of cultivation, compared to registered at 40 days of cultivation. However, statistically showed no significant difference ($P<0.05$) among them.

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Table 3: Maximum photosynthetic efficiency (Fv/Fm) at three levels of PFD and 40 and 90 days of cultureby NFT hydroponic culture of *S. rebaudina*.

Hydroponic culture (days)	Levels of PFD (µmol m^{-2} s^{-1})		
	440.8	383.9	273.2
40	0.75 ±0.15[a]	0.76 ±0.02[a]	0.80 ±0.014[a]
90	0.74 ±0.040[a]	0.72 ±0.055[a]	0.79 ± 0.016[a]

Difference letters indicate significant difference (P<0.05), Tukey test

3.5. $PSII_{RC}$ ENERGETIC EFFICIENCY

The data in Table 4 show those 90 days of cultivation, the values of the variables ϕPo, Ψo and ϕEo had an increase as the irradiance decreased, while ϕDo had an opposite behaviour, that is, it increased with high PFD. This means that with low luminosity the light energy is absorbed, transferred to the reaction center and used in the transport of electrons more efficiently than in high PFD. Regarding the total flux of photons absorbed by the antenna pigments of the PSII (ABS) and the photons partially trapped by the PSII reaction center (TR0), it was observed that their values were higher with the PFD of 383.9 mol m-^{2} s-^{1}. The same happened in the cases of the energy of the absorbed photons used for the separation of charges and stabilization of the PSII as P680.bQA (ET0) and the dissipation of energy in the form of heat and fluorescence (DI).

To increase the efficiency of primary photochemistry and photochemical efficiency of photosynthetic electron transport associated with an increased DI_o/RC on *Stevia rebaudiana* it result necessary using a shadow to protect of the direct light incidence. This study showed in the maximum yield of primary photochemistry of PSII (ϕ_{Po}) of *S. rebaudiana* is affected by the levels of PFD and only slight difference were found with the lower irradiance (273.2 µmol $m^{-2}s^{-1}$.

It was observed that at regret maintaining greater energy absorption towards the reaction centers (ABS/RC) for 383.9 µmol $m^{-2}s^{-1}$ the rate of photon entrapment by the reaction centers (TRo/RC) was not affected in all levels of PFD. However, the absorbed energy was not fully utilized and was dissipated in the form of heat (DIo/RC), obtaining a greater dissipation at 383.9 µmol $m^{-2}s^{-1}$ suggesting that the lower PFD (273.2 µmol $m^{-2}s^{-1}$) the absorbed energy was harnessed more efficiently by the $PSII_{RC}$, when less heat dissipation was observed.

Table 4: Effect of PFD (µmol $m^{-2}s^{-1}$) on specific flux photon absorbed by $PSII_{RC}$ for *S. rebaudiana* under NFT hydroponic system at 90 days of culture.

NFT-Hydroponic culture	PFD	Energetic efficiency parameters of $PSII_{RC}$							
(days)	(µmol m^{-2} s^{-1})	ϕ_{Po}	Ψo	ϕ_{Eo}	ϕ_{Do}	ABS	TRo	ETo	Dio
90	**440.8**	0.74a	0.48a	0.36a	0.26a	2.34a	1.73b	0.83b	0.61a
	383.9	0.72a	0.53a	0.38a	0.28a	2.83a	2.03a	1.07a	0.81a
	273.2	0.79a	0.61a	0.48a	0.21a	2.20a	1.73b	1.06a	0.46a

Difference letters indicate significant difference (P<0.05), Tukey test

(φ)= quantum yield; ϕ_{PSII}, actual PSII efficiency; ϕ_{Eo}= ET_0/ABS, quantum yield of electron transport; ϕ_{Do}, quantum yield of dissipation; Ψ_o= ET_0/TR_0, yield of electron transport per trapped exciton; ϕ_{Po}= TR_o=ABS¼1Fo=FM= Maximum quantum yield of primary PSII photochemistry; ϕ_{ETo} = Electron transport flux from Q_A to Q_B ; ϕ_{DIo}= Rate of energy dissipation in all the PSIIs, in processes other than trapping – denoted as *dissipated energy flux;* **ABS/RC =** absorbed photon flux per $PSII_{RC}$ (or also, apparent antenna size of an active $PSII_{RC}$; **TRo/RC =** Maximum (initial) trapped exciton flux; **ETo/RC =** Electron transport flux from Q_A to Q_B; **Dio(RC =** Rate of energy dissipation in all the $PSII_{RC}$s, in processes other than trapping – denoted as *dissipated energy flux).*

3.6. EFFECT OF THREE LEVELS OF PFD ON STEVIOL GLYCOSIDES SYNTHESIS IN *STEVIA REBAUDIANA* VAR. MORITA II BY NFT HYDROPONIC SYSTEM.

Regarding the yield of steviol glycosides, values between 1.4 and 3.5 mg g^{-1}db of steviosides were obtained, while for rebaudioside A, it ranged from 0.2 to 2.2 mg g^{-1} db. The light level had a positive effect for stevioside, with a statistically significant difference only between the treatment of 273.2 µmol m^{-2}s and 440.8 µmol m^{-2}s at 90 days of culture. In the case of rebaudioside A, no statistically significant differences were found (Table 5).

In general, it is observed that the stevioside yields in both cultivation times tended to decrease as the lighting decreased from 3.0 to 2.9 mg g^{-1}db in 40 days and from 3.3 to 1.4 mg g^{-1}db in 90 days. cultivation days. In contrast, rebaudioside A tended to increase with low illumination; from

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0.5 to 2.2 mg g^{-1}db (440.0 – 383.9 µmol m^{-2}s) in 40 days and from 0.6 to 1.1 mg g^{-1}db (440.0 – 272.2 µmol m^{-2}s)

Table 5: Effect of three levels of PFD and time of culture at 40 and 90 days on leaf steviol glycosides yielding (mg g^{-1}) in *Stevia rebaudiana* growth by NFT hydroponic system.

Leaf content	NFT-HC	Levels of PFD (µmol m^{-2}s)		
(mg g^{-1}db)	days	440.8	383.9	273.2
Estevioside	**40**	3.0[a]	2.8[a]	2.9[a]
	90	3.3[a]	3.5[a]	1.4[b]
Rebaudiosido A	**40**	0.5[b]	2.2[a]	0.2[b]
	90	0.6[a]	1.0[a]	1.1[a]

Difference letters indicate significant difference (P<0.05), Tukey test

The results were similar to those reported by Jarma *et al*., (2012), who reported that the production of Reb-A had a negative response to the increase in light. The higher values of GSt production, compared to those of Reb-A at 40 and 90 days of treatment, could be an indication of an affectation in the enzymatic process for its synthesis; however, since the enzymes participating in this work were not evaluated, it is suggested that the enzymatic action could be a limiting factor for the synthesis of Reb-A.

UGT determines the glycosylation of steviol (intermediate) to form the different steviol glycosides (Jarma O.*et al*., 2010). Therefore, it is suggested that the enzymatic activity favored the synthesis of steviosides. However, in general, the GSt were observed decreased, which could be due to the flowering that occurred in both cultivation times. Munz *et al*., (2018) reported that the reduction of GSt is marked just before flowering, so the treatment with the highest light (T1: 440.8 µmol m^{-2}s), showed a significant difference with respect to the treatment of medium luminosity (T2: 383.9 µmol m^{-2}s) in the production of Reb-A. This was due to the decrease in GSt synthesis in the higher light treatment (T1: 440.8 µmol m^{-2}s) by maintaining the flowering stage at 40 days of treatment, while the medium light treatment (T2: 383.9 µmol m^{-2}s) was maintained at the beginning of floral sprouting at 40 days of treatment, so according to Martínez (2015), the maximum increase in GSt content occurs at the beginning of the sprouts floral, because the plant has reached its physiological maturity.

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Steviol glycosides can be considered reserve metabolites, therefore, upon entering the physiological stage of flowering, the GSt were possibly hydrolyzed for the use of simpler molecules. It has been shown that for the synthesis of isopentenyl pyrophosphate IPP, a precursor molecule in the elongation of the terpene chain, for the synthesis of GTs, light plays an important role (Lemus-Mondaca *et al*., 2012). However, IPP is also a precursor in the synthesis of a phytohormone, gibberellin (GAs), which is involved in several growth processes.

The phenomenon of avoiding the shade in the 90 days of treatment, *S. rebaudiana* could have increased the synthesis of GAs in response to the low availability of light, therefore, according to Table 5, significant differences were observed in the synthesis of steviosides for the treatment with the highest light (T1: 440.8 µmol m^{-2}s) compared to the one with the lowest light (T3: 273.2 µmol m^{-2}s), where an increase in the elongation of the internodes was observed, and it is the GAs that they are involved in this process, by participating in cell division and elongation, in addition to promoting flowering.

Yoneda *et al*., (2017) mentions that the concentration of gibberellin acts as a regulator in the synthesis of GSt by converging in the same biosynthetic pathway through the formation of ent-kaurenoic acid. Even though in the present work the concentration of GAs was not determined, it can be suggested that the decrease in GSt could be attributed to a high concentration of gibberellins. On the other hand, Jarma *et al*., (2012) mentions the importance of NADPH and molecular oxygen for the hydroxylation of ent-kaurenoic acid to steviol in the synthesis of GSt. Therefore, the photosynthetic efficiency benefited the synthesis of steviol glycosides in a positive way.

It is important to consider that performance is affected by the extraction method used. Hinojosa-González *et al.*, (2017) obtained 5.52±0.31 mg Reb A/100 g of leaf from the extracts of *S. rebaudiana* B. Morita II variety cultivated in Yucatán, by the supercritical fluid method without cosolvent, under temperature of 90°C, Pressure: 400 bar, and a time of 60 min. and 11.78±0.20 mg Reb A/100 g of leaf with supercritical fluid with 20.0% cosolvent water: ethanol (70:30v/v), 400 bars, 75 ºC for 45 min. Erkucuk *et al*., (2009) report 41.10 mg stevioside/g of leaf and 18.80 mg rebaudioside A/g of leaf under conditions 250 bar, 80°C, using 20% cosolvent (ethanol-water (70:30 v/ v) However, Aranda-González *et al*., (2014) obtained 15.15 ± 0.2 g of rebaudioside A/100 g of dry leaf. The yields obtained are above those reported by Hinojosa-González *et al*., (2017), but very low compared to those determined by Erkucuk et al., (2009) and Aranda-González *et al*., (2014).

In this work it can be concludedthat the increased of level of ligth as well as hydroponic culture time, provide significant effect on the plant biomass. The assimilation of CO2 determined the emission of fluorescence, due to the use of energy in the photosynthetic process, decreasing the

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emission in the treatment with greater luminosity. On the other hand, the values of the maximum photosynthetic efficiency (Fv/Fm) were similar in all treatments and culture times, which indicates that their photosynthetic apparatus works in the same way without being modified by the effect of light. Regarding energy efficiency, it was observed that with low luminosity the light energy is absorbed, transferred to the reaction center and used in the transport of electrons more efficiently than in high PFD.The yields of stevioside, were affected by light conditions and culture time, registering the highest yields with the intermediate PFD values and longer cultivation time. While rebaudioside A was produced in greater quantity with intermediate luminosity in both culture times.

Future studies will be aimed at optimizing nutrition and extraction methods, to obtain maximum yields of biomass and steviol glycosides.

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