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Effect of sucrose on the fatty acid metabolism of adventitious root cultures *in vitro* of *Stevia rebaudiana*

Efeito da sacarose no metabolismo dos ácidos graxos de culturas de raízes adventícias de *Stevia rebaudiana*

Efecto de la sacarosa sobre el metabolismo de los ácidos grasos de cultivos de raíces adventicias de *Stevia rebaudiana*

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Abstract
In this study, the effect of sucrose on the neutral lipid profile of adventitious root cultures of *Stevia rebaudiana* was evaluated. The cultures were obtained employing a roller bottle system. In this system, Schott-type flasks were used, which contained Murashige and Skoog liquid medium at 33.3% strength (MS/3) supplemented with 30, 60, and 80 g L^{-1} of sucrose, respectively, and 10.7 mM 1-naphthaleneacetic acid (NAA). The spectroscopic analyzes showed that the portion of polyunsaturated fatty acids (PUFAs) was highest in roots treated with 30 g L^{-1} of sucrose. The spectrometric analyzes showed that the palmitic acid was found to be present in relatively higher amounts in the roots submitted to the MS/3-30 g L^{-1} (31.9%) and MS/3-60 g L^{-1} (29.5%) sucrose treatments, and lower in the treatment with MS/3-80 g L^{-1} (28.8%) of sucrose. Also, the treatment using 30 g L^{-1} of sucrose was the best for obtaining unsaturated fatty acids (UFAs) in the culture, with a relative percentage of 62.9%. Our results indicate that the MS medium that received 30 g L^{-1} of sucrose induced a lesser abiotic stress condition, which favored PUFAs production in the adventitious root cultures of *S. rebaudiana*.
Keywords: Adventitious root cultures; Fatty acids; *Stevia rebaudiana*; Sucrose.

Resumo
Neste estudo, o efeito da sacarose no perfil lipídico neutro de culturas de raízes adventícias de *Stevia rebaudiana* foi avaliado. As culturas foram obtidas usando um sistema de garrafa giratória. Neste sistema, foram utilizados frascos do tipo Schott que continham meio líquido de Murashige e Skoog de 33,3% de força (MS/3), suplementado com 30, 60 e 80 g L^{-1} de sacarose, respectivamente, e 10.7 mM de ácido 1-naftalenoacético (ANA). As análises espectroscópicas dos extratos das raízes demonstraram que a porção de ácidos graxos poli-insaturados (AGPIs) foi mais alta nas raízes tratadas com 30 g L^{-1} de sacarose. As análises espectrométricas mostraram que o ácido palmítico estava presente em quantidades relativamente maiores nas raízes submetidas com tratamento de sacarose de MS/3-30 g L^{-1} (31.9%) e MS/3-60 g L^{-1} (29.5%), e em quantidades menores no tratamento de sacarose de MS/3-80 g L^{-1} (28.8%). No tratamento de sacarose de MS/3-80 g L^{-1}, o ácidos esteárico foi o ácido graxo prevalente (56.8%). Além disso, o tratamento usando 30 g L^{-1} de sacarose foi o melhor para obtenção de ácidos graxos insaturados nas culturas com porcentagem relativa de 62,9%. Nossos resultados indicaram que o meio MS que recebeu 30 g L^{-1} de sacarose induziu

uma condição de menor estresse abiótico o que favoreceu a produção de AGPIs nas culturas de raízes adventícias de *S. rebaudiana*.
Palavras-chave: Ácidos graxos; Culturas de raízes adventícias; Sacarose; *Stevia rebaudiana*.

Resumen

En este estudio, se evaluó el efecto de la sacarosa sobre el perfil de lípidos neutros de cultivos de raíces adventicias de *Stevia rebaudiana*. Los cultivos se obtuvieron utilizando un sistema de botella giratoria. En este sistema se utilizaron matraces tipo Schott (con tapón), que contenían medio líquido Murashige y Skoog al 33,3% de fuerza (MS/3) suplementado con 30, 60 y 80 g L^{-1} de sacarosa, respectivamente, y 10,7 mM ácido de 1-naftalenoacético (ANA). Los análisis espectroscópicos de los extractos de raíces mostraron que la porción de ácidos grasos poliinsaturados (AGP) fue mayor en las raíces tratadas con 30 g L^{-1} de sacarosa. Los análisis espectrométricos mostraron que el ácido palmítico estaba presente en cantidades relativamente mayores en las raíces sometidas a los tratamientos con sacarosa MS/3-30 g L^{-1} (31.9%) y MS/3-60 g L^{-1} (29.5%), y menor en el tratamiento con MS/3-80 g L-1 (28,8%) de sacarosa. En el tratamiento con MS/3-80 g L^{-1} de sacarosa, el ácido esteárico fue el ácido graso predominante (56,8%). Además, el tratamiento con 30 g L^{-1} de sacarosa fue el mejor para la obtención de ácidos grasos insaturados (HUFA) en el cultivo, con un porcentaje relativo de 62,9%. Nuestros resultados indicaron que el medio MS que recibió 30 g L-1 de sacarosa indujo una condición de menor estrés abiótico, lo que favoreció la producción de HUFAs en los cultivos de raíces adventicias de *S. rebaudiana.*
Palabras clave: Ácidos grasos; Cultivos de raíces adventicias; Sacarosa; *Stevia rebaudiana.*

1. Introduction

Stevia rebaudiana (Bertoni) Bertoni is a species of perennial shrub belonging to the family Asteracea (Asterales). This plant (in Guarani language Ka'ahe'ê) is native to South America (Tavarini, & Angelini, 2013), and popularly known "*sweet herb of Paraguay, sweet leaf, candy leaf, or honey leaf*" (Soejarto, Kinghorn, & Farnsworth, 1982; Brandle, & Rosa, 1992; Madan et al., 2010).

S. rebaudiana is a species of nutritional importance, it has been applied industrially since the 1970s, during which Japanese researchers optimized techniques to extract glucoside steviosides (Dacome et al., 2005). The main producers of *Stevia* are China and Southeast Asian countries. Currently, this plant has been gaining importance since 2011 when steviol glucosides obtained from the leaves were recognized as a sweetener by some important regulatory agencies around the world (Tavarini, & Angelini, 2013). In addition, recently, studies have shown that its roots are a rich source of inulin, a compound used as a prebiotic source (Lopes et al., 2016).

The use of plant tissue cultures and micropropagations in plant biotechnology are techniques that maintain the genetic stability of plants, which aim to produce primary and secondary metabolites for pharmacological and nutritional purposes (Wu et al., 2009; Thiyagarajan, & Venkatachalam, 2012; Lopes et al., 2016).Thus, with the fast root growth that happens through *in vitro* procedures, it is possible to acquire sufficient raw material for the extraction and commercial production of bioactive metabolites without the destruction of natural biodiversity. The adventitious roots of *S. rebaudiana* represent an important and promising biotechnological alternative for obtaining bioactive substances, given their good propagation, conservation of the species can be achieved, as shown in our previous work using adventitious roots cultured *in vitro* (Reis et al., 2011; Lopes et al., 2016).

Some environmental stresses, such as temperature, sunlight, and variations in the composition of nutrients, can induce changes in the lipid composition of these root cells (Oksman-Caldentey et al., 1994; Singer, Zou, & Weselake, 2016; Zhai et al., 2017). One reason is that neutral lipids(NLs), made up mainly of triacylclycerols (TAGs) and free fatty acids (FAs), provide not only energy and the building blocks for secondary metabolite biosynthesis by plants, but also play important roles in plant growth/survival when submitted to biotic and abiotic stresses. Thus, understanding all the physiological attributions of TAGs and FAs will be a crucial point for the future design of biotechnological strategies to overcome bottlenecks in increasing

the production of metabolites of interest. Therefore, the present study aimed to evaluate the effect of sucrose concentration on the neutral lipid profile in the adventitious root cultures of *S. rebaudiana* by gas-liquid chromatography coupled to mass spectrometry (GC-MS) and nuclear resonance spectroscopy (NMR).

2. Methodology

The work corresponded to laboratory activities of semi-quantitative and qualitative character of the effect of sucrose on adventitious roots of *Stevia rebaudiana* cultured in vitro. The first one related to the establishment of the cultivation process of adventitious roots of *S. rebaudiana*. The second, in turn, is linked to the qualitative and quantitative assessment of the effect of different concentrations of sucrose on the pattern of neutral lipids and fatty acids. The research was carried out experimentally based on the procedures proposed by Jacomini et al. (2015) and using a comparative research method as described by Pereira et al. (2018), took into account the observation, recording and analysis of the objects of study and their relationship with other phenomena.

2.1 Plant material

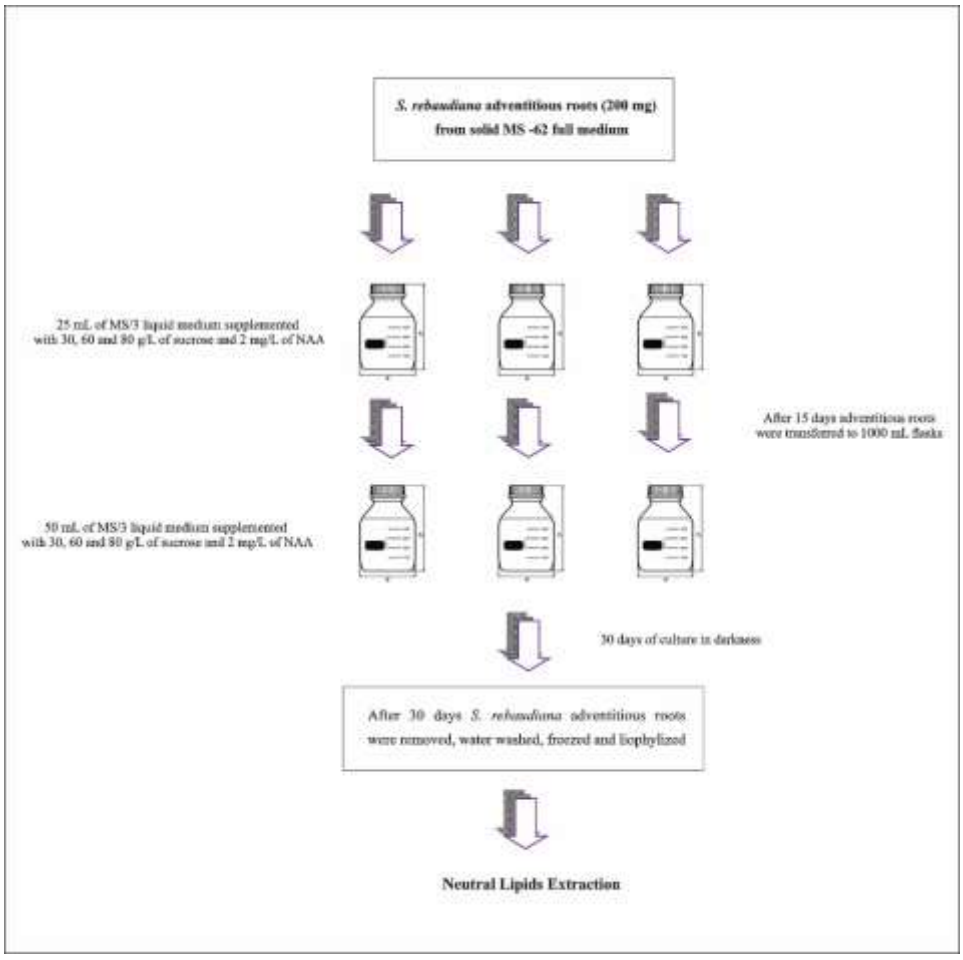
Stevia rebaudiana (Bertoni) specimens were identified by Dr. Jimi Naoki Nakagima (Federal University of Uberlândia) in March 2008. An exsiccate was deposited at the Herbarium of the State University of Maringá, Brazil (14301-HUEM). The seeds of *S. rebaudiana* were acquired from the Iguatemi Research Station of the State University of Maringá (FEI/UEM).

2.2 Adventitious root cultures of *Stevia rebaudiana*

Aseptic plants of *S. rebaudiana* were obtained according to the protocol of Reis et al. (2011). Root cultures of *S. rebaudiana* were then started from root tip explants about 1.5 cm long derived from *in vitro* rooting nodal explants on solid MS media supplemented with 10.7 mM of 1-naphthaleneacetic acid (NAA) at 25±1 ºC, with 2 months of cultivation. The MS media (pH 5.8) was incorporated with 0.8% (w/v) agar. These cultures were successfully grown and maintained with regular subcultures every 4 weeks.

After 2 months of cultivation, roots were aseptically isolated from the explants, and the nutrient medium present was completely eliminated. Approximately 0.2 g of fresh roots were withdrawn and transferred to 500 mL Schott-type flasks containing 25 mL of MS liquid medium (pH 5.8), with 33.3% strength (MS/3) that was supplemented, respectively, with 30, 60 and 80 g L^{-1} of D-sucrose and 10.7 mM 1-NAA. The adventitious roots of *S. rebaudiana* were cultivated in a roller bottle system at 2 rotations per minute (RPM) at 25±1ºC under dark conditions. These roots were maintained under this culture condition for 15 days, after which they were transferred to 1 L Schott-type flasks containing 50 mL of MS/3 liquid medium that was supplemented, respectively, with 30, 60 and 80 g L^{-1} D-sucrose and 10.7 mM 1-NAA. The roots were then cultivated for another 30 days in the same conditions described above, as shown in Figure 1.

Figure 1. Procedures of adventitious root cultures of *Stevia rebaudiana* trials.



Source: Authors.

The Growth Index (GI) was determined by the dry weight of the root inoculated in the liquid MS medium and the final dry weight of the root obtained, after the lyophilization process (adapted from Sivanandhan et al., 2012), according to the equation described below, Eq. (1):

$$\text{Growth Index } (GI) = \frac{\text{Dry root weight obtained}}{\text{Dry root of inoculated root}} \quad (1)$$

2.3 Neutral lipids extraction

The lyophilized adventitious roots obtained from the different treatments (SRR-30, SRR-60, and SRR-80) were extracted under reflux with hexane for 4 h. After the extracts were filtered, the solvent was evaporated in a rotary evaporator (35 °C), yielding the crude extracts of neutral lipids (NLs). These samples were stored at -20 °C until the respective analyzes.

2.4 Neutral lipid analysis

Fatty acids were chemically transformed into fatty acid methyl esters (FAMEs) by the transesterification of the NL samples (SRNL-30, SRNL-60, SRNL-80) with 1 mL of 1% NaOH in MeOH, with subsequent heating for 15 min at 55 °C. Two milliliters of 5% methanolic HCl was then added, followed by heating for 15 min at 55 °C and the addition of 1 mL H_2O

ultrapure. FAMEs were extracted with hexane (3×1 mL) and evaporated to dryness in an evaporator. Samples were redissolved in 200 µL hexane and stored at -4 °C in glass vials until analysis by GC-MS (Kumari, Reddy, & Jha, 2011).

2.4.1 Gas-liquid chromatography coupled to mass spectrometry (GC-MS) analysis

Gas-liquid chromatography coupled to mass spectrometry (GC-MS) analysis was carried out using a Gas Chromatography Agilent 7890B coupled with a Mass Spectrometer Agilent 5977A MSD and an HP5-MS UI-Agilent fused silica capillary (30 × 0.25 mm × 0.25 mm; Agilent Technologies). The He was carrier gas used at a flow rate of 1 mL min^{-1}. The temperature of the split injector (split ratio 1:30) was 250 °C and temperature of the transfer lines was maintained at 250 °C. The operating specifications of the column were as follows: the initial temperature of the oven was 60 °C maintained for 4 min, then 60 to 250 °C at 10 °C min^{-1}, and the final temperature was sustained for 28 min. The mass spectrometer was operated in the electron impact mode, with electron energy of 70 eV for the positive mode. Ionization source and quadrupole temperature were maintained at 230 °C and 150 °C respectively (Jacomini et al., 2015). The verification of the identity of the compounds was performed by checking their mass spectra with data from NIST 11.0 libraries (National Institute of Standards and Technology, US).

2.4.2 Nuclear magnetic resonance spectroscopy (NMR)

Nuclear magnetic resonance spectroscopy (NMR) spectra were recorded on a Bruker Avance HD III spectrometer operating at 500.13 MHz for ^{1}H and 125.0 MHz for ^{13}C nucleus. Samples of the NL crude extract (10 mg) were run in deuterated chloroform ($CDCl_3$; at 294.7 K). The polyunsaturated fatty acid (PUFA) Eq. (2), saturated fatty acid (SFA) Eq. (4), and monounsaturated fatty acid (MUFA) Eq. (3), a content of *S. rebaudiana* adventitious roots was determined using spectra of ^{1} H NMR (Figure 2 A), according to the methodology proposed by Usman et al. (2016).

$$\text{PUFA} = \frac{I_z}{I_Y} \quad (2)$$

Where: (I_Y) integration of acyl group proton environment; (I_Z) integration of the bis-allylic proton environment.

$$\text{MUFA} = \left(\frac{I_X}{I_{2Y}}\right) - \text{PUFA} \quad (3)$$

Where: (I_{2Y}) integration of acyl group proton environment; (I_X) integration of the allylic proton environment.

$$\text{SFA} = 1 - \left(\frac{I_X}{I_{2Y}}\right) \quad (4)$$

2.5 Statistical analysis

The results were presented as mean ± standard deviation (SD), followed by the statistical analysis employing the statistical software SISVAR version 5.3. ANOVA and Tukey's test (p<0.05).

3. Results and Discussion

3.1 Effect of sucrose on the accumulation of biomass in adventitious root cultures *in vitro* of *S. rebaudiana*

One of the main arguments to invest time and effort into the search for biotechnological alternatives for the production of bioactive metabolites is because the yields of these are normally low in the wild specimens, and often only found in specific plant tissues or species. However, natural production is rarely a viable option for the pharmaceutical and food industry. Therefore, several methodologies are being used to optimize the production of plant-derived metabolites, such as two-part culture systems, genetic engineering, and the use of bioreactor systems (Tocci et al., 2011; Georgiev, & Weber, 2014; Wilson, Cummings, & Roberts,2014; Simonetti et al., 2016). The elicitation technique, used to enhance the synthesis of metabolites in order to ensure plant survival, persistence or competitiveness, has proven to be a competent approach both in the *ex vitro* and in *in vitro* root systems (Tocci et al., 2012; Yin et al., 2012).

This bioreactor allows the flow of the culture medium to be uniform within the flasks, allowing the roots to properly absorb nutrients from the medium. This system was successfully used in the adventitious root cultures of *S. rebaudiana* by Reis et al. (2011) and Lopes et al. (2016).In this work, the effect of increasing concentrations of sucrose of 30 g L^{-1}, 60 g L^{-1} and 80 g L^{-1}onroot growth and the production of NLs in the adventitious root cultures of *S. rebaudiana* was evaluated.

The MS nutrient medium containing 30 g L^{-1} of sucrose resulted in the greatest root biomass, as the values of fresh weight (FW), dry weight (DW), and the growth index (GI) were better when compared to other sucrose treatments, as shown in Table 1.The lower root biomass in the treatments with 60 and 80 g L^{-1} of sucrose may be associated to the osmotic stress caused in the plant cell by the increased sucrose concentration. This osmotic effect could be causing an increase in the viscosity of the culture medium thus restricting the use of nutrients by the plant cells (Lee et al., 2006).The same observation of the osmotic effect of sucrose in adventitious root cultures was made by Cui et al. (2010) for *Hypericum perforatum*, Baque et al. (2012) for *Morinda citrifolia* and Wu et al. (2018) for *Echinacea pallida* and *Echinacea purpurea*, and also in cell cultures by Lee et al. (2006).

3.2 Effect of sucrose stress on the neutral lipid composition of *S. rebaudiana adventitious* root cultures *in vitro*

In this study, the yields of the hexane extracts, basically the neutral lipids (free FAs, TAGs, DAGs and MAGs), increased as the supply of sucrose in the nutrient medium increased, see Table 1. It was already expected that the increased supply of sucrose would increase levels of NLs, as the sugar conversion (ATP) obtained through photosynthesis is closely related to the production of FAs in plant cultures, providing basic carbon skeletons (Xu, & Shanklin, 2016; Zhai et al., 2017).

Table 1. Growth in dry weight (g ± SD), growth index (GI), and neutral lipid (NL) extract yield of adventitious roots after 30 days of cultivation in a bioreactor from 0.022 g of root biomass (dry weight), under different sucrose conditions.

Treatment	Growth (g)	GI	NL yield (%)
MS/3-30 g L^{-1}	0.139±0.067	6.317±3.084	5.155
MS/3-60 g L^{-1}	0.203±0.040	7.731±2.540	6.671
MS/3-80 g L^{-1}	0.229±0.009	10.423±0.417	7.046

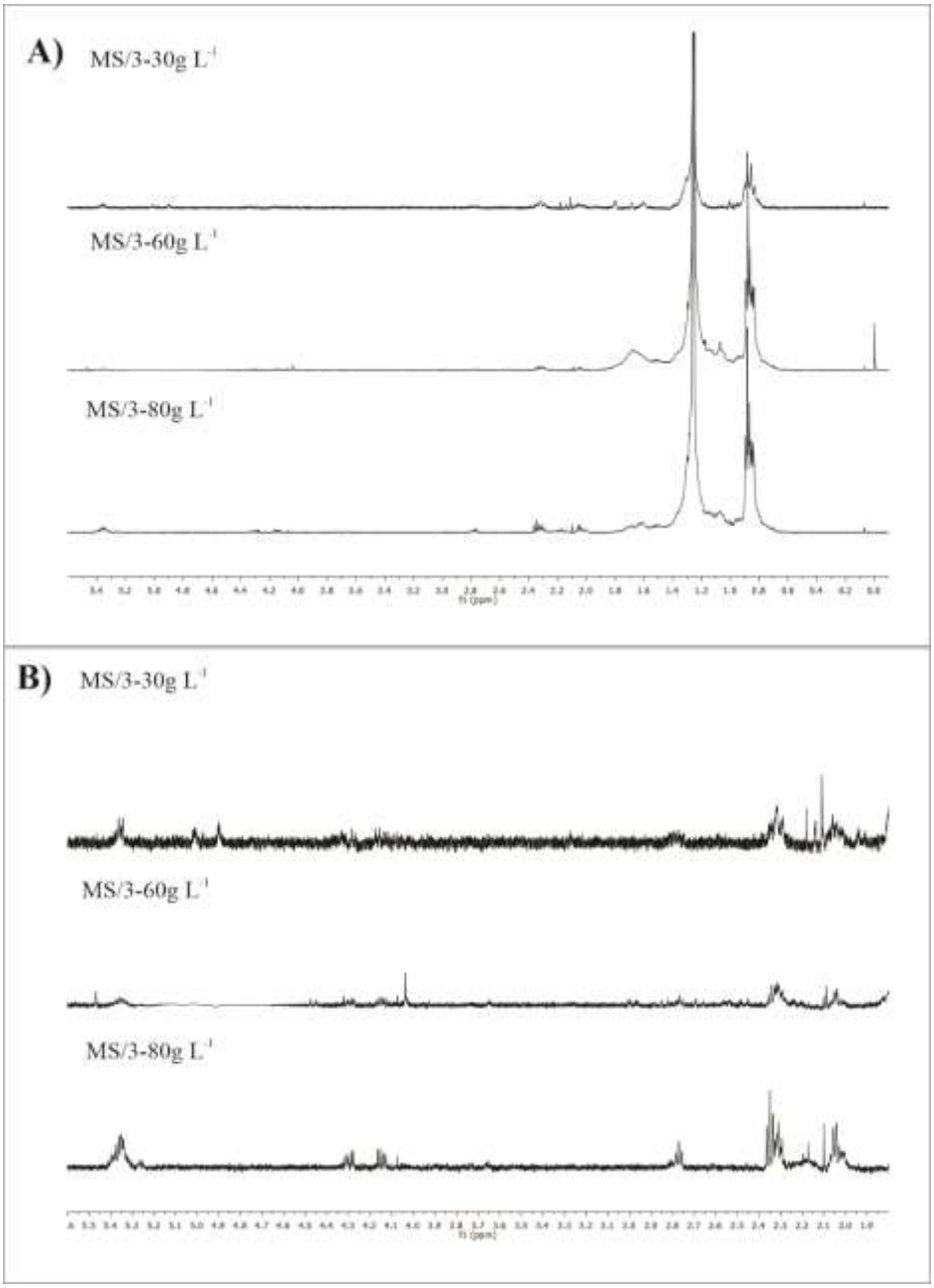
* Statistically different by Tukey's test $p<0.05$. Source: Authors

However, our results demonstrate that the sucrose concentration of the culture medium did not significantly influence the production of FAs. Oksman-Caldentey et al. (1994) also demonstrated that FA production was not greatly affected by the increment of sucrose concentration in root cultures *in vitro* of *Hyoscyamus muticus*.

3.3 ^{1}H and ^{13}CNMR neutral lipid analysis

Through the analysis of ^{1}H NMR spectra of NLs from adventitious root cultures of *S. rebaudiana*, it was possible to detect the presence of FAs, TAGs, and DAGs, as shown in Figure 2 A, B, and Table 2. Table 3 shows the relative percentages of the PUFAs, MUFAs, and SFAs through the integration of the signals from the different proton environments. All signals were attributed by comparison with literature data (Thoss et al., 2012; Jacomini et al. 2015).

Figure 2A. ^{1}H NMR spectral profile of NL extracts of adventitious root cultures of *Stevia rebaudiana* in MS/3 medium supplemented with 2 mg L^{-1} of NAA and 30, 60 g, and 80 g L^{-1} of sucrose. **B.** ^{1}H NMR spectral region between 5.60-1.80 ppm of NL extracts of adventitious root cultures of *Stevia rebaudiana* in MS/3 medium supplemented with 2 mg L^{-1} of NAA and 30, 60, and 80 g L^{-1} of sucrose.



Source: Authors.

Table 2. Chemical shifts and assignments of the characteristic resonance in the ^{1}H and ^{13}C NMR of NL extracts from adventitious root cultures of *S. rebaudiana.*

Position	δ_H		
	NL 30 g L^{-1} sucrose	NL 60 g L^{-1} sucrose	NL 80 g L^{-1} sucrose
Terminal methyl	0.88	0.88	0.88
Methylene	1.26	1.26	1.26
All acyl chains	1.61	1.53	1.62
Allylics	2.05	2.05	2.04
All acyl chains	2.35	2.35	2.32
Bisallylics	2.78	2.78	2.77
Glycerol (triglycerides)	4.04-5.28	3.75-5.26	4.05-5.27
Olefinic (all unsaturated chains)	5.37	5.37	5.35
Position	δ_C		
	NL 30 g L^{-1} sucrose	NL 60 g L^{-1} sucrose	NL 80 g L^{-1} sucrose
Terminal methyl	14.1	14.1	14.1
Methylene	29.7	29.7	29.7
All acyl chains	22.7	22.7	22.6
Allylics	27.0	27.0	27.2
All acyl chains	31.9	31.9	31.9
Bisallylics	24.4	24.4	24.4
Glycerol (triglycerides)	62.1-68.8	62.1	62.1-68.9
Olefinic (all unsaturated chains)	127.9-130.2	130.2	127.9-130.0
Carboxyl	177.1	169.2	173.1

Source: Authors.

In addition to the ^{1}H NMR spectrum, the^{13}C NMR spectra were also obtained, as shown in the Figure 2B and Tablr 2, further confirming the presence of NLs in the extracts. The chemical shifts to the triacylglycerides carbon atoms of NLs from *S. rebaudiana* adventitious roots were similar to literature data described for NLs from other plants (Thoss et al., 2012; Jacomini et al., 2015).

After the spectroscopic analysis, it was determined that SFAs predominate in all extracts regardless of the sucrose concentration to which the roots were submitted. Furthermore, the fraction of PUFAs was higher in roots treated with 30 g L^{-1} of sucrose and lower in roots treated with 60 g L^{-1} of sucrose. The fraction of MUFAs was lower in roots treated with 60 g L^{-1} of sucrose than in the other treatments, Table 3.

Table 3. Relative percentage (%) of polyunsaturated fatty acids (PUFAs), monounsaturated fatty acids (MUFAs) and saturated fatty acids (SFAs) obtained by ^{1}H NMR in the NLs extracts from adventitious root cultures of *S. rebaudiana* in different sucrose concentrations.

Treatment	PUFAs	MUFAs	SFAs
MS/3 30 g L^{-1}	20.79	15.39	63.82
MS/3 60 g L^{-1}	15.74	7.18	77.08
MS/3 80 g L^{-1}	19.35	11.70	68.95

Source: Authors.

3.4 GC-MS analysis

The FAME profile of *S. rebaudiana* adventitious root cultures was established by GC-MS analysis (Figure 3, Table 4). The peaks corresponding to the FAME content of the samples were compared and identified using the NIST library match software.

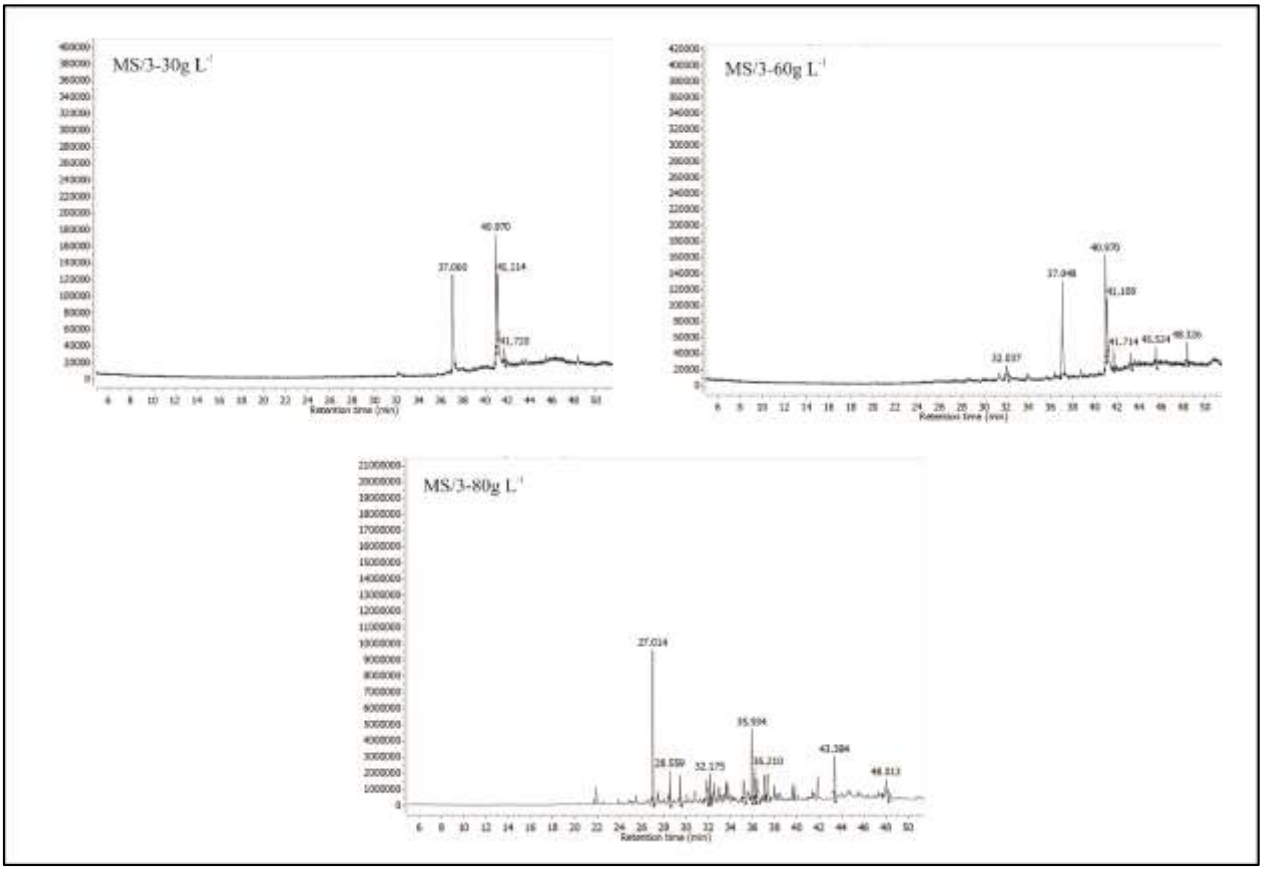
All mass spectra of FAMEs showed the peak at *m/z* 74 responsive to saturated FAMEs, monounsaturated FAMEs show a base peak at *m/z* 55 and polyunsaturated fatty acids was confirmed by the characteristic base peak at *m/z* 67 compared to literature data (Tariq et al., 2011; Gunawan et al., 2013).

GC-MS data analysis showed that SFAs were the major FAMEs in all extracts (Table 4), which palmitic acid was the main FA detected. Interestingly, the relative content of stearic acid, a SFA, increased significantly in the NL extract of roots grown in medium supplemented with 80 g L^{-1} of sucrose, with a relative percentage of 56.8%, in contrast to those grown with the opposite treatments of 60 g L^{-1} (5.90%) and 30 g L^{-1} of sucrose (5.10%). This means there was a gradual reduction in the relative concentration of UFAs with sucrose increase in the culture medium (Table 4).

The medium supplemented with 30 g L^{-1} of sucrose was the most favorable for UFA production in the roots when compared with the results of the other treatments, with a relative percentage of 62.9% as describe in the Table 4.

The application of osmotic stress by an increment of sucrose concentration did not appear to possess a negative influence on the relative amount of PUFA production; however, the sucrose concentration did not have a cumulative boosting effect on PUFA production either.

Figure 3. FAME profiles obtained by GC/MS analysis from NL extracts of adventitious root cultures of *Stevia rebaudiana* in MS/3 medium supplemented with 2 mg L^{-1} of NAA and 30, 60, and 80 g L^{-1} of sucrose.



Source: Authors.

TAGs in cytosolic lipid droplets are not only involved in membrane remodeling, but they also provide a source for the production of bioactive compounds derived from PUFAs involved in the plant defense mechanism. According to Shimada et al. (2014) and Shimada, Takano, & Hara-Nishimura (2015) during fungal infections, leaf TAGs accumulate in lipid droplet types. These TAGs contain an abundance of PUFAs, which are consecutively arranged for the formation of oxylipins as defense compounds (Shimada, & Hara-Nishimura, 2015). Equivalent to the chloroplast plastoglobules, the profusion of cytosolic lipid droplets also increases during adverse environmental conditions and senescence (Singer, Zou, &Weselake, 2016). The amount of TAGs in the leaves appears to be linked to the stress tolerance of plants. Experiments that induced the suppression of TAG and FA degradation pathways, using mutant plants, resulted in an increment in leaf TAG content, but also caused necrosis and bleaching during dark and age-induced senescence (Slocombe et al., 2009). Together, this highlights the essentiality of TAG homeostasis in plant tissue.

Table 4. Relative percentage (%) of FAMEs in the adventitious root cultures of *S. rebaudiana* under different sucrose concentrations determined by GC-MS.

Fatty acid	MM	Group	R_t	RP
		NL 30 g L^{-1} sucrose		
Palmitic	256.43	SFA	37.1	31.9
Linoleic	280.44	PUFA	40.9	26.7
Oleic	282.47	MUFA	41.1	36.2
Stearic	284.48	SFA	41.7	5.1
Total SFA				37.1
Total UFA				62.9
		NL 60 g L^{-1} sucrose		
Myristic	228.20	SFA	32.0	7.7
Palmitic	256.43	SFA	37.0	29.5
Linoleic	280.44	PUFA	40.9	23.8
Oleic	282.47	MUFA	41.1	30.8
Stearic	284.48	SFA	41.7	5.9
Total SFA				43.1
Total UFA				54.6
		NL 80 g L^{-1} sucrose		
Palmitic	256.43	SFA	37.1	28.8
Linoleic	280.44	PUFA	40.9	11.5
Oleic	282.47	MUFA	41.1	6.4
Stearic	284.48	SFA	41.7	56.8
Total SFA				85.6
Total UFA				17.9

MM: Monoisotopic molecular weight (g mol^{-1}); R_t: Retention time; RP: Relative Percentage. Source. Authors.

The effect of sucrose in the nutrient medium has been to increase the osmotic pressure causing oxidative stress in the plant cell with the formation of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2). ROS, in turn, cause lipid peroxidation of PUFAs with the formation of by-products such as malondialdehyde (MDA) (Baque et al., 2012; Cui et al. 2010). This phenomenon could be occurring in our study and would explain the reduction of PUFAs in the roots treated with a higher concentration of sucrose. Thus, to prove the oxidative stress described it would be interesting in future studies to evaluate the production of these compounds. In addition, changes in sucrose concentration also alter the production of secondary metabolites in plants, such as phenolic (Baque et al., 2012; Cui et al. 2010) and terpenoid compounds (Yin et al., 2013; Manuhara et al., 2015). Future perspectives of this work would be to evaluate the production of other groups of substances like phenolic and terpenoid compounds in the adventitious roots of *S.rebaudiana*, as well as their respective biological activities.

4. Conclusion

The results of this work demonstrated that the MS/3 medium supplemented with 30 g L^{-1} of sucrose, for inducing a minor abiotic stress condition, is the more adequate for PUFA production/accumulation using the adventitious root cultures of *S. rebaudiana*.

Using this adventitious root culture methodology, new results can be achieved by studying the effects of different carbon sources on the production of polyunsaturated fatty acids as well as other primary and secondary metabolites. Abiotic stress conditions that can improve the results presented here will also be used.

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