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INITIAL STUDY ON EFFECT OF SUBTRATES, LIGHT CONDITIONS ON SOME PHYSIOLOGYCAL PARAMETERS OF SACHA INCHI (*Plukenetia volubilis* L.) AND FATTY ACID COMPOSITION OF SEEDS

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Abstract: Primarily results on effect of subtrates, light conditions on growth of sacha inchi (*Plukenetia volubilis* L.) and fatty acid composition of seed showed that ratio soil : sand is 50:50 and natutal light condition are favorable to the height of seedling of sacha inchi plant. Though these factors did not affect to chlorophyll a content but they affected possitively to chlorophyll b content as well as vitamin C content in leaves. Beside that, exogenous application of BA converted male flowers on most of the inflorescences to female flowers, and 20 mg/L of BA increased the number of fruit up to 3 fruits per inflorescences in comparing to 1 fruit in the control. After 8 month of growing, the Oleic acid - omega 9 was high, occupied 55.64% of total fatty acid in seed of sacha inchi. Thus, the cultivation of sacha inchi in Vietnam is feasible allowing to meet the needs of high seed quality of sacha inchi byVietnamese's people.

Keywords: Benzyladenin (BA), fatty acid, physiological parameter, sacha inchi.

1. INTRODUCTION

Finding plants that high economic, suitable for the climate and soil of Vietnam as well as benefit on both agricultural development and industrial development is very important. Sacha inchi (*Plukenetia volubilis* L.) is a native to Peruvian Amazon plant (Sunan Wang, 2018) that perfectly meets these requirementsis gaining attention of many country, and researched by many scientists around the world. Sacha inchi is a precious food considered as the world's green gold because sacha inchi seeds (SIS) are rich oil content (Rosana, 2013; Natalie, 2012) which is very good for human heath (Gustavo, 2017). Once sacha inchi plant has acclimatilized to high-light growing conditions (Sunan et al., 2018), the effect of substrate and light requirements for seed germination and for seedings survival is needed to evaluate. *P. volubilis* plants flower continuously throughout the growing season, the capsule fruits consist four-to-seven pods, with one seed per pod. Sacha inchi plants, whose seeds contain a high content of polyunsaturated fatty acids, produces approximately 60 male flowers but only 1–2 female flowers per inflorescence (Qiantang Fu et al., 2014). Therefore, increasing the number of female flowers is critical for yield improvement of *P. volubilis*.

If Vietnam can expand this plant, it will become raw material for food, cosmetic industry, and drug industry. Sacha inchi was growth in Vietnam in 2012 by Sachi Vina

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Company. At present, sacha inchi trees are grown in some provinces of Vietnam such as: Ninh Binh, Hoa Binh, Dak Lak… Local people have basic knowledge to grow this plant. However they don't know the best way to grow under weather, soil conditions of Vietnam to get the higher yeild and seed quality. A few information from Vietnamese researchers about this plant such as a study on suitable medium for *in vitro* culture of stem of sacha inchi (Thuy LL, 2019; Hong NTB, 2018). Therefore, a study titled: "Initial study on effect of substrates, light conditions on some physiological parameters of sacha inchi (*Plukenetia volubilis* L.) and fatty acid composition of seeds" is necessary.

2. MATERIALS AND METHODS

Sacha inchi seed is provided by Dien Tran company, Bac Ninh province. Scientific name of sacha inchi plant is *Plukenetia volubilis* L. and is known by other names such as "Inca Peanut", "wild peanut", "Inca inchi", "mountain peanut".

This study was conducted at Department of Plant Physiology and Application and Experimental garden of Faculty of Biology, HNUE from 3/2017 to 4/2018.

Seeds were treated by soaking in warm water for 24 hour (ratio of hot water and cold water is 2:5) before sowing.

To assess the effect to the substrate on germination, seed were sowed on 4 different substrates as following:

CT 1: 100% sand	**CT3**: 30% soil, 70% sand (in volume)
CT2: 50% soil, 50% sand (in volume)	**CT4:** 70% soil, 30% sand (in volume)

Soil was taken from the Experimental garden of Faculty of Biology, HNUE and sand was taken from Hong River.

Seedling has 3 real leaves were assessed in different light conditions at the end of 7 days as following treatment:

- CT I: Natural lighting

- CT II: Continuous light in the light room. The light was provided by four 30 W-lamps

- CT III: Dark condition

This experiment was set up based on a suggestion of Amanda *et al.*, 2015. Each treatment was repeated 10 times.

Benzyladenin (BA) application on floral sex in sacha inchi (followed by method of Qiantang et al., 2014). This experiment started from May 2017 using 45 days old seeding plants (the plants which were grown in 50% soil, 50% sand substrate for germination (CT 2 treatment) and under natural lighting for seedling period (CT I treatment) to grow in soil of Experimental gardent at Faculty of Biology. As a liana species, *P. volubilis* plants were supported by bambooscaffold with a height of 1.8 m.

BA was sprayed on November, 2017 and repeated BA spraying one time after one week with the following treatments:

- CTA: Concentration of 0 mg/L

- CT B: Concentration of 5 mg/L

- CT C: Concentration of BA is 10 mg/L

- CT D: Concentration of BA is 20 mg/L

- CT E: Concentration of BA is 40 mg/L

A stock solution (1 mg/mL) of BA was prepared by dissolving 1g BA in 8 ml 1M NAOH and bringing the final volume to 1L with distilled water. Tween - 2 was added to BA working solution at a final concentration of 0.05% (v/v) as a wetting agent. Working solutions of various concentration of BA (5, 10, 20 and 40 mg/L) were sprayed onto top with a hand sprayer, wetting the tree to the point of run-off.

Analytical sampling method

- Leaf sampling method: take the third leaf from the top at 3 periods:

Period 1: After 14 days of sowing

Period 2: After 44 days of sowing

Period 3: After 74 days of sowing

Each treatment was repeated 4 times.

- Fruit sampling method: The fruit and seed at treatment CT D were collected and analyzed after 6 and 8 months after sowing (unmature fruit - the outer husk is vibrant green and fully mature fruit - the outer husk turns to a dark brown, respectively AAS) to analyze composition and fatty acid content in sacha inchi seed .

Determination of growth indicators

The height of plant, chlorophyll content (according to the equation of Mac – Kinney), vitamin C content (by titrate Iodine method), Fe, Ca, K content in leaf (by Atomic absorption spectrometry AAS), unsaturated fatty acid content (followed by ISO/FDIS 5509:1997 and analysed by Institute of BioTechonology) were determined.

Data were analyzed using the Statistical Product and Service Solution (SPSS) version 20.0 software. The significance of differences among means was determined using one way ANOVA with Tukey's post hoc tests at $\alpha = 0,05$.

3. RESULTS AND DISCUSION

3.1. Effect of different substrates and light conditions on height of sacha inchi

The height of plant is very important parameter to appraise the impact of experimental elements on growth of plant. The height dependent on many factors like: nutrients, temperature, humidity, soil,… The nutrient in the soil is the decisive factor because soil provides the essential mineral for plants.

Base on the result Table 3.1 we can see the height of sacha inchi in treatment CT1 and CT2 were not statistically significant. The height of sacha inchi in treatment CT3 and treatment CT4 were significantly lower than that in treatment CT1 and CT2. The height of sacha inchi depends on rate of sand and soil. In treatment CT2, the height of this plant was increased 10.36% in comparison to that in treatment CT1. In treatment CT3 and CT4 lower than CT1 4.43% and 6.58% respectively. We can explain as follows: although sand is very poor of nutrient, but it's absorbent and drained. Because the seeds have stored nutrients itself when seed germinated, so in this period the plant needs more water. Treatment CT2 has suitable rate of sand and soil, so the height of sacha inchi of CT2 is the highest.

Table 3.1. *Effect of different substrates and light conditions on height of sacha inchi*

Treatments	Height (cm)	Compare to control (%)
CT1	21.9^{b} ± 0.32	100
CT2	24.17^{b} ± 0.44	110.36
CT3	20.93^{a} ± 0.53	95.57
CT4	20.46^{a} ± 0.29	93.42
CTI	19.70^{c} ± 0.26	100
CTII	17.27^{b} ± 1.19	87.66
CTIII	15.73^{a} ± 0.53	79.85

(Values with the same letter in the same column in the same experiment are not statistically significant at $\alpha = 0.05$)

Light is a factor that has a great influence on plant height. The table showed the effect of light on the seedling's height (Table 3.1). At different lighting intensities, the height of sacha inchi plant varies. The seedlings at CTI obtained the highest value and that was significant different to that in treatment CTII and CTIII. The height was decreased from 12.34% to 20.15% (at treatment CTII, CTIII, respectively) compared to that in treatment CT1. Because light is one of important factors that effect to plant height. With normal condition (CTI) photosynthesis and respiration are rhythmic, plant synthesis of organic substances for plant growth and the height is higher. If plants were continuously illuminated, they continue to photosynthesis. Since *P. volubilis* plants are high light demanding species so when they grown in high light, they exhibited better growth compared with plants grown in the shade, which mainly attributed to its superior physiological performance. This result is consisted with data reported by Amanda et al., 2015.

3.2. Effect of different substrates and light conditions on chlorophyll content in sacha inchi leaf

Chlorophyll is an important pigment for photosynthesis. The chlorophyll content in the leaves determines the photosynthetic efficiency that affects the growth, development and yield of crop. Thus, determination of chlorophyll content help to evaluate the response

of plant to external factors. The Table 3.2A and 3.2B showed the result of chlorophyll content in sacha inchi leaves under different conditions.

Effect of different substrates and light condition to chlorophyll a content in sacha inchi leaf

Table 3.2A. *Effect of different substrates and light conditions to chlorophyll a content in leaf*

Treatments	Chlorophyll a content (mg/g)		
	Period 1	Period 2	Period 3
CT1	0.62^{a} ± 0,02	0.66^{a} ± 0.01	0.65^{a} ± 0.02
CT2	0.68^{a} ± 0.01	0.65^{a} ± 0.11	0.65^{a} ± 0.04
CT3	0.69^{a} ± 0.01	0.67^{a} ± 0.01	0.65^{a} ± 0.16
CT4	0.7^{a} ± 0.01	0.66^{a} ± 0.01	0.65^{a} ± 0.12
CTI	0.68^{a} ± 0.01	0.67^{a} ± 0,01	0.65^{a}± 0.03
CTII	0.7^{a} ± 0.01	0.67^{a} ± 0,01	0.65^{a}± 0.02
CTIII	0.64^{a} ± 0.05	0.67^{a} ± 0,01	0.65^{a}± 0.02

(Values with the same letter in the same column in the same experiment are not statistically significant at α = 0,05)

We can see from Table 3.2A that the chlorophyll a content in leaves of sacha inchi was constant through the time (3 periods) and even there was different in soil and sand ratio in substrates but it did not affect to the content of chlorophyll a. Similar of tendency of chlorophyll a under diffirent light conditions was aslo observed. Cai Z.Q et al., (2011) found that shade decreased photosynthesis of sacha inchi during an 8-month period.

Effect of different substrates and light conditions on chlorophyll b content in leaf

Table 3.2B. *Effect of different substrates and light condition on chlorophyll b content in leaf*

Treatments	Chlorophyll b content (mg/g)		
	Period 1	Period 2	Period 3
CT1	0.38^{a} ± 0.03	0.43^{a} ± 0.05	0.94^{a} ± 0.02
CT2	0.43^{b} ± 0.03	0.43^{a} ± 0.09	0.94^{a} ± 0.04
CT3	0.31^{a} ± 0.02	0.43^{a} ± 0.11	0.94^{a} ± 0.01
CT4	0.24^{c} ± 0.01	0.43^{a} ± 0.02	0.94^{a} ± 0.02
CTI	0.53^{b} ± 0.03	0.43^{a} ± 0.01	0.94^{a} ± 0.04
CTII	0.21^{b} ± 0.01	0.43^{a} ± 0.01	0.94^{a} ± 0.01
CTIII	0.54^{a} ± 0.04	0.43^{a} ± 0.05	0.94^{a} ± 0.01

(Values with the same letter in the same column in the same experiment are not statistically significant at α = 0,05)

As can be seen from the Table 3.2B, the chlorophyll b content was different between treatments at period 1 and these differences were significant statistically. The chlorophyll b content at treatment CT2 was highest (0.43 mg/g) and that at treatment CT4 was lowest (0.24 mg/g). However, at the period 2 and period 3, the chlorophyll b content at all treatments had similar result (0.43 mg/g, 0.94 mg/g, respectivelly).

When sacha inchi plant grew in the dark condition, the chlorophyll b content was higher than another light treatments (period 1). After 7 days, in different light conditions, the chlorophyll b content of treatment CTII was lowest (0.21 mg/g), the chlorophyll b content of treatment CTIII was highest (0.54 mg/g) and significant different to that in other treatments. At the period 2 and period 3, chlorophyll b content at all of treatments decreased compared to CTI, however these differences were not significant different. It could be explained that under different light conditions, at period 1, sach inchi plants in treatment CTIII did not received enough the light so plant have to increase number of chlorophyll b pigment in order to receive as much as possible of short – wavelengths.

3.3. Effect of different substrates and light conditions on vitamin C content

Vitamin C also known as L - ascorbic acid. Vitamin C is already known to be an antioxidant, which helps plants deal with stresses from drought to ozone and UV radiation. The vitamin C content is a parameter important to determine nutrient of vegetables when the leaf of sacha inchi is used to make instance tea or even fresh vegatable by farmer (Sunan et al., 2018).

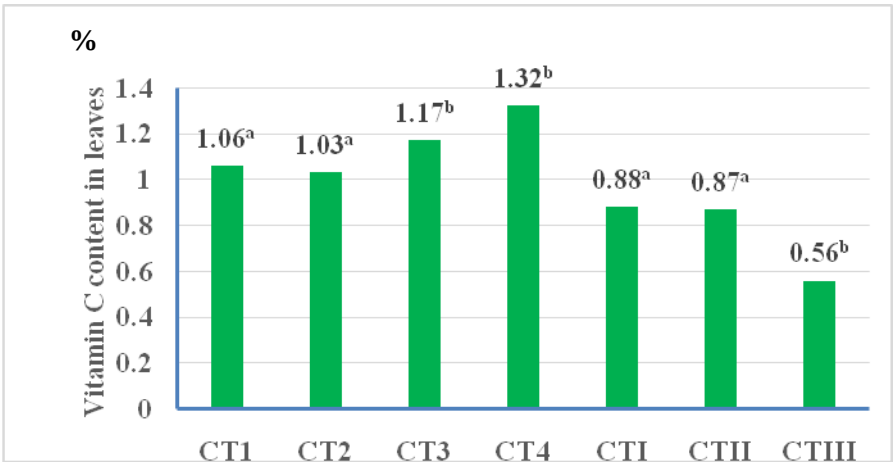

Figure 1. *Effect of different substrates andlight conditions to vitamin C content in leaves*

The vitamin C content in leaves under different substrates was varied from 1.03% to 1.32%. Treatment CT4 with ratio of sandy and soil is 7:3 had highest vitamin C content, and significant different in comparing to other treatments. In the natural light condition (treatment CTI), sacha inchi tree had vitamin C content lower than other light conditions. Once leaf of sacha inchi was used as vegetable by farmer or making instant tea by Sachi Vina company, this primarily data on vitamin C content in leaves may suggest to farmer for substrates would be soil and supply sufficient light radius to sachi inchi's growth.

3.4. The concentration of BA effect to the number of female flower

Table 3.4. *The number of female flower in inflorescence of different BA concentration*

Concentration of BA (mg/l)	After 20 days spray BA	After 50 days Spray BA	After 70 days spray BA

0	1 female flower/ inflorescence	1 female flower/ inflorescence	1 fruit/ inflorescence
5	7.2 female flowers/ inflorescence	6.4 female flowers/ inflorescence	Wilt
10	3.7 female flowers/ inflorescence	3.5 female flowers/ inflorescence	Wilt
20	9.5 female flowers/ inflorescence	8.2 female flowers/ inflorescence	3 fruits/ inflorescence
40	12.7 female flowers/ inflorescence	11.9 female flowers/ inflorescence	Wilt

As shown in Table 3.4, in different concentration of BA made the number of female flower were different. The number of female flowers per inflorescence was significantly higher on BA-treated control.

Figure 2. *Effect of BA to conversion male flowers into female flowers on sacha inchi inflorescences after 20 days. (A) Inflorescences of control top; (B) Inflorescences of top treated with 40 mg/L BA; (C) Inflorescences of top treated with 20 mg/L BA*

At treatment by BA concentration 40 mg/L, the number of female flowers was highest (12.7 female flowers/inflorescence) while in treatment by BA 20 mg/L, the number of female flowers was only 3.17 female after 20 days of spraying BA. However, after 50 and then 70 days of spraying, the number of fruit per inflorescence decreased remarkly (Figure 3). In this stage, the control and BA 20 mg/L treatment, flowers developed normal but at other treatments, they stopped growing. However, after 70 days BA sprayed, at BA 40 mg/L, BA10 mg/L and BA 5 mg/L treatment, all flowers were

wilted, might be due to of too high or too low concentration of BA. Sacha inchi flower developed well at BA 20 mg/L treatment by making 3 fruits/inflorescence. So, BA 20 mg/L may be suitable to sacha inchi grown in this study. Qiantang Fu et al., 2014 reported that exogenous applicationof BA converted male flowers on most of the inflorescences to female flowers, and approximately 8-20% of the induced female flowers further developed into fruits. Treatment with various concentrations of BA resulted in 3-41 female flowers per inflorescence. The average number of fruits per infructescence was 3.3 in the trees treated with the optimalconcentration of BA (20 mg/L), compared with 1.3 for infructescences of the control plants. Then, we could say that BA is a plant growth regulator which has the potential to induce floral feminization andpromote fruiting of *P. volubilis*.

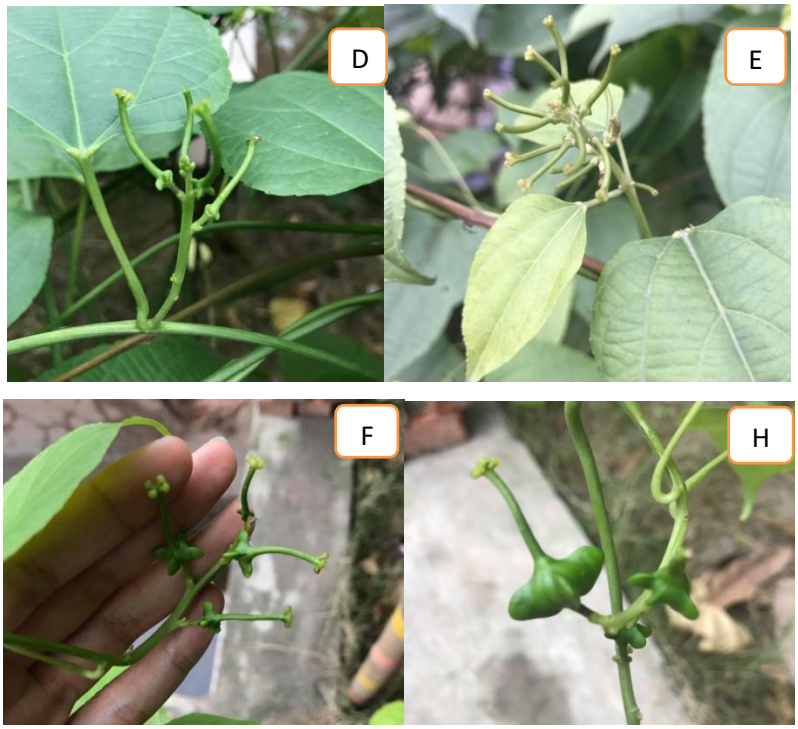

Figure 3. *The development of inflorescence after 50 days of sprayed BA*

(D) Inflorescences of top treated with 10 mg/L BA. (E) Inflorescences of top treated with 40 mg/L BA. (F) and (H) Inflorescences of top treated with 20 mg/L BA

3.5. The composition and fatty acid content of sacha inchi seed at different time of ripeness

Table 3.5. *The fatty acids content in fruits at age of 6 months and 8 months since sowing*

No.	Fatty acids	Scientific name	Common name	Content % (after 6 months)	Content % (after 8 months)	References
1	14:0	Tetradecanoic acid	Myristic acid	4.71	4.27	

2	16:0	Hexadecanoic acid	Palmitic acid	0.49	0.55	
3	18:2n-6	cis-9,12-octadecadienoic acid	Linoleic acid	91.85	33.41	
4	18: 1n-9	9-octadecenoicacid	Oleic acid	1.48	55.64	1
5	18: 1n-7	11-Octadecenoic acid	Vaccenic acid	0.05	1.63	1
6	18:3n-3	cis-9,12,15-octadecatrienoic acid	Anpha Linolenic acid	0.17	0.04	1
7	18:0	Octadecanoic acid	Atearic acid	0.1	0.1	
8	18: 1n-7	11-Octadecenoic acid	Vaccenic acid	0.21	0.22	
9	19:0	Nonadecanoic acid	Nonadecylic acid	0.15	0.08	
10	20:0	Eicosanoic acid	Arachidic acid	0.22	0.05	
11	22:0	Docosanoic acid	Behenic acid	0.68	0.14	
12			Lipid	35.97	41.0	

The data of Table 3.5 showed that after 6 months and 8 months growing, lipid content increased from 35.97 to 41% in seed of sacha inchi plant (plants at treatment CT D- BA 20 mg/L). It is the same value with other research (Sunan Wang et al., 2018). Sacha inchi fruits was unripened at the time of 6 months from sowing day: linoleic acid - omega 6 content was highest (91.85%). Other fatty acid ranged from 0.05% to 4.17%. Oleic acid - omega 9 was 1.48% content and alpha acid linolenic - omega 3 was very low, just 0.17%. However, after 8 months from sowing day, omega 6 content dropped 33.41%. On another hand, omega 9 increased to 55.64% and was highest but omega 3 decreased to 0.04%. Compare with research of Luis - Felipe Gutiérrez et al., 2013; Natalie et al., 2012; Rosana et al., 2015, omega 3 content in seed of this experiment was much lower but omega 9 content was higher. It might be due to the soil's characteristics and climate in Vietnam (especially during winter time) is different to those in Amazon. This is the preliminary data on seed quality, so we need to conduct further experiments in order to better understanding about soils, climate, farming seasonal and seed quality to have in detail results.

4. CONCLUSIONS

The different substrates and light conditions did not effect to some physiological – biochemical parameters such as: the height of plant, chlorophyll a content however they had positive affect to chlorophyll b content and vitamin C content in leaves. The exogenous application of BA concentration (20 mg/L) increased the number of female flower to 9.5 female flowers/inflorescence, which forming 3 fruits/inflorescence. Fatty acid content in the seed changed during the process of ripening of sacha inchi and when fruits turned into ripen period, the omega 6 content decreased from 91.85 to 33.41% but the omega 9 content increased markly up to 55.64%. It shows a potential of growth *P. volubilis* in Vietnam to get high seed quality.

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BƯỚC ĐẦU NGHIÊN CỨU ẢNH HƯỞNG CỦA CƠ CHẤT, CHẾ ĐỘ ÁNH SÁNG ĐẾN MỘT SỐ CHỈ TIÊU SINH LÝ CỦA CÂY SACHI INCHI (*Plukenetia volubilis* L.) VÀ THÀNH PHẦN AXIT BÉO TRONG HẠT

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Tóm tắt: Số liệu bước đầu về ảnh hưởng của cơ chất, điều kiện ánh sáng đến một số chỉ tiêu sinh lý của cây sacha inchi (*Plukenetia volubilis* L.) và thành phần axit béo trong hạt cây này đã chỉ ra rằng tỉ lệ cơ chất đất : cát là 50 : 50 và điều kiện chiếu sáng tự nhiên là thích hợp cho sự tăng trưởng chiều cao của cây con. Tuy những nhân tố này không ảnh hưởng đến hàm lượng diệp lục a trong lá cây nhưng lại có tác động tích cực đến hàm lượng diệp lục b cũng như hàm lượng vitamin C trong lá. Bên cạnh đó, việc phun chất điều hòa sinh trưởng ngoại sinh BA đã chuyển giới tính hoa đực thành hoa cái và hàm lượng BA 20 mg/L đã làm tăng số lượng quả trên một cành lên thành 3 quả thay vì 1 quả như ở công thức đối chứng (không phun BA). Sau 8 tháng kể từ khi gieo hạt thì hàm lượng axit béo trong hạt khá cao, đặc biệt là hàm lượng axit Oleic, hay còn gọi là Omega 9 chiếm đến 55,64% lượng axit béo tổng số trong hạt. Như vậy, việc trồng sacha inchi ở Việt Nam là khả thi cho phép đáp ứng được nhu cầu tiêu dùng hạt sacha inchi có chất lượng cao của người dân.

Từ khóa: Axit béo, benzyladenin (BA), chỉ tiêu sinh lý, sacha inchi.

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