Effect of Plant Growth Regulators on Shoot induction of Sacha Inchi (*Plukenetia volubilis* L.) *in vitro*

ผลของสารควบคุมการเจริญเติบโตต่อการชักนำยอดใหม่ของดาวอินคา (*Plukenetia volubilis* L.)

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ABSTRACT

Sacha inchi (*Plukenetia volubilis* L.) is rich in oil and accumulate for medicinal substance that contains a high content of polyunsaturated fatty acids such as 45.62% ω3 and 32.66% ω6. Increasing the number of shoot bud induction is critical for its rapid propagation. In this study, the hypocotyl explants were cultured on MS medium supplemented in the different concentrations of individually and in combinations of 0.5-1 mg/l 6-benzylaminopurine (BAP), kinetin (Kn) and thidiazuron (TDZ) to find an optimal concentration of plant growth regulators for shoot induction for 4 weeks. The best number of shoot buds (5.00±0.19) and number of leaves (12.00±0.52 leaves) were observed on MS medium supplemented with 1 mg/l BAP. The highest length of shoot (7.33±0.31 cm) was observed from MS medium supplemented with 0.5 mg/l BAP. All treatments responding were significantly affected.

บทคัดย่อ

ดาวอินคา (*Plukenetia volubilis* L.) เป็นพืชที่เมล็ดมีการสร้างน้ำมันและสะสมสารที่มีฤทธิ์ทางยา โดยพบกรดไขมันที่จำเป็นในปริมาณสูง คือ โอเมก้า 3 ร้อยละ 45.62 และ โอเมก้า 6 ร้อยละ 32.66 ทำการศึกษาผลของสารควบคุมการเจริญเติบโตต่อการชักนำให้เกิดยอดใหม่ด้วยการใช้ชิ้นส่วนใต้ใบเลี้ยง (hypocotyl) เลี้ยงในอาหารสังเคราะห์สูตร Murashige and Skoog Medium (MS) ร่วมกับสารควบคุมการเจริญของพืชกลุ่มไซโตไคนิน คือ 6-benzylamino purine (BAP) Kinetin (Kn) Thaidiazuron (TDZ) ความเข้มข้น 0.5 และ 1 มิลลิกรัมต่อลิตร เป็นเวลา 4 สัปดาห์ พบว่าอาหารสังเคราะห์สูตร MS เติม BAP 1 มิลลิกรัมต่อลิตร สามารถชักนำให้เกิดจำนวนยอดเฉลี่ยสูงสุดเท่ากับ 5.00±0.19 ยอด และจำนวนใบเฉลี่ยสูงสุด 12.00±0.52 ใบ ตามลำดับ และอาหารสูตร MS เติม BAP ความเข้มข้น 0.5 มิลลิกรัมต่อลิตร ให้ความสูงยอดเฉลี่ยสูงสุดเท่ากับ 7.33±0.31 เซนติเมตร โดยมีความแตกต่างอย่างมีนัยสำคัญทางสถิติ

Keywords: Sacha inchi, Shoot induction, Plant growth regulators

คำสำคัญ: ดาวอินคา การชักนำยอดใหม่ สารควบคุมการเจริญเติบโต

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Introduction

Sacha Inchi (*Plukenetia volubilis* L.), also known as Inca peanut, Sacha peanut or mountain peanut is an oleaginous plant that belongs to the Euphorbiaceae family. It grows in the lowlands of the Peruvian Amazon, having been cultivated for centuries by the indigenous population. In Asia, Sacha Inchi was introduced to Xishuangbanna, Yunnan Province of China. It is a biodiversity hotspot and is plenty in tropical plant resources. (Cao et al., 2006) Sacha Inchi seeds are considered to be a rich source of proteins and oil. The oil is characterized by its high content of polyunsaturated fatty acids mainly α-linolenic fatty acid (ω3) 12.8–16.0 g/100 g seed followed by linoleic fatty acid (ω6) 12.4–14.1 g/100 g seed. A wide range consumption of bioactive is important in terms of health. Both of α-linolenic and linoleic are important for the prevention of coronary heart disease and hypertension, showing a hypocholesterolemic effect (Simopoulos, 2011). The presence of other bioactive compounds, such as tocopherols, carotenes, polyphenolic compounds and phytosterols have been previously reported in Sacha Inchi oil. Phytosterols have been reported to reduce blood cholesterol and to decrease the risk of cancer. Tocopherols have vitamin E properties and display a strong antioxidant activity by conferring protection against lipid peroxidation (Chirinos et al., 2015). In addition, the amino acid profile showed a relatively high level of cysteine, tyrosine, threonine and tryptophan.

Sacha inchi is an encouraging species because many products from the plant can be made useful and profitable. Especially, Seed oil is traditionally used as skincare oil applied regularly on the skin in order to preserve skin softness. The whole seed is ground to a floury paste or made a day-care skin cream with other ingredients for rejuvenation purposes. The use of oil on dermal cells is safe and efficient in the inhibition of *S. aureus* adherence (Gonzalez et al., 2015). The acceptability and side-effects of consumption of Sacha Inchi oil is safety. Biochemical markers of hepatic and kidney function were maintained unchanged. Serum total cholesterol and LDL cholesterol levels and arterial blood pressure were lowered. Higher HDL-cholesterol was observed with Sacha Inchi oil at 4 month (Gonzalez and Gonzalez, 2014). The interest in Sacha Inchi has increased during the last years and this is appreciated in the evolution of exportations from Peru. During 2005 was exported to Europe and United State approximately USD 28,811.90 and in 2012 USD 168,285.43 (Gonzalez et al., 2015).

Conventional propagation of Sacha Inchi utilizes seeds and cuttings. But due to poor seed viability, lower germination rate, lower disease resistance and delayed rooting of seedling. *In vitro* regeneration offers a significant opportunity for operative mass propagation and genetic enhancement of plants within a restricted time period. Sacha inchi seeds can usually be harvested one year after planting. It is best to harvest the fruits when these have turned dark brown when the capsules have split open. Plant tissue culture offers an alternative approach to the plants which are difficult to cultivate, low yield and low cultivation period (Cai et al., 2013). Most of these problems can be overcome by using specific type and definite concentration of plant growth regulators while developing efficient micropropagation protocols. The effects of different concentrations and combinations of plant growth regulators on regeneration from various explants such as stem, leaf, epicotyl, hypocotyl and petiole have been reported. The explants were cultured on various cytokinin types including 0.1-3 mg/l BAP, Kn and TDZ (Behera et al., 2014; Kumar et al., 2011; Mubashar et al., 2015; Rathore et al., 2015)

Objective of the study

The objective of this research was to evaluate the effect of plant growth regulators in shoot induction of Sacha Inchi (*Plukenetia volubilis* L.)

Materials and methods

Plant material and source of explants

The seeds of Sacha inchi were obtained from Sanokkao sub-district, Phonthong district, Roi-Et (16˚17”N, 103˚58”E). Seed coats were removed and then surface sterilized with 70% Ethyl alcohol for 1 minute followed by 20% sodium hypochlorite solution (NaOCl) for 15 minutes and rinsed five times in sterile distilled water. The sterilized decoated seeds were germinated on MS medium supplemented with 3% w/v Sucrose and 1% w/v Agar. The plant embryo that develops in the four kinds of vegetative parts: cotyledon, epicotyl, hypocotyl and radicle after 4 weeks of germination. Hypocotyl explants were collected from germinated seedlings. Uniform culture conditions were maintained in all experiments. The pH of the medium was adjusted to 5.8 using 1M NaOH or HCl, prior to autoclaving at 1.05 kg/ cm^2 pressure at 121 °C for 15 minutes. The cultures were maintained at 25±2 °C under a 16 hours photoperiod with light intensity 35-40 µmol$m^{-2}s^{-1}$ (Phillip, Thailand).

Shoot bud induction

Hypocotyl explants were collected from 30 days post-germination. The hypocotyl segments were prepared by dividing hypocotyls into two parts as apical part and lower part. Explants were cultured on MS medium supplemented with different concentrations of individually and in combinations of 0.5 and 1 mg/l BAP, Kn and TDZ to find an optimal concentration of plan growth regulators for induction of shoot buds. The number of shoot buds per explant, the length of shoot and the number of leaves were recorded after 4 weeks.

Data analysis

All the experiments were repeated three times with 10 replicates, with one explant per bottle. The results were expressed as mean±SE. The data were analyzed using One-way analysis of variance (ANOVA). The difference between the mean of samples was analyzed by Duncan’s multiple range test using SPSS versions 22.0 at the $p < 0.05$.

Results

The surface sterilization of Sacha Inchi explants treating with 20% sodium hypochlorite solution for 15 minutes was the optimal condition (Table 1). However, the effective treatment should not harm the explants. The explants cultured on MS medium with incorporation of BAP, Kn and TDZ were significantly improved response. The results showed that highest number of shoot buds (5.00±0.19) was found in the MS mediums supplemented with 1 mg/l BAP, followed by the combination of 1 mg/l BAP and 1 mg/l Kn (4.00±0.57), and 0.5 mg/l BAP (2.68±0.37) (Table 2).

Table 1 The effect of sodium hypochlorite (NaOCl) concentration and duration times on seed germination of Sacha Inchi

Treatments			
Concentration (%)	**Duration (minutes)**	**% Contamination**	**% Establishment**
10	10	30.15±0.28	69.85±0.34
15	10	22.74±0.47	78.26±0.27
20	10	14.93±0.21	86.07±0.49
10	15	20.58±0.67	79.42±0.13
15	15	11.21±0.41	89.79±0.22
20	15	5.34±0.36	94.66±0.18

* 100 explants per treatment, Observations after 4 weeks.

Table 2 The effect of different plant growth regulators on shoot induction from hypocotyl explants of Sacha Inchi

Treatment				No. of shoot	Length of shoot (cm.)	No. of leaves
No.	**BAP (mg/l)**	**Kn (mg/l)**	**TDZ (mg/l)**			
T1	0	0	0	2.67±0.33c	5.00±0.48b	7.33±0.31b
T2	0.5	0	0	2.68±0.37c	7.33±0.31a	9.00±0.56b
T3	1	0	0	5.00±0.19a	4.67±0.39b	12.00±0.52a
T4	0	0.5	0	1.00±0.28d	5.67±0.34b	5.33±0.34c
T5	0	1	0	1.33±0.32d	4.33±0.33b	5.00±0.57c
T6	0	0	0.5	callus	callus	callus
T7	0	0	1	callus	callus	callus
T8	1	1	0	4.00±0.57b	4.67±0.28b	8.33±0.20b
T9	1	0	1	callus	callus	callus
T10	0	1	1	callus	callus	callus

* Values represent means±SE of 10 explants per treatment in three repeated experiments, Observations after 4 weeks.

** The different letters of the letter above of columns should be note under the table to significantly different at $p<0.05$.

Length of shoot was highly affected by shoot induction mediums. The highest value of shoot height was observed in MS medium supplemented with 0.5 mg/l BAP (7.33±0.31 cm) followed by MS mediums supplemented with the 0.5 mg/l Kn (5.67±0.34 cm), and MS mediums without plant growth regulators (5.00±0.57 cm). Significant differences in number of leaves were observed at different concentrations of plant growth regulators. The best number of leaves (12.00±0.52 leaves) was observed on a medium containing 1 mg/l BAP followed by 0.5 mg/l BAP (9.00±0.56 leaves), and the combination of 1 mg/l BAP and 1 mg/l Kn (8.33±0.20 leaves) (Table 2).

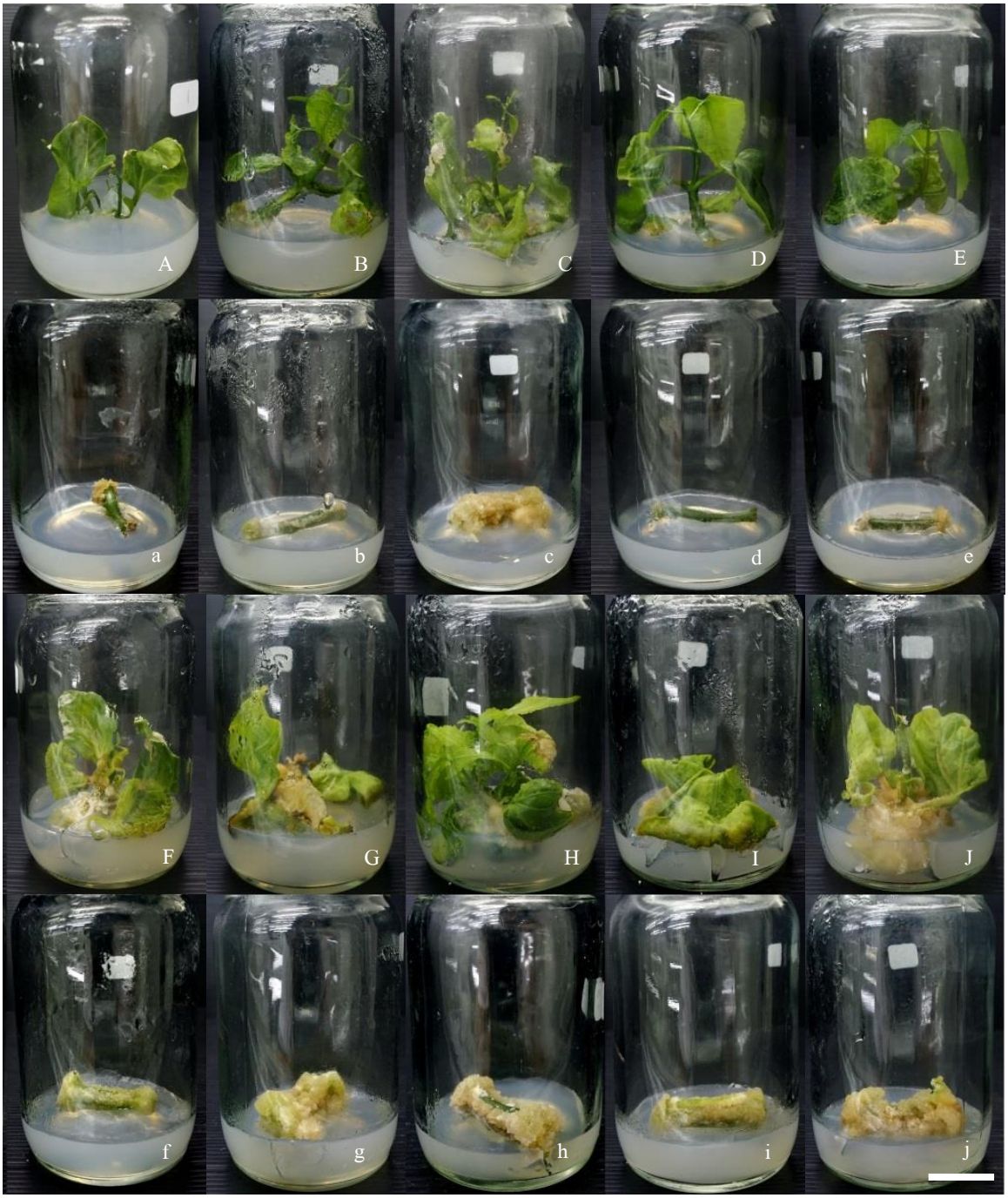

Figure 1 Shoot induction from hypocotyl explants at different concentrations of plant growth regulators after 4 weeks of culture: T1-T5 into apical part (A-E) and lower part (a-e); T6-T10 into apical part (F-J) and lower part (f-j).Scale bar = 3 cm.

In this investigation, Explants from hypocotyl apical part were efficiently regenerated, whereas explants from hypocotyl lower part were still juvenile and formed callus easily (Figure 1).

Conclusion and Discussion

In the present study, MS medium supplemented with BAP concentration 0.5 and 1 mg/l was found to be best for shoot induction and suggested that BAP plays an important role in the induction of shoot regeneration especially from hypocotyl explants for woody plant tissue culture.

The appropriate composition of the culture media is one the most important factors for the successful plant tissue culture. Addition of cytokinin (BAP, TDZ and Kn) to the medium is considered to promote cell division and cell expansion leading to induce the large number of multiple shoots. In addition, BAP at lower concentrations (0.1 - 1 mg/l) in the MS medium was the most effective for inducing shoot bud as reported by several woody plant species. The stimulating effect of BAP on shoot induction has been reported: Mulberry, Cassava, Physic nut and Para rubber (Chang, Aroonpong, 2015; Kumar, Reddy, 2012; Sharma et al., 2011). On the contrary, TDZ is known to bud break dormancy, stimulate growth and induce multiple shoots in various explants. It is very effective in amounts as low concentrations as 0.001- 0.01 mg/l which alone is more efficient than various combinations of other cytokinins. However, it also induces various physiological disorders on prolonged exposure or over dosage. In the present investigation, MS supplement with TDZ induced high frequency of compact callus that could not be generated (Singh et al., 2010). Moreover, in the future study, the combined effects of auxin and cytokinin were also proved to be essential for Sacha Inchi micropropogation.

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