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Effects of photoperiod and storage temperature on inulin and fructo-oligosaccharides accumulation in *In vitro* microtubers of kaentawan (*Helianthus tuberosus* L.)

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ABSTRACT

Kaentawan (*Helianthus tuberosus* L.) is an important tuberous crop accumulated high content of inulin and fructo-oligosaccharides (FOS) which are currently used as prebiotics in various food products. The aims of this research were to induce microtubers of Kaentawan under *in vitro* condition and study of photoperiod and storage temperature effects on inulin and FOS accumulation in obtained microtubers. The microtubers were induced on tuber induction medium with some modifications. Microtubers induced under long day photoperiod (16h light/ 8h dark (LD)) and darkness (D) condition gave higher fresh weight than those induced under short day photoperiod (8h light/ 16h dark (SD)). The different applied photoperiods had an effect to inulin and FOS production in microtubers. High amount of inulin was determined in LD induced microtubers while FOS (nystose and 1- kestose) was highly produced in microtobers induced under SD and D conditions. In addition, inulin content of LD induced microtubers storing in low temperature (4°C) for 15 days was degraded into FOS and sucrose while the inulin and FOS contents of SD and D induced microtubers was unchanged. These results gave an advantage for inulin and FOS production by means of plant organ cultures.

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INTRODUCTION

Kaentawan or Jerusalem artichoke (*Helianthus tuberosus* L.) is a tuberous plant rich in inulin-type fructans (Judprasong *et al*., 2011). Inulin and fructo-oligosaccharides (FOS); long and short chain fructans are currently used as prebiotics in various food products. They are major reserved carbohydrates found in Kaentawan tuber (Van Loo *et al*., 1995). Due to the variation of inulin and FOS contents derived from natural grown Kaentawan depends on geography and climate, plant varieties and plant diseases etc., many researches have paid attention to search for the alternative methods to produce inulin and FOS with homogenous quality and quantity from this plant. Microtubers are small tubers of tuber-producing plants which are induced under *in vitro* condition. They are the best plant material for germplasm conservation of tuber-producing plants because they are pathogen-free and are suitable for prolonged preservation at low temperature (Gamburg *et al*., 1999). Additionally, the carbohydrate levels in microtubers may give insight on the possible relation to carbohydrate content of mature tuber in the field (Pesce *et al*., 2011). The microtubers of Kaentawan were successfully established under *in vitro* conditions (Gamburg *et al*., 1999; Pesce *et al*., 2011). However, the production of inulin and FOS in induced microtuber has not been investigated. The production of inulin in calli and shoots of Kaentawan induced under *in vitro* conditions was reported by Taha *et al*. (2004). The present study was therefore aimed to evaluate the accumulation of inulin and FOS in induced microtuber. Additionally, the effects of photoperiod and storage temperature on inulin and FOS accumulation in obtained microtubers were also conducted. It has been reported that environmental factors including light intensity and temperature had an effect to the culture of microtubers of potato (Dobránszki *et al*., 2008). This may also influence to the culture of Kaentawan microtubers which has same pattern of tuber development as potato. The obtained results will be beneficial for inulin and FOS production under *in vitro* conditions.

MATERIALS AND METHODS

The establishment of microtubers

The microtubers were induced on tuber induction medium (Estrada *et al*., 1986) with some modifications. Murashige and Skoog (MS) basal medium supplemented with 0.4 mg/L thiamine HCl, 5.0 mg/L benzyladenine (BA), 500 mg/L chlorochlorine chloride (CCC), 80 g/l sucrose and 7.5 g/L agar, pH 5.6 was used as tuber induction medium. The 2 cm long nodal segments with axillary bud were excised from 1 month old sterile plant and aseptically transferred to bottle containing tuber induction medium. Three pieces of explants were cultured in one culture bottle. Experiments were duplicated with at least 9 bottles (replications) in each treatment. The design of all experiments was completely randomized. The obtained data were statistically analyzed using One-way ANOVA by SPSS program version 17.0. Significant difference was assessed at 5% level of probability ($P \leq 0.05$).

Effects of photoperiods and storage temperature on microtubers induction and accumulation of inulin and FOS

The cultures were maintained at 25±2°C and illuminated under fluorescent light with different photoperiods; long day photoperiod (16h light/ 8h dark (LD)), short day photoperiod (8h light/ 16h dark (SD)) and darkness (D) for 45 days. Three cultured bottles were taken to determine the growth of microtubers as fresh weight and the production of inulin and FOS. In order to study the relation of storage temperature to the changes of long chain (inulin) and short chain (FOS) fructans and other carbohydrate contents of induced microtubers, at least three of cultured bottles were kept at the same previous conditions for additional 15 days. The left cultured bottles were transferred to low storage temperatures at 4±2°C in darkness for additional 15 days.

Extraction of inulin and FOS

Inulin and FOS were extracted by distilled water (Sinngam and Ngampanya, 2012). The ratio of plant samples and distilled water was ½ (w/v). The homogenate was extracted three times at room temperature for 10 min and filtered through 3M filter paper with diameter of 0.2 mm. The filtrates were separated from plant residues by centrifugation. The residual extracts were stored in freezer for further analysis.

Determination of inulin, FOS and other carbohydrate contents

Inulin, FOS and other carbohydrate contents were analyzed by HPLC. The types and contents of inulin, FOS and other carbohydrates were analyzed by comparison to HPLC chromatograms of the known inulin, FOS (nystose, 1-kestose), sucrose, glucose and fructose.

RESULTS AND DISCUSSION

Induction and growth of microtubers

Microtubers were induced from explants cultured on tuber induction medium at 25±2°C and illuminated under fluorescent light with different photoperiods; long day photoperiod (LD), short day photoperiod (SD) and darkness (D) for 45 days (Figure 1).

Photoperiods (LD and SD) and light intensity (LD/SD and D) had effects to color and fresh weight of microtubers. The color of those induced under LD and SD were pale yellow to green while that induced under darkness was bleach. Light affected tuber morphology was reported by Dobránszki *et al*. (2008), color of potato tuber varied from green to blackish green due to the light illumination. Considering the growth of microtubers which expressed as fresh weight, those induced under LD and D conditions gave higher fresh weight than microtubers induced under SD photoperiod as shown in Table 1.

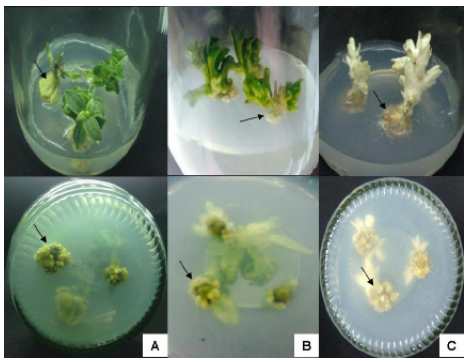

Figure 1 Microtubers were illuminated under fluorescent light with different photoperiods; long day photoperiod (A), short day photoperiod (B) and darkness (C) for 45 days. Black arrows depicted induced microtubers.

Table 1 Fresh weight of plantlets induced under different photoperiods for 45 days.

Plant part	Fresh weight (g/bottle)*		
	Long day (LD)	Short day (SD)	Darkness (D)
Aerial part (leave and stem)	0.35±0.15	0.31±0.16	0.30±0.13
Underground part (microtubers)	0.65±0.27	0.26±0.18	0.70±0.24

* Mean±SD of 3 bottles in each treatment

The fresh weight of aerial part (leave and stem) of induced plants under LD, SD and D was not difference. It suggested photoperiods had an effect to tuberization of Kaentawan while the growth of tubers did not result from effect of different light intensities (LD and D). Dobránszki *et al*. (2008) have reported that tuberization of potato was induced on medium with a layer of 8% sucrose solution poured onto 4-week-old plantlets cultures grown under long day (16h) whereas applying of high intensity of short day (8h) light delayed or inhibited tuber initiation.

Effects of induced photoperiods and storage temperatures on inulin and FOS accumulation

The different applied photoperiods had an effect to inulin and FOS production in microtubers as shown in Table 2. The significant difference (*P<0.05*) of inulin content in microtubers derived from different induction photoperiods was detected. High amount of inulin (long chain fructan) was determined in LD induced microtubers (34.18±1.69 mg/g fresh weight) while the amount of inulin found in those induced under SD and D was not difference. FOS (nystose and 1-kestose) was highly produced in microtubers induced under SD (6.28±1.04 and 8.71±2.12 mg/g fresh weight) and D (5.10±1.32 and 10.29±4.94 mg/g fresh weight) conditions. Although the inulin and FOS content detected in 45 days old microtubers was lower than those detected in natural grown tubers derived from 120-150 day olds plant (Judprasong *et al.*, 2011) but the ratio of inulin and FOS content found in both type of tubers were same. It suggests the occurring of similar synthesis pathway of fructans in *in vitro* and natural grown tubers.

Considering to the storage temperature, inulin content of LD induced microtubers storing in low temperature (4°C) for additional 15 days was degraded into nystose and sucrose while inulin content of those maintained under the same previous condition for additional 15 days was increasing (Table 2). There has reported that total FOS of burdock roots (*Arctium lappa* L.) increased during the first three weeks of storage at 15 and 20°C and then decreased, while at 0°C FOS increased progressively during storage. Inulin content of burdock roots decreased during storage and lower content was observed at 20°C. This suggests the carbohydrate metabolism in stored burdock depends partly on temperature (Ishiguro *et al.*, 2010). In this study, the inulin content of microtubers obtained from LD increased from 34.18±1.69 mg/g fresh weight to 57.16±12.99 mg/g fresh weight when stored at the same condition while inulin decreased from 34.18±1.69 mg/g fresh weight to 12.58±2.51 mg/g fresh weight when stored in darkness at 4°C (Table 2). In case of SD induced microtubers, inulin and FOS contents did not change when they were transferred to store at low temperature. The continuous production of inulin was observed in D induced microtubers which maintained at the same condition and stored at low temperature (Table 2).

FOS in plants may have functions other than carbon storage; they have been implicated in protecting plants against water deficit by drought or low temperature (Hendry and Wallace, 1993) and osmoregulators (Hincha *et al*., 2000). FOS may be a prefer fructan used for maintaining osmotic potential of plant cells when stress condition from low temperature occurred. Hence long chain fructan (inulin) was metabolized to short chain fructan (FOS). According to the microtubers induced under short day and darkness and kept at low temperature accumulated high content of FOS therefore they may be suitable material for short chain fructan production under *in vitro* condition. To explain the change of inulin and FOS accumulation during low temperature storage, the analysis of fructans-metabolizing enzymes activity should be investigated.

Table 2 Inulin, FOS (Nystose and 1-Kestose) and other carbohydrate contents of microtubers induced in different photoperiods and stored at 25ºC and 4 °C

Types of carbohydrate (mg/g fresh weight)*	Microtubers induction	Storage Condition		Microtubers induction	Storage Condition		Microtubers induction	Storage Condition	
	LD at 25±2ºC, 45 days	LD at 25±2ºC, 15 days	D at 4±2ºC, 45 days	SD at 25±2ºC, 45 days	SD at 25±2ºC, 15 days	D at 4±2ºC, 45 days	D at 25±2ºC, 45 days	D at 25±2ºC, 15 days	D at 4±2ºC, 45 days
Inulin	34.18±1.69b	57.16±12.99a	12.58±2.51d	18.00±2.00d	15.11±0.58d	15.58±8.11d	22.00±2.54cd	35.52±6.18b	31.05±7.54bc
Nystose	-	-	3.74±0.24b	6.28±1.04a	6.11±1.31a	4.67±1.09b	5.10±1.32ab	-	-
1-Kestose	5.39±1.66b	7.37±1.63ab	7.02±1.40ab	8.71±2.12ab	9.30±1.81ab	7.43±1.11ab	10.29±4.94a	6.27±2.70ab	7.30±1.83ab
Sucrose	8.29±3.91c	9.93±2.81c	25.32±2.75b	36.34±6.47a	32.10±9.14ab	37.80±7.54a	13.99±0.72c	8.18±1.51c	10.73±8.89c
Glucose	3.68±1.97b	6.30±0.44ab	6.84±0.25a	7.93±0.35a	8.64±1.25a	6.43±1.31ab	7.67±1.86a	3.98±0.72	7.63±2.49a
Fructose	3.61±0.31c	6.44±0.22bc	9.41±3.85b	10.08±2.52b	7.59±0.36bc	9.86±1.31b	4.68±2.05c	5.67±0.63b	15.36±3.87a

LD, SD and D were long day, short day and darkness in respectively.
*Mean±SD of 3 analyses in each treatment (n=3)
Different letter in the same row indicated the significant difference at *P≤0.05*

CONCLUSION

Photoperiods applying had some effects to growth of Kaentawan microtubers. High inulin content was derived from microtubers induced under long day photoperiod whereas higher FOS content was gained from those induced under short day and darkness conditions. The low storage temperature had an effect to the degradation of inulin content in LD induced microtubers into FOS and other carbohydrates. These results gave an advantage for inulin and FOS production by means of plant organ cultures.

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