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Research article

Effect of Sucrose on Microtuber Induction and Inulin Accumulation in Jerusalem Artichoke (*Helianthus tuberosus* L.)

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Abstract The effect of sucrose concentrations and photoperiod applying on microtuber induction and inulin accumulation of Jerusalem artichoke (*Helianthus tuberosus* L.) have conducted under *in vitro* condition. Numbers, lengths and weights of microtubers induced from the single node explants with 0.50 cm above stem node- and stem node- cutting was not significant difference. Concentration of sucrose (51.70, 60, 80, 100 and 108.20 g/l) containing in microtuber induction medium (MST) and photoperiod applying (10.30/13.70, 12/12, 16/8, 20/4 and 21.60/2.40 h light/dark) significant effected to numbers of microtubers ($P \leq 0.05$). The optimized sucrose concentration and photoperiod applying for highest numbers of microtubers was 100 g/l and 20/4 h light/dark, respectively. The significant difference of inulin content ($P \leq 0.05$) in microtuber induced from various conditions was determined. The microtubers induced on MST medium supplemented with 80 g/l sucrose under 16/8 h light/dark accumulated highest inulin content (324.84 ± 40.78 mg/ g dry weight) when compared with others. Data suggested that sucrose and light duration played role in microtuber induction and inulin accumulation of Jerusalem artichoke.

Keywords: Inulin, Jerusalem artichoke, Microtuber, Photoperiod, Sucrose

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INTRODUCTION

Jerusalem artichoke (*Helianthus tuberosus* L.) is one of important tuberous crops. It accumulates high amount of inulin in tuber as a reserve carbohydrate (Judprasong et al., 2018). Inulin is a fructan which consists of linear chains of β - 2, 1 - linked D - fructofuranose molecules terminated by a glucose residue through a sucrose-type linkage at the reducing end (Chi et al., 2011). Approximately 50% inulin is accumulated in the dried materials of the tubers (Pandey et al., 1999; Judprasong et al., 2011; Judprasong et al., 2018). It is currently used in food and pharmaceutical industry owing to its numerous benefits to human health and low caloric values when compared to other carbohydrates (Takeachi and Nagashima, 2011). In addition, it also has prebiotic property which can decrease the level of pathogenic bacteria and stimulate the growth of probiotic bacteria in the intestine. Jerusalem artichoke is therefore a choice of plants for inulin production. This plant can grow and yield in various conditions including cold and drought areas (Chi et al., 2011; Takeachi and Nagashima, 2011; Tanjor et al., 2012). Tubers from field- grown plant are major material for inulin production. However, those of field- grown tubers took at least four months for high yielding and may face plant disease and pest harmful. An alternative approach for tuber production of plant without abiotic and biotic interference is plant tissue culture technique. *In vitro* tuberization of plants such as potato (*Solanum tuberosum* L.) (Estrada et al., 1986; Dobránski et al., 2008; Mokshin et al., 2008; Nistor et al., 2010; Motallebi-Azar and Kazemiani, 2012; Fufa and Diro, 2014; Al-Hussaini et al., 2015; Rahman et al., 2015; Hossain et al., 2017; Li et al., 2020), steroid yam (*Dioscorea composite*) (Alizadeh et al., 1998) and Jerusalem artichoke (*Helianthus tuberosus* L.) (Gamburg et al., 1999; Polska and Ngampanya, 2015) has been reported. *In vitro* developing tubers called microtubers of those plants were induced and taken their advantage as disease-free germplasm for vegetative micrpropagation and experimental tools in basic research. Tuberization under *in vitro* condition is a complex process relied on many factors such as environmental factors (light duration, light quality and temperature) (Dobránski et al., 2008; Al-Hussaini et al., 2015; Li et al., 2020), composition of medium (sucrose, nitrogen, gelling agents) (Dobránski et al., 2008; Motallebi-Azar and Kazemiani, 2012; Al-Hussaini et al., 2015; Hossain et al., 2017), genotype and explants used for tuberization (Estrada et al., 1986; Dobránski et al., 2008; Nistor et al., 2010; Fufa and Diro, 2014). There has reported that photoperiod and storage temperature influenced on microtuber induction and inulin accumulation in *in vitro* microtuber of Jerusalem artichoke (Polska and Ngampanya, 2015). Apart from light and temperature, medium composition particularly sucrose has also reported as an important factor playing role in tuberization of potato under *in vitro* condition (Dobranski et al., 2008; Nistor et al., 2010; Fufa and Diro, 2014; Al-Hussaini et al., 2015; Hossain et al., 2017). Sucrose is a carbon source that serves as energy for *in vitro* plant growth and development. There has evident that it can stimulate tuber formation and numbers in potato (Nistor et al., 2010; Fufa and Diro, 2014; Al-Hussaini et al., 2015; Hossain et al., 2017). Like potato, Jerusalem artichoke is in a group of tuberous crop and has a same pattern of development. Therefore, it may be possible to stimulate tuber formation via sucrose signaling as previously reported in potato (Dobranski et al., 2008; Nistor et al., 2010; Al-Hussaini et al., 2015; Hossain et al., 2017). Microtuber of potato did not induce and form when cultured on medium without sucrose supplementation. Minimum concentration of sucrose for microtuber formation in potato was 8% (Hossain et al., 2017). Additionally, light duration and quality is also reported as an environmental factor effected to tuber formation of potato (Dobranski et al., 2008; Al-Hussaini et al., 2015; Li et al., 2020). This research aimed to evaluate effects of sucrose concentration and photoperiod applying on the formation and numbers of microtubers of Jerusalem artichoke under *in vitro* condition. Additionally, inulin production and accumulation were also determined. The successful of Jerusalem artichoke microtuber induction under *in vitro* condition together with its high accumulation of inulin would be an alternative way for inulin production instead of inulin production relied on field- grown tuber.

MATERIALS AND METHODS

Effect of explant cutting on microtuber induction

Microtuber induction from single node explants (the 2nd- 4th stem node) with different cutting types (0.50 cm above stem node- and stem node- cutting) were compared. The 1.50 - 2 cm long nodal segments with axillary bud were excised from one-month old sterile plant and aseptically transferred to bottle containing tuber induction medium (MST; MS basal salts + MS vitamins + 5.00 mg/l benzyladenine (BA) + 500 mg/l chlorochlorine chloride (CCC) + 0.40 mg/l Thiamine HCl + 1% activated charcoal + 80 g/l sucrose, pH 5.60) (Polsa and Ngampanya, 2015). The cultures were cultivated under fluorescent light with long day photoperiod (16/8 h light/dark) at 25 ± 2°C for 45 days. Formation time, numbers, lengths and weights of microtubers were recorded. Three pieces of explants were cultured in one culture bottle. At least three bottles with 9 explants in total were investigated per treatment. Experiments were conducted three times with three replications (bottles).

Effect of sucrose on microtuber induction and inulin accumulation

The 2 cm long nodal segments with axillary bud (0.50 cm above stem node-cutting) were excised from sterile plant and aseptically transferred to bottle containing MST medium (Polsa and Ngampanya, 2015) supplemented with 60, 80 or 120 g/l sucrose. The cultures were cultivated under fluorescent light with long day photoperiod (16/8 h light/dark) at 25 ± 2°C for 45 days. Ten pieces of explants were cultured per treatment. Experiments were conducted three times, and each experiment contained 10 explants). After 45 days of culturing, the formation and number of microtubers were recorded. The determination of fresh weight and the production of inulin was also recorded.

Optimization of sucrose concentration and photoperiod applying on microtuber induction and inulin accumulation

To obtain high microtuber numbers, the experimental treatment of sucrose concentration and photoperiods treatments was optimized by central composite design (CCD). The cultures were cultivated at 25 ± 2°C for 45 days. Ten pieces of explants were cultured per treatment. Experiments were conducted three times, and each experiment contained 10 explants). After 45 days of culturing, the formation and number of microtubers were recorded. The determination of dry weight and the production of inulin was also recorded.

Determination of inulin content by HPLC

Inulin content was determined according to HPLC analysis procedure described by Bampensin et al (2019). The obtained microtubers from each treatment were extracted by distilled water. The ratio of microtubers to distilled water was 1 : 2 (w/v). The homogenate was extracted three times for 10 min at room temperature. The supernatant was collected by centrifugation and pooled. Inulin contents were analyzed by HPLC. The types and contents of inulin were analyzed by comparison to HPLC chromatograms of the known inulin.

Statistical analysis

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 ($P \leq 0.05$).

RESULTS

Effect of different explant cutting on microtuber induction

Formation of microtubers from the single node explants with 0.50 cm above stem node- and stem node- cutting cultured on MST supplemented 80 g/l sucrose was observed after 15 days of induction and size of those microtubers was larger upon time course of culturing until 45 days as shown in Figure 1.

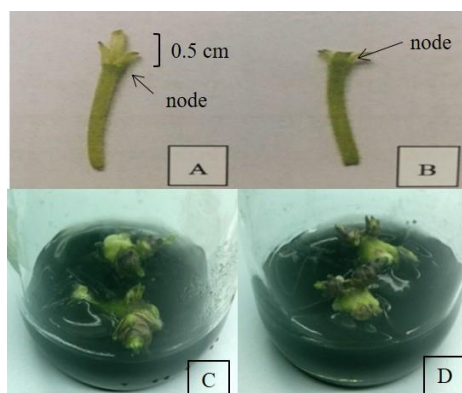


Figure 1. The single node explant with 0.50 cm above stem node- cutting (A) cultured on MST medium supplemented with 80 g/l sucrose under long day photoperiod (16/8 h light/dark) for 45 days (C) and the single node explant with the stem node- cutting (B) cultured on MST medium supplemented with 80 g/l sucrose under long day photoperiod (16/8 h light/dark) for 45 days (D).

Table 1 indicated numbers, lengths and weights of microtubers induced from the single node explant with 0.50 cm above stem node- (type I) and stem node- cutting (type II) was not significant difference. The numbers of microtubers obtained from that of the type I cutting was higher than type II, therefore the type I cutting explant was selected for further experiments because of its numbers of microtubers was higher than those recorded in type II cutting explant.

Table 1. Numbers, lengths and weights of microtubers induced from different cutting explants cultured on MST supplemented 80 g/l sucrose under long day photoperiod (16/8 h light/dark) for 45 days.

Explant cutting	Numbers of induced microtubers* (tuber)	Lengths of induced microtubers* (cm/microtuber)	Weights of induced microtubers* (g/microtuber)
Type I	14.0 ± 1.0 ^a	0.62 ± 0.18 ^a	0.075 ± 0.02 ^a
Type II	11.0 ± 2.0 ^a	0.59 ± 0.09 ^a	0.076 ± 0.02 ^a

Note: Type I is the single node explant with 0.5 cm above stem node- cutting.

Type II is the single node explant with stem node- cutting.

*Mean ± SD (n=9)

Different letters in the same column indicated the significant difference at $P \leq 0.05$

Effect of sucrose on microtuber induction and inulin accumulation

Microtubers were successfully induced on the tuber induction medium (MST) supplemented all tested sucrose concentrations as shown in Figure 2. In case of explants cultured on MST with 60 g/l sucrose, shoot elongation and leaves development were clearly seen. In agreement with other reports (Gamburg et al., 1999; Polsa and Ngampanya, 2015), microtubers formation was observed on MST supplemented 80 g/l sucrose. The size of microtubers induced on MST supplemented with 80 g/l sucrose was larger than those induced on MST supplemented with 120 g/l sucrose.

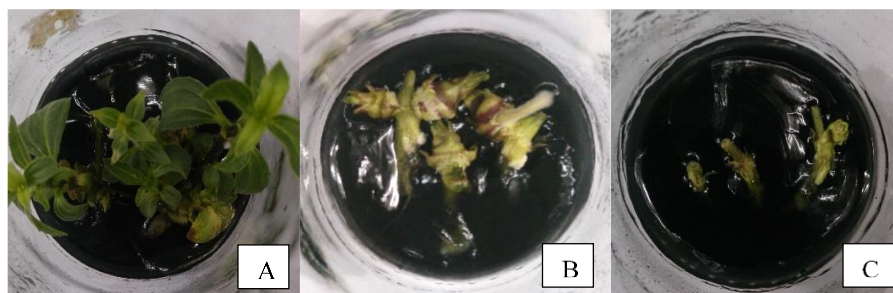


Figure 2. Microtubers were induced on tuber induction medium supplemented with different sucrose concentrations; 60 (A), 80 (B) and 120 g/l (C) under long day photoperiod (16/8 h light/dark) for 45 days.

There was significant difference ($P \leq 0.05$) of microtuber numbers and fresh weight as shown in Table 2. High numbers of microtubers was determined in the MST medium supplemented with 80 g/l sucrose while those induced on MST with 60 and 120 g/l sucrose gave less microtubers numbers and fresh weight.

Table 2. Numbers and fresh weight of microtubers induced on MST medium supplemented with 60, 80 and 120 g/l sucrose for 45 days.

Sucrose (g/l)	Numbers of microtubers ^{*,**}	Fresh weight (g/microtuber)
60	6.0 ± 2.6 ^b	0.053 ± 0.05 ^{ab}
80	14.3 ± 2.0 ^a	0.106 ± 0.03 ^a
120	6.0 ± 2.6 ^b	0.042 ± 0.01 ^b

Note: *Mean ± SD (n=10), ** Number of microtubers is amount of all in each treatment., Different letters in the same column indicated the significant difference at $P \leq 0.05$

After microtuber induction for 45 days, the obtained microtubers were extracted to determine the inulin content using HPLC technique by comparison to HPLC chromatograms of known inulin and other standard sugars. HPLC chromatograms revealed that peak pattern of microtuber extract was same as the peak pattern of extract from field-grown tuber as shown in Figure 3. The extract consisted of 6 carbohydrates which were inulin, nystose, 1-kestose, sucrose, glucose and fructose.

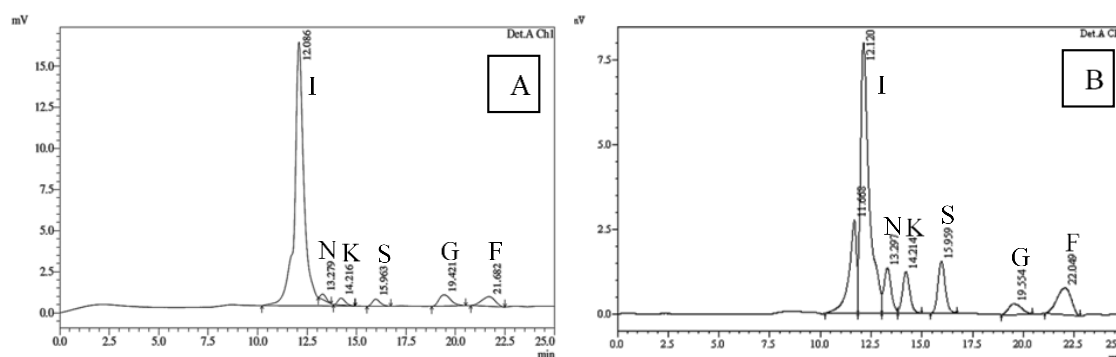


Figure 3. HPLC chromatograms of extract from field-grown tuber of Jerusalem artichoke (A) and microtubers induced on MST medium with 120 g/l sucrose (B) I = inulin, N = nystose, K = 1-kestose, S = sucrose, G = glucose and F = fructose.

The inulin content in microtubers induced from MST medium with different concentrations of sucrose (60, 80 and 120 g/l) were significant difference ($P \leq 0.05$) as shown in Table 3. The microtubers induced on MST medium supplemented 80 g/l sucrose accumulated higher inulin content (73.56 ± 16.46 mg/ g fresh weight) than those cultured on medium supplemented 120 (44.93 ± 23.80 mg/ g fresh weight) and

60 g/l sucrose (7.53 ± 3.24), respectively. Although, the inulin content in microtubers obtained from MST medium with 80 and 120 g/l were not significant difference ($P \leq 0.05$) but the maximum yield of inulin content was obtained from induced microtubers cultured on MST medium supplemented with 80 g/l sucrose (Table 2). Additionally, this condition also gave higher values of fresh weight and number of obtained microtubers.

Table 3. Inulin, Fructo-oligosaccharides (Nystose and 1-kestose) and other sugars content of microtubers induced in different sucrose concentrations for 45 days.

Sucrose (g/l)	Type of sugar (mg/g fresh weight)					
	Inulin	Nystose	1-kestose	Sucrose	Glucose	Fructose
60	7.53 ± 3.24^c	0.56 ± 0.79^a	0.86 ± 0.58^c	1.74 ± 1.18^b	1.96 ± 1.24^b	2.97 ± 1.95^b
80	73.56 ± 16.46^b	3.28 ± 1.89^a	3.10 ± 0.34^b	3.10 ± 0.34^b	-	3.27 ± 0.475^b
120	44.93 ± 17.37^{bc}	3.16 ± 0.78^a	6.91 ± 0.71^a	17.25 ± 5.73^a	9.32 ± 4.19^a	20.03 ± 7.09^a
Field- grown tuber	132.29 ± 23.8^a	1.18 ± 0.71^a	1.76 ± 0.06^c	2.15 ± 0.04^b	4.30 ± 4.19^{ab}	6.69 ± 0.14^b

Note: *Mean \pm SD (n=2), Different letters in the same column indicated the significant difference at $P \leq 0.05$

Optimization of sucrose concentration and photoperiod applying on microtuber induction and inulin accumulation

Sucrose concentration and photoperiod applying was optimized by central composite design (CCD). As shown in Table 4, sucrose concentrations in microtuber induction medium and photoperiod applying significantly affected to numbers of microtubers ($P \leq 0.05$). The optimum condition for numbers of microtubers was 100 g/l sucrose with 20/4 h light/dark photoperiod while inulin content was highest in microtubers induced in 80 g/l sucrose with 16/8 h light/dark photoperiod.

Table 4. Numbers and inulin content of microtubers induced from different sucrose concentrations and photoperiod applying

Sucrose (g/l) and photoperiod apply (h light/dark)	Numbers of microtubers ^{*,**}	Inulin content (mg/g dry weight)*
51.7 (16/8 h)	3.66 ± 0.6^c	232.00 ± 90.58^{ab}
60 (12/12 h)	9.0 ± 1.0^{ab}	276.83 ± 30.22^{ab}
60 (20/4 h)	5.7 ± 0.6^{bc}	192.47 ± 55.59^b
80 (10.3/13.7 h)	8.3 ± 3.2^{abc}	250.68 ± 40.71^{ab}
80 (16/8 h)	7.7 ± 2.0^{abc}	324.84 ± 40.78^a
80 (21.6/2.4 h)	6.7 ± 0.6^{bc}	250.47 ± 53.65^{ab}
100 (12/12 h)	9.7 ± 2.3^{ab}	191.81 ± 56.44^b
100 (20/4 h)	12.0 ± 1.7^a	287.33 ± 19.66^{ab}
108.2 (16/8 h)	8.3 ± 1.2^{abc}	191.70 ± 22.85^b

Note: *Mean \pm SD (n=10), ** Number of microtubers is amount of all in each treatment., Different letters in the same column indicated the significant difference at $P \leq 0.05$

DISCUSSION

In vitro tuber is a complex process relied on many factors such as environmental factors (light and temperature), composition of medium (sucrose, nitrogen, gelling agents), genotype and explants used for tuberization (Dobrński et al., 2008; Motallebi-Azar and Kazemiani, 2012; Fufa and Diro, 2014; Al-Hussaini et al., 2015; Hossain et al., 2017; Li et al., 2020). In potato, microtubers were induced from over 50 different genotypes. The induced microtubers of potato were morphologically similar to field-

grown tuber. However, different microtuber numbers and weight among genotypes tested were observed (Estrada et al., 1986; Nistor et al., 2010; Fufa and Diro, 2014; Hossain et al., 2017). Explant used for tuberization is an important factor. The physiological age, type of explants and explant density effected to tuber development. Stolons or axillary buds has documented to use as explant for tuber formation. Nodal segments were suitable explant for tuberization of plant under *in vitro* condition and explants with one node is induced and initiated higher microtubers than that explant with two-five nodes (Dobránski et al., 2008). One bud cutting was successful for microtuber induction in potato (Mokshin et al., 2008; Motallebi-Azar and Kazemiani, 2012; Fufa and Diro, 2014) and Jerusalem artichoke (Gamburg et al., 1999; Polsa and Ngampanya, 2015). To control hormonal balance, the single node from the 2nd -4th stem node was selected as explant for tuberization in this study. Both cutting type was not significant difference in microtuber formation and size. It may suggest that the hormone level at axillary bud of both explants was not difference and bud dormancy was terminated leading to tuber development. Apart from effect of explant used for tuberization, carbon source in medium also strongly influenced on tuber formation. In this study, microtubers were successfully induced on medium with exogenously supplemented plant growth regulators (BA and CCC as cytokinin) and sucrose at 60, 80 and 120 g/l. In case of explants cultured on MST with 60 g/l sucrose, shoot elongation and leaves development were clearly seen. It dues to sucrose concentration is not high enough for extra organs formation. Hossain et al. (2017) has reported that stem segments of potato cultured on medium without sucrose supplementation did not form microtuber. Sucrose concentration at 8% was minimum level for microtuber formation. In current study, microtubers formation of Jerusalem artichoke was also observed on MST supplemented 80 g/l sucrose. This result was in agreement with other reports of microtubers induction in Jerusalem artichoke (Gamburg et al., 1999; Polsa and Ngampanya, 2015). The size of microtubers induced on MST supplemented with 80 g/l sucrose was larger than those induced on MST supplemented with 120 g/l sucrose. As generally known that sucrose serves as energy source for *in vitro* plant growth and development, but high level of soluble sugars can cause the formation of extra organ in different plant species such as potato (Gibson, 2005). Although high sucrose concentration can induce microtuber but higher level may effect to size of microtuber as well. There has reported in potato tuberization that increasing of sucrose concentration up to 12% caused a delay in tuber initiation and resulted in smaller tuber (Dobránski et al., 2008; Hossain et al., 2017). In this study, number and size of microtubers obtaining from medium supplemented 120 g/l sucrose was also less than those induced on 80 g/l sucrose supplementation. In order to optimize sucrose concentration for microtuber production, sucrose concentrations at 51.70, 60, 80 and 100 g/l also tested in this study. The results indicated that 80-100 g/l sucrose is optimal for microtuber production. Additionally, microtuber numbers induced under long or short day photoperiod was not significant difference when 80-100 g/l sucrose was supplemented while short day photoperiod (12/12 h light/dark) effected to number of microtubers induced on medium supplemented 60 g/l sucrose. In potato, light duration significantly affected to tuber initiation only in high carbon source concentration (Al-Hussaini et al., 2015). Other sugars such as glucose, fructose and alcohol sugars (mannitol and sorbitol) has also reported to use for tuber induction of potato (Mokshin et al., 2008; Motallebi-Azar and Kazemiani, 2012). Among carbon sources used for tuberization, sucrose is a sugar normally used in plant tissue culture medium. It plays role in plant development and morphogenesis. It is essential for *in vitro* plant as energy source or osmotic potent agent. Additionally, it also serves as a signal for *in vitro* tuberization of plant. There has reported that high sucrose concentration (8%) in medium without plant growth regulators supplementation can induce microtuber of potato (Alix et al., 2001; Dobránski et al., 2008; Al-Hussaini et al., 2015; Hossain et al., 2017). For inulin accumulation, results indicated that microtubers of Jerusalem artichoke induced in this study can produce same carbohydrates of field- grown tuber. The optimum condition for high inulin accumulation is 80 g/l sucrose supplementation. All data obtained from this study could be a choice for inulin production by means of organ culture techniques. Microtuber production is an efficient method for obtaining a healthy material that can produce same valuable substances as found in natural grown

plant. In the same time the microtubers are important because they could be produced in any period of the year and they are easy to be transported and stored (Nistor et al., 2010). However, cost of high sucrose supplemented in culture medium for microtuber and inulin production should be further feasibility study.

CONCLUSION

High sucrose concentrations significantly affected to microtubers numbers of Jerusalem artichoke ($P \leq 0.05$). The high numbers of microtuber was obtained when 80-100 g/l sucrose was supplemented to MST medium. Microtubers can produce inulin that was similar to that was found in field- grown tuber. In conclusion, MST medium supplemented 80-100 g/l sucrose was optimal for microtuber induction and inulin accumulation.

AUTHOR CONTRIBUTIONS

Nalinee Homsuwan conducted all of the experiments excepted the part of optimization of sucrose concentration and photoperiod applying on microtuber induction and inulin accumulation, performed the statistical and wrote the manuscript. Kajorn Mapiyaphun conducted the experiments in the part of optimization of sucrose concentration and photoperiod applying on microtuber induction and inulin accumulation, performed the statistical. Budsaraporn Ngampanya assisted in designed and gave the advices in all of the experiments, wrote and improve the manuscript. All authors have read and approved of the final manuscript.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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