CHAPTER III

RESULTS

Plant tissue culture

1. Seed germination efficiency

Safflower's seeds to be used as a source of the explants for tissue culture were periodically tested for germination efficiency (section 5.1). The germination efficiency of seeds was gradually decreased even they were stored in the cold room (5-10 ^{o}C) (Fig. 5). However, storage of seeds under this condition was good enough to use along the whole period of this research work since the percentage of germination was not less than 70% after 15 months.

2. Seed surface sterilization

Seeds were sterilized by clorox treatment. The clorox concentration and period of treatment were varied. The percentage of seed contamination in each treatment obtained according to Table 2. It was cleared that either the increment in clorox concentration or time of immersion could reduce percentage of seed contamination. In the mean time the percentage of abnormal seedlings was also calculated. Therefore, the condition selected for seed sterilization in next experiments carried out in this research was 15 minutes immersion in 10% clorox containing 0.05% (v/v) triton X-100.

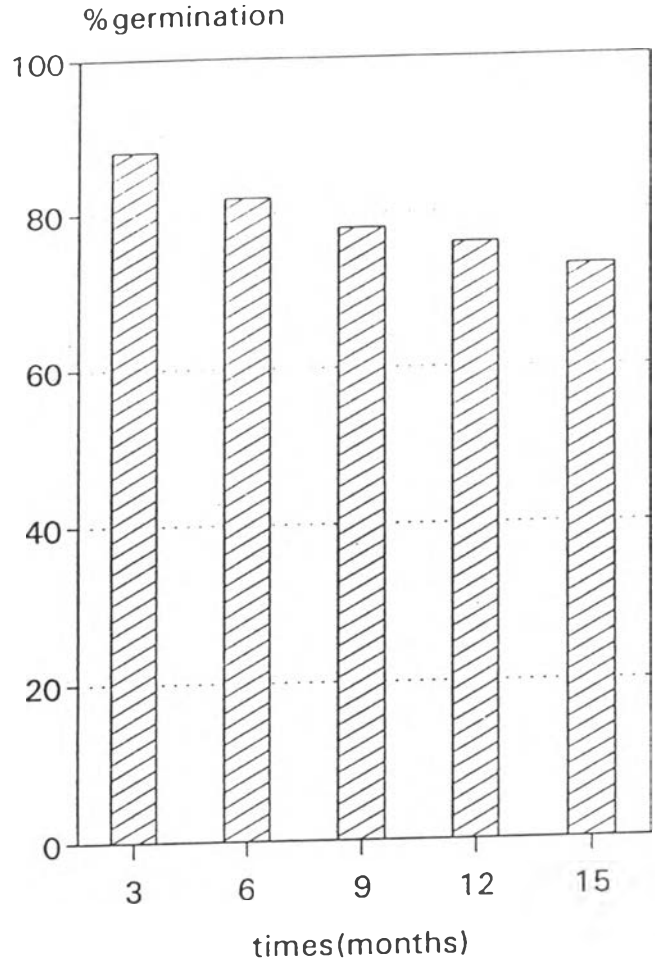


Figure. 5 The percentage of *Carthamus tinctorius* Linn. seeds germination after being stored in the cold room (5-10°C) for a long period.

Table. 2 The percentage of contamination of *Carthamus tincrius* Linn. seeds at various surface sterilized conditions.

conditions*	No. of explants	No. of contaminated explants (4 weeks cultured)	Contamination (%)
(10,10)	50	19	38
(10,15)	50	9	18
(10,20)	50	12	24
(10,30)	50	17	34
(15,10)	50	13	26
(15,15)	50	10	20
(15,30)	50	10	20
(20,10)	50	8	16
(20,15)	50	9	18
(20,20)	50	11	22
(30,10)	50	8	16
(30,15)	50	9	18

*- Clorox concentration (% v/v) and immersion time (min.) used for seed surface sterilization.

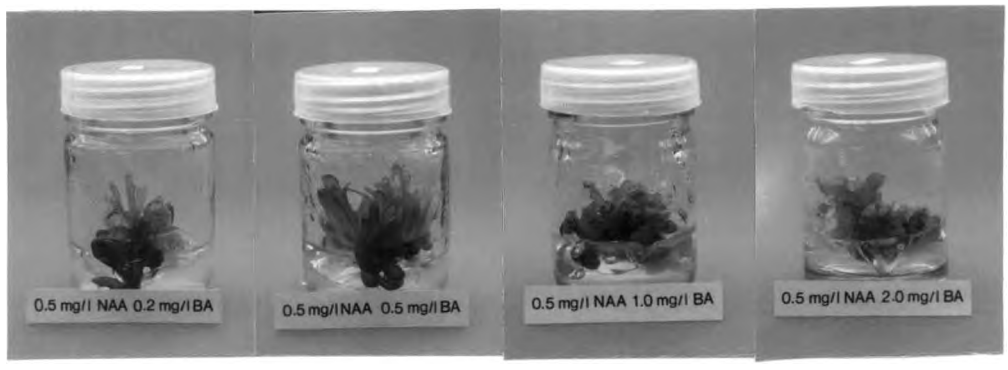
Callus induction and regeneration

1. Light and growth regulators

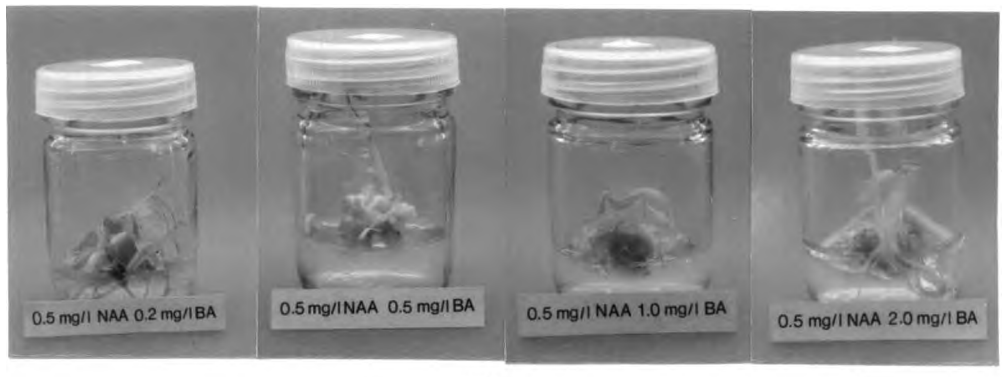
After the excised cotyledons had been previously cultured in the dark at 2 and 4 weeks interval, both groups were incubated in the light under 16/8 hrs. photoperiod. The earliest visible signs of callus growth was noticed within 4-5 days. Further cultivation for 14 days, some callus could be regenerated to form leaves and roots like structures (Fig. 6). Table. 3 , 4 and Fig. 7 summarized the effect of growth regulators (NAA and BA) and light incubation periods on the external morphology of excised cotyledons after 4 weeks cultivation. The callus induction efficiency were highly observed (90-98%) in all of the media tested. The explants previously incubated in the dark for 2 weeks period obviously showed a higher percentage of regeneration than that of 4 weeks period in the medium supplemented with NAA and BA the same concentration of 0.5 mg/l. (Fig. 7). However, continuous subculturing of the regenerated calluses which was carried out in the same media for a few cycle leading to the increment of callus mass within the cultivated samples.

2. Light and types of the explants

The effect of dark pre-incubation periods and types of the explants on callus induction and shoot regeneration were studied. The excised cotyledons and hypocotyl segments were cultured on MS medium supplemented with 0.5 mg/l NAA and 0.5 mg/l BA at 25±2^{o}C. They were previously incubated in the dark condition at various periods (0, 1, 2, 3 and 4 weeks) before transferring to the light at 16/8 hrs. photoperiod. Under these conditions, the ability of excised cotyledons and hypocotyl segments to form callus was found to be different. On the fifth day, the excised cotyledons and hypocotyl segments began to swell unevenly and produced small callus mass emerged from the abaxial surface and edges of tissues. After 14 days of



(A)



(B)

Figure. 6 Induction of callus regeneration from cotyledon explants of *Carthamus tinctorius* Linn. on MS medium supplemented with different levels of plant growth regulators (NAA, BA).

(A) - 2 weeks pre-incubation in the dark

(B) - 4 weeks pre-incubation in the dark

Table. 3 Effect of plant growth regulators on callus induction efficiency of *Carthamus tinctorius* Linn. cotyledons.

Growth regulators (mg/l)		Callusing (%) Dark pre-incubation period	
NAA	BA	2 weeks	4 weeks
0.5	0.2	98	98
0.5	0.5	98	98
0.5	1.0	96	98
0.5	2.0	95	98

Table. 4 Effect of light and plant growth regulators on the external morphology of *Carthamus tinctorius* Linn. cotyledon callus.

Growth regulators (mg/l)		Morphogenetic response Dark pre-incubation period	
NAA	BA	2 weeks	4 weeks
0.5	0.2	Leaf - like organs developed on callus surface	Root formation on callus surface
0.5	0.5	Leaf - like organs developed on callus surface	Shoot formation on callus surface
0.5	1.0	Leaf - like organs with bud development	Shoot and root formation on callus surface
0.5	2.0	Leaf - like organs with bud development	Shoot and root formation on callus surface

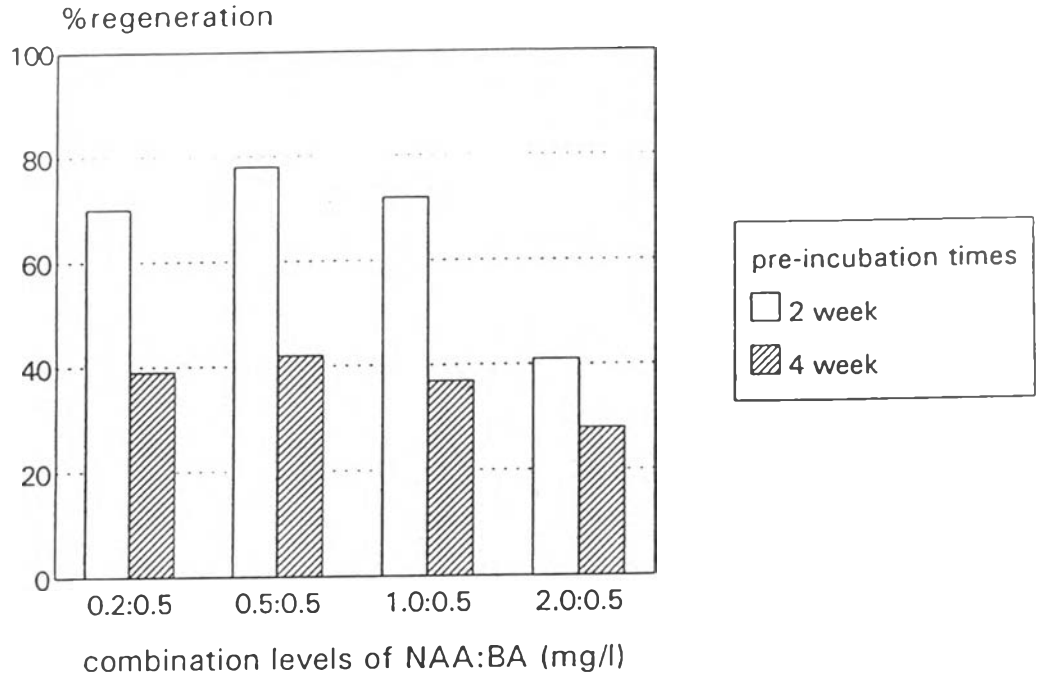


Figure. 7 Effect of light pre-incubation time and plant growth regulators (NAA and BA) on plant regeneration.

culture, some calli of both excised tissues were started to regenerate. Details on characteristics of callus and morphogenetic response of regenerated tissues were observed at various cultivation conditions (Table. 5, 6). Therefore, excised cotyledons with 2 weeks dark pre-incubation were used as the source of explants for the following experiments (Fig. 8).

3. Direction of excised explants.

To determine the appropriate direction of excised cotyledons and hypocotyl segments for the optimal callus growth and regeneration, both explants were cultured on MS medium supplemented with 0.5 mg/l each of NAA and BA containing 2.0 % (w/v) sucrose in 0.7 % (w/v) agar, the frequency of callus induction and plant regeneration from callus was shown in Figure. 9. The callus induction was first observed in all treatments of the cotyledons within 4 days after culturing while the longer time for callus formation was found in all treatments of hypocotyls (14 days). The friable light-green hypocotyl calli were initially much smaller than calli proliferated from the cut surface cotyledons. It was also found that the cotyledons with upper cut transversely yielded the highest percentage of callus induction (96 %). Nevertheless, only 20% of root-like organs were developed by hypocotyl segments. The regenerated calli which were developed from cotyledons produced leave-liked structures on the callus surface. The differentiate structures produced in culture including a variety of abnormal organs that failed to produce true leaves or shoot were also noticed (Table 7).

4. Maturity of seedlings

Different ages of seedlings were used for callus initiation and regeneration. The original callus derived from the abaxial surface and edges of the excised cotyledons was noticed after 5 days in culture. The callus incubated for 4 weeks in the dark was consisted of whitish, wet and loosely-packed cells, which was different

Table. 5 Effect of dark pre-incubation periods on callus induction and shoot regeneration of explants from *Carthamus tinctorius* Linn.

Explants	Dark pre-incubation periods (weeks)	Callusing* (%)	Regeneration* (%)
Cotyledons	0	96	41
	1	98	50
	2	98	75
	3	98	68
	4	95	42
Hypocotyls	0	90	3
	1	85	0
	2	88	3
	3	83	0
	4	86	32

*MS medium supplemented with 0.5 mg/l NAA and BA at 16/8 hrs. photoperiod.

Table. 6 Effect of dark pre-incubation periods and types of explants on the callus and external morphology of *Carthamus tinctorius* Linn. tissue culture.

Explants	Dark pre-incubation penod (weeks)	Callus growth*	Morphogenetic response
Cotyledons	0	++, green compact	leaf-like organs developed on callus surface
	1	+++, green compact	leaf-like organs developed on callus surface
	2	+++, pale-green, compact & nodular	leafy shoot developed on callus surface
	3	++, pale-green, friable	Nodular structure on callus surface
	4	++, pale-yellow, friable	leave and roots-like structure developed on callus surface
Hypocotyls	0	++, pale-green friable	no morphognetic response
	1	+, pale-green friable	no morphogenetic response
	2	+, pale-green friable	a few nodular green on callus surface
	3	++, pale-yellow, friable	no morphogenetic response
	4	++, whitish, friable	a few nodular green on callus surface

*callus growth + less (1-5 mm.)
++ modulate (5-10 mm.)
+++ profuse (>10 mm.)

Figure. 8 Shoot regeneration of *Carthamus tinctorius* Linn. cotyledons after culturing for 2 weeks in the dark on MS medium supplemented with 0.5 mg/l NAA and 0.5 mg/l BA and transferring to 16/8 hrs. photoperiod for 50 days.

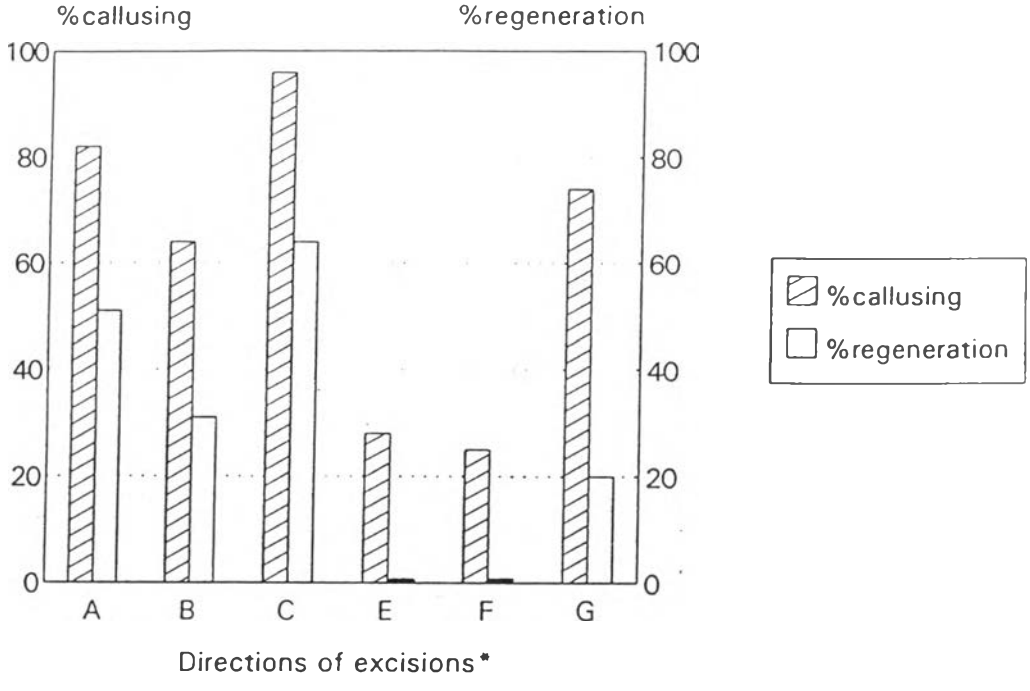


Figure. 9 Effect of direction of excision explant segments on callus induction and plant regeneration at the period of 4 weeks after culturing on MS medium containing 0.5 mg/l NAA and 0.5 mg/l BA.

* Direction of excisions

Excised cotyledons

A - longitudinal sections

B - lower cut transversely

C - upper cut transversely

** Hypocotyl segments

E - top up-bottom down

F - bottom up-top down

G - outer surface horizontally

Table. 7 Effect of direction of excised cotyledons and hypocotyl segments on growth and external morphology of *Carthamus tinctorius* Linn. callus.

Types of excisions*	Callus growth**	Morphogenetic response***
Excised cotyledons		
a	pale-green, wet, friable, xxx	leaf-like organs with bud primordia developed on callus surface
b	pale-green, wet, friable, x	leaf-like organs developed on callus surface
c	green, wet, xxx mix compact & friable	leaf-like organs and root liked structure developed on callus surface
Hypocotyls segments		
e	-	no morphogenetic response
f	-	no morphogenetic response
g	yellowish, wet, friable, xx	a few bud primordia and some root-like organs developed on callus surface

* Types of excisions

Excised cotyledons
- a - longitudinal sections
- b - lower cut transversely
- c - upper cut transversely

Hypocotyl segments
- e - top up-bottom down
- f - bottom up-top down
- g - outer surface horizontally

** callus gowth

x	- small	(1-5 mm.)
xx	- medium	(5-10 mm.)
xxx	- large	(>10 mm.)

***MS medium supplemented with 0.5 mg/l NAA and 0.5 mg/l BA

from the callus of the same age when exposed to light. The morphological appearence was pale green, wet and more dense cells looked like a cluster of compact. The relative size of cotyledon callus from 14 and 17 days old seedlings was bigger than that of callus in the other treatments. The average number of callus induction was 90 % of the original explants. Some regenerated callus was occurred from the 14 and 17 days old seedlings which was incubated in both light and dark. Some differentiated structures of callus tissues could be observed such as, a variety of leaf-like organs, small roots with root hairs (Fig. 10). The highest plant regeneration (76%) efficiency was achieved with transversely cut-upper part of 14 day-old cotyledons cultured in the light.

For hypocotyl segments, the initial callus was first observed after 9 days of cultivation. Callus cells were readily proliferated from the cut surface.The light grown callus was characterized by the common pale green, wet and friable in comparison to the dark grown callus which was mostly consisted of yellowish, wet and friable. Only the calli which were exposed to the light for 21 days can be regenerated. Organs development (leaf and root-liked structures) and a few bud primordia were occured on the callus surface. The highest level of regeneration was found in the light grown callus from 14 day-old seedlings, while the dark grown callus did not regenerate any organs.

From these results, it was found that cotyledon explants from 14 day-old seedling yielded best callus formation and plant regeneration (Table. 8).

The production of shoot was found only in the cotyledon callus from 14 days-old seedling after subculturing for 22 days. There was no multiplication in the shoot number when cultured in the established condition. When the regenerated shoots were transferred to the rooting medium (full-strength MS medium without plant growth regulators), callus development was observed instead of root formation at

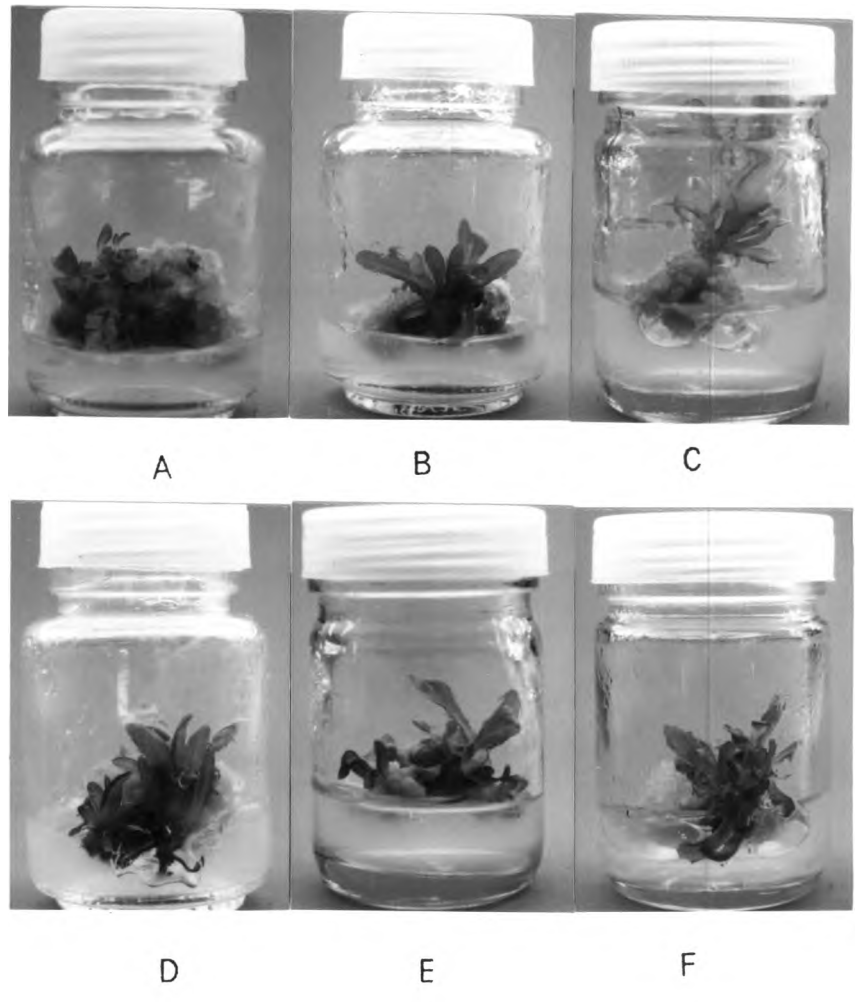


Figure. 10 Induction of callus from various ages of cotyledon explants cultured on MS medium supplemented with 0.5 mg/l NAA and 0.5 mg/l BA at the period of 4 weeks after culturing.

A - callus from 4 days old-seedlings

B - callus from 7 days old-seedlings

C - callus from 10 days old-seedlings

D - callus from 14 days old-seedlings

E - callus from 17 days old-seedlings

F - callus from 21 days old-seedlings

Table. 8 Effect of seedling maturity on callus induction and shoot regeneration of *Carthamus tinctorius* Linn. callus.

Age of explants (days)	Callusing (%)		Regeneration (%)	
	dark	light	dark	light
Cotyledons:				
4	96	95	21	42
7	98	94	37	40
10	98	98	12	20
14	98	98	27	76
17	97	98	25	57
21	98	98	3	42
Hypocotyls:				
4	58	66	0	13
7	50	70	0	4
10	82	80	0	16
14	85	86	0	21
17	82	84	0	5
21	86	64	0	5

*culture on MS medium supplemented with 0.5 mg/l NAA, 0.5 mg/l BA and 0.7%(w/v) agar.

the cut ends of the excision (Fig. 11).

5. Comparison of various media

Effect of various types of media on callus induction was also studied. When the excised cotyledons were cut from 2 weeks-old seedlings and inoculated on various media i.e. MS (Murashige & Skoog 1962), B_5 (Gamborg 1970), N_6 (Chu 1966), LS (Linsmaier & Skoog 1965) and HM (Hildebrandt 1962). Each medium was supplemented with 1.0 mg/l 2,4-D for callus induction. As shown in Table 9 and Fig. 12 , it was noticed that the callus induction efficiency of certain explants cultured on several media was found to be different. As early as 8 days after culturing, the explants began to swell unevenly and callus tissues were produced at the cut surface. The hypocotyl explants yielded a similar type of callus (yellowish, wet and friable) in all kinds of media used except for LS medium. The growth rate of cotyledons callus was relatively faster than the callus developed from hypocotyl. The growth index of cotyledons callus was found to be highest in MS medium followed by B_5 medium while lowest growth index was observed in LS medium (1.26). All tested media contained 1.0 mg/l of 2,4-D.

6. Types of plant growth regulators

The influence of growth regulators on callus initiation and shoot regeneration including shoot formation was shown in Table 10 and Figure. 13 and 14. Callus initiation was first observed within 7 days after culturing in all treatments. The concentration of two types of cytokinins which were BA and Kn in each medium was fixed at 0.5 mg/l. The fixed concentration at 0.5 mg/l of various types of auxins was supplemented in combination with BA and kinetin. The highest percentage of callus induction was observed on MS medium supplemented with BA and NAA giving biggest size of callus. The regenerated callus was first observed in some explants after 18 days of culturing. Leaf- and root-like structure were exhibited

Figure. 11 Shoot regeneration from the 14 days old-seedlings cotyledon callus when cultured on hormone free full-strength MS medium for root induction.

Table. 9 Influence of growth media on growth index and morphogenetic response of *Carthamus tinctorius* Linn. cotyledon callus.

Medium*	Initial fresh wt. (Mean±SD)	Final fresh wt. (Mean±SD)	GI**
MS	0.128±0.059	0.909±0.129	7.09
N6	0.115±0.064	0.283±0.063	2.45
B5	0.114±0.049	0.675±0.130	5.93
HM	0.106±0.061	0.412±0.089	3.89
LS	0.109±0.043	0.138±0.046	1.26

*Each medium contained fixed concentration of 2,4-D (1.0 mg/l) and 2.0% (w/v) of sucrose. The light intensity was 5000 lux cool white fluorescent and the photoperiod was 16 hrs. at 25±2°C.

**$GI = \frac{\text{Final mean value of fresh weight}}{\text{Initial mean value of fresh weight}}$

GI (Growth Index) was determined at the period of 4 weeks.

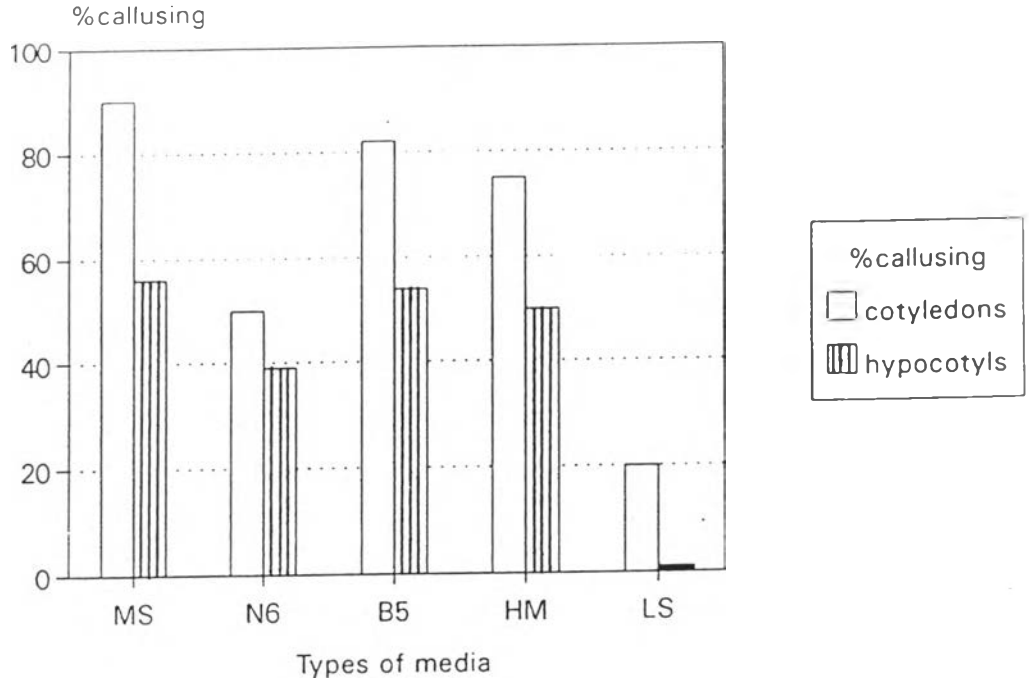


Figure. 12 Influence of the types of media on callus induction and plant regeneration of excised cotyledons and hypocotyl segments.

Table. 10 Effect of types of plant growth regulators on the morphogenetic response of *Carthamus tinctorius* Linn. cotyledon callus.

Types of Hormones		Size of callus*	Morphogenetic response of callus
Cytokinin	Auxins		
BA	NAA	xxxx	green, wet, compact
	IAA	xxx	pale-green, wet, friable
	IBA	xxx	pale-green, wet, friable
	2,4-D	xx	white-yellowish, wet, friable
Kn	NAA	xxx	pale-green, wet, friable
	IAA	xx	green-yellowish, wet, friable
	IBA	xx	green-yellowish, wet, friable
	2,4-D	x	yellowish, wet, friable

size of callus x = small (1-5 mm.)

xx = medium (5-10 mm.)

xxx = large (10-15 mm.)

xxxx = very large (>15 mm.)

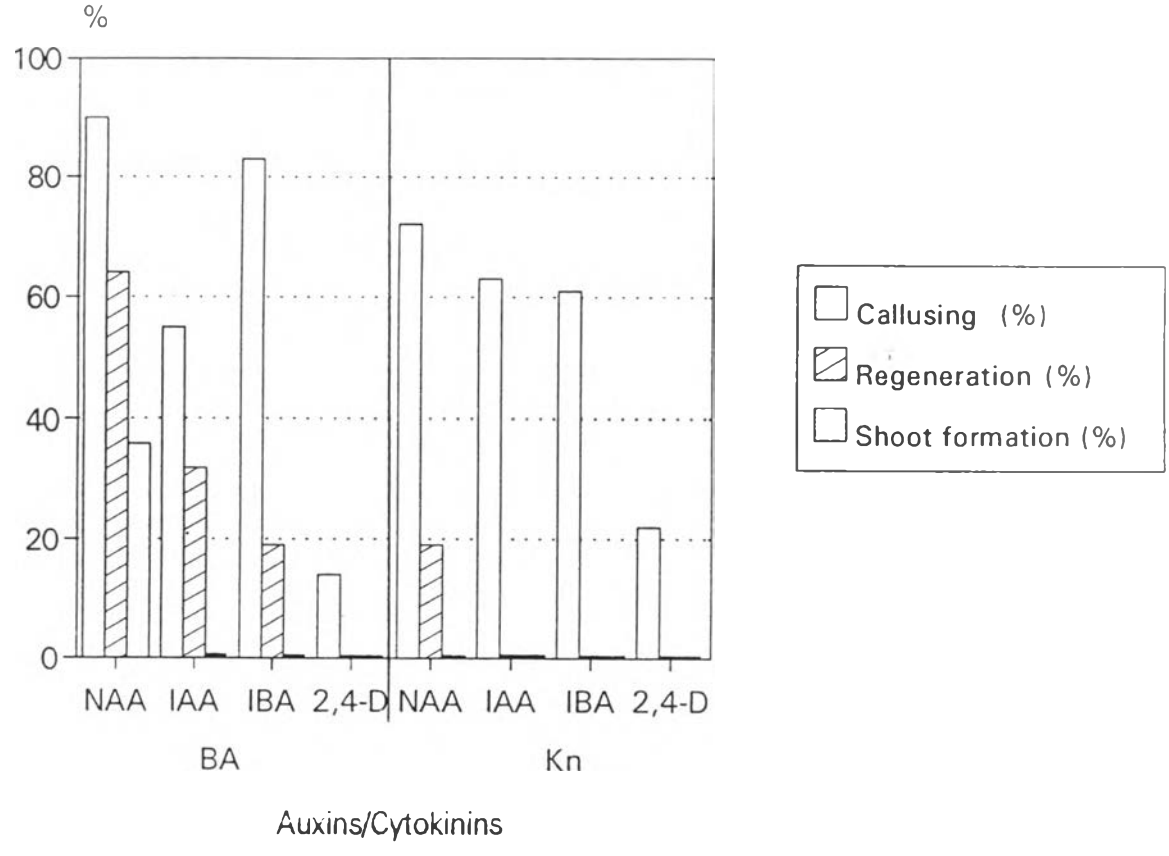


Figure. 13 Callus induction and plant regeneration from 2 week-dark pre-incubation periods of *Carthamus tinctorius* Linn. cotyledon callus cultured on MS medium supplemented with fixed concentration (0.5 mg/l) of various types of plant growth regulators.

(A) (B)

Figure. 14 Characteristics of shoots regenerated from *Carthamus tincorius* Linn. cotyledon callus cultured on MS medium supplemented with 0.5 mg/l NAA and 0.5 mg/l BA after 60 days of culturing and transferring to the media promoting root production:

(A) hormone free full-strength MS medium

(B) half strength MS medium supplemented with 0.5 mg/l NAA

on callus surface. The highest levels of regeneration was also occured in MS medium supplemented with BA and NAA (Fig. 13). The combination of IAA, IBA, 2,4-D with Kn effectively generated callus formation but very low in regeneration. Continuous subculturing of these regenerated callus onto the fresh media, only the regenerated callus being cultured on BA and NAA could form a new shoot after 50 days of culturing (36%). Each regenerated callus generally formed 1-2 shoots at the cut end of pieces (Fig. 14). Under other experimental conditions, no shoot was developed from regenerated callus, even though the cultured times was extended to 60 days. When shoot with 4 leaves (10 mm. length), was excised from regenerated callus, and transferred to various types of root induction medium such as 1/2 MS medium without growth regulators or 1/2 MS medium supplemented with 0.5 mg/l NAA etc, callus initiation was observed at the edge of cut surface after 8-10 days instead of root formation. The original shoot began to swell unevenly and the edge of leaves turned brown, wilting and it was dead finally.

From the above experiment, it was found that the optimum conditions for plant and shoot regeneration of *Carthamus tinctorius* Linn. was established on MS medium supplemented with 0.5 mg/l NAA, 0.5 mg/l BA, 2.0 %(w/v) sucrose in 0.7 % (w/v) agar. In order to improve the efficiency of plant regeneration, the concentrations of NAA in combination with BA were tested for appropriate proportions. Under those conditions, callus induction efficiency and plant regeneration was found to be different, depending on the hormonal ratios. Callus formation could be observed within 6 days after culturing. The morphology of callus was exhibited as the pale green, wet and friable. The overall frequency of callusing in all treatments was 95.2% (Fig. 15 (a), (b)). The first noticeable of plant regeneration from callus surface was observed within 17 days of culture. Green meristematic spots were visible in some calli at all treatments, and after 22 days of

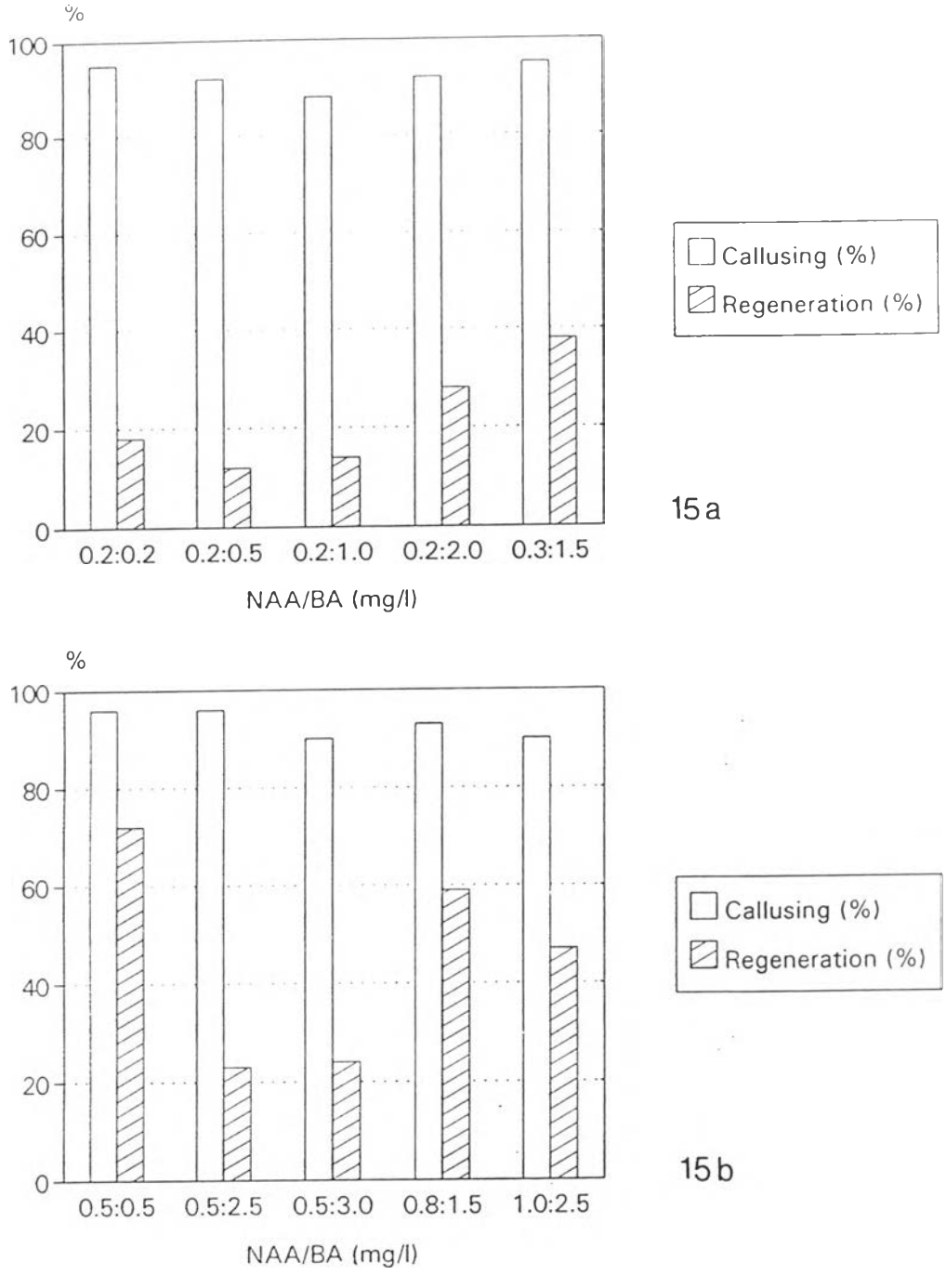


Figure. 15(a), 15(b) Callus induction and plant regeneration of *Carthamus tinctorius* Linn. cotyledon callus cultured on MS medium supplemented with various concentration of NAA and BA.

culturing, some green meristematic spots on callus became organ-like structures (leaves and roots). Only a few of regenerated calli could further develope into a proper shoot on callus surface (Fig. 16). The developing shoot had 7 leaves, about 5 mm. long, and rather steady growth. In conclusion, it was found that the highest plant regeneration (72%) was best achieved when the explants were cultured on MS medium supplemented with 0.5 mg/l NAA and 0.5 mg/l BA. However, when the regenerated shoot was transferred to rooting medium containing 1/2 MS medium supplemented with 0.5 mg/l NAA and 0.1 mg/l BA, the original shoot began to swell and develope into a callus tissues after one or two continuous subculturing

7. The major inorganic nutrients (NH_4NO_3 and KNO_3)

Nutritional components required as a source of nitrogen for shoot initiation and regeneration were tested. Nutritional studies were based on main criteria of nutritional adequacy. The results shown in Tables 11, 12, 13 Figures 17 and 18 indicated that the highest percentage of callus induction (98%) was established at 1.9 g/l KNO_3 and/or 1.65 g/l NH_4NO_3 MS salts. The first regeneration was observed after 20 days of culturing (Fig. 17 and 18). The callus could regenerate only on the medium supplemented with addition of 1.9 or 3.0 g/l KNO_3 and 1.65, or 3.0 g/l NH_4NO_3. The highest regeneration efficiency (26%) was obtained on MS medium supplemented with 0.5 mg/l NAA, 0.5 mg/l BA and1.65 g/l NH_4NO_3(Table 12). This medium condition was also the only one giving high shoot regeneration (27%) efficiency. The shoot was then excised and transferred to various rooting media as follows: 1) 1/2 MS medium supplemented with 0.5 mg/l NAA, 0.1 mg/l BA and 6.0 %(w/v) sucrose and 2) 1/2 MS medium supplemented with 0.5 mg/l NAA, 0.5 mg/l GA_3 and 1.0 g/l charcoal. No rooting was observed but callus formation was induced at the cut end surface after 12 days of culturing then the shoot began to swell and developed into callus as mentioned before.

Figure. 16 Characteristics of regenerated shoot from cotyledon callus of *Carthamus tinctorius* Linn. cultured on MS medium supplemented with 0.5 mg/l NAA and 0.5 mg/l BA after 60 days of culturing.

Table. 11 Effect of ammonium nitrate and potassium nitrate on the external morphology of *Carthamus tinctorius* Linn. cotyledon callus.

NH_4NO_3 (g/l)	KNO_3 (g/l)	Size of callus*	Morphogenetic response
0	1.9	xxx	pale green, wet, friable and compact
0	3.0	xx	pale green, wet, compact
0	5.0	x	yellow-green, wet, compact
0	7.0	xx	yellowish, wet, compact
1.65	0	xxxx	pale green, wet, friable and compact
3.0	0	x	green, wet, friable and compact
5.0	0	xxx	green, wet, compact
7.0	0	xx	yellow-green, wet, compact

size of callus*

x - small (1-5 mm.)

xx - medium (5-10 mm.)

xxx - large (10-15 mm.)

xxxx - very large (>15 mm.)

Table. 12 Effect of ammonium nitrate and potassium nitrate concentrations on plant regeneration of *Carthamus tinctorius* Linn. cotyledon callus.

NH_4NO_3 (g/l)	KNO_3 (g/l)	Regeneration (%)	Shoot formation (%)	Root formation (%)
1.65	1.9	13	0	0
1.65	3.0	10	0	0
1.65	5.0	0	0	0
1.65	7.0	0	0	0
1.65	1.9	26	27	43
3.0	1.9	12	0	0
5.0	1.9	0	0	0
7.0	1.9	0	0	0

Table. 13 Effect of ammonium nitrate on the external morphology of *Carthamus tinctorius* Linn. regenerated callus.

Callus no.	Morphogenetic response*
1,2	shoots proliferated from callus surface, with 2 leaves and no petiole, 2.0 cm. in length of whitish stem, was differentiated from pale green and friable callus.
3	two groups of leaf-like structures directly differentiated from callus surface, 0.5-1.5 cm. in length with no petiole. Callus was pale green, wet and friable.
4,5	groups of leaf-like structures were originated from callus surface, with 4-7 leaves and no petiole, 0.5-1.0 cm. in length look like translucent leaves.
6	two groups of leaf-like structures with bud primordia were occurred on callus surface, 3-5 leaves of each group of leaf-like structure with no petiole was 0.5 cm. in length, thickness and translucent leaves.
7	shoot elongation was occurred in among leaf- like structures on callus surface, with 4 leaves, 1.0-1.5 cm. in length of pale green stem. Regenerated leaves were similar to normal leaves of seedlings.

*MS medium supplemented with 0.5 mg/l NAA, 0.5 mg/l BA and 1.65 g/l NH_4NO_3 for 6 weeks culture period.

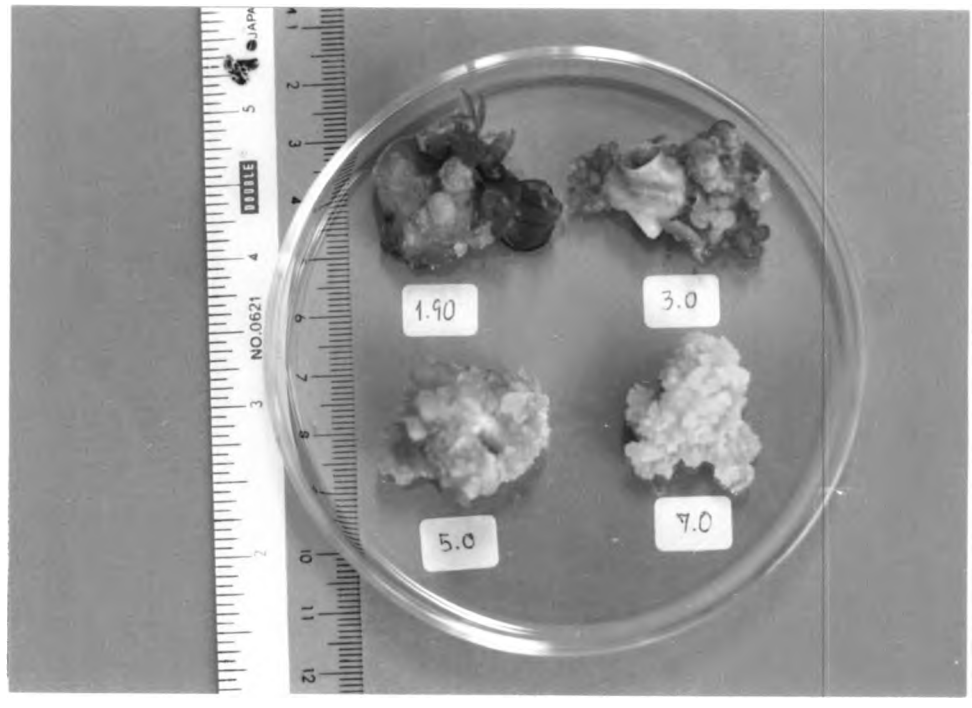


Figure. 17 Regenerated callus derived from *Carthamus tinctorius* Linn. cotyledon explants cultured on MS medium supplemented with 0.5 mg/l NAA and 0.5 mg/l BA and various levels of KNO_3 (g/l) for 4 weeks.

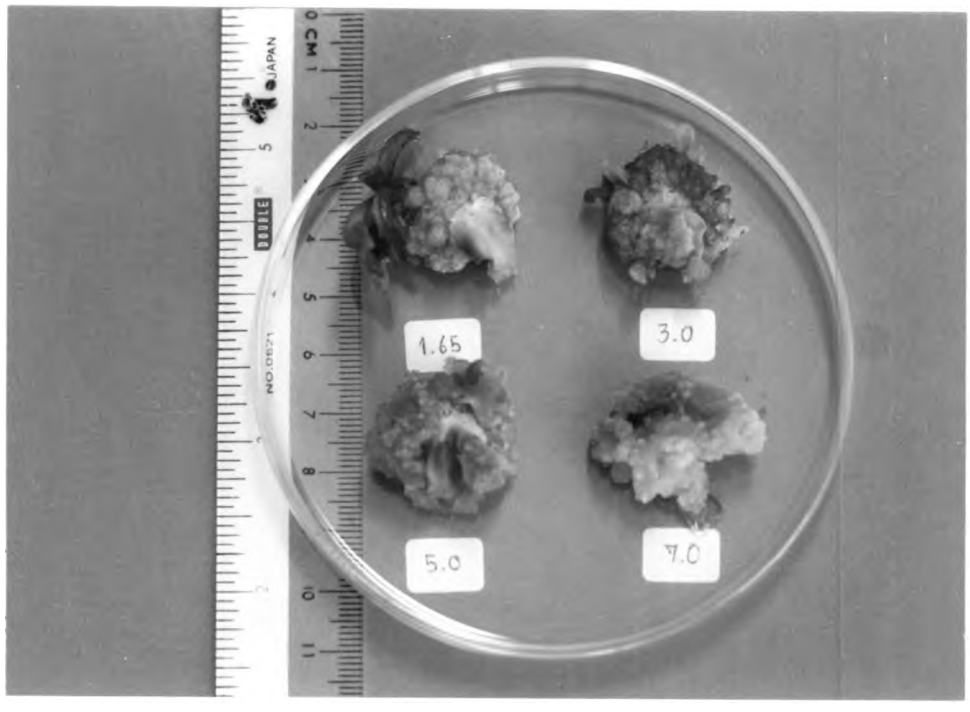


Figure. 18 Regenerated callus derived from *Carthamus tinctorius* Linn. cotyledon explants cultured on MS medium supplemented with 0.5 mg/l NAA and 0.5 mg/l BA and various levels of NH_4NO_3 (g/l) for 4 weeks.

Further rooting experiments were performed by transferring the regenerated shoot into the MS culture medium without plant growth regulators. It was finally found that roots were able to proliferate from the previously formed callus at the cut end of the shoots. Figure 19 showed characteristic of white color and adventitious roots (4-5 cm. length). There was no hairy root observed. Finally the whole plant was transferred to the potting soil for growth in the green house to compare with normal seedling plants (Fig. 20).

8. Silver nitrate and cobalt chloride

The average frequency of callusing of both treatments with optimal concentrations of $AgNO_3$ and $CoCl_2$ were as high as 93-98%. The largest size of calli were observed in the medium containing 3.0 mg/l $AgNO_3$ and 5.0 mg/l $CoCl_2$. However, at higher concentration of $AgNO_3$ and $CoCl_2$ (5.0 and 10.0 mg/l), several calli were shown necrosis after 18 days of culturing due to the slight toxic effect of both inorganic salts. The external morphology of calli which percentage of regeneration and shoot formation were recorded and summarized in Table 14, 15, 16 and 17. The first regeneration of cotyledon explants was noticed after culturing for 22 days. The highest level of regeneration also occurred at 3.0 mg/l $AgNO_3$ and 5.0 mg/l $CoCl_2$. Continuous subculturing of regenerated calli into fresh medium containing the same concentration of $AgNO_3$ and $CoCl_2$ yielded an elongated shoot after 50 days of culturing. The elongated shoots obtained from this regenerated medium were excised for *in vitro* rooting.

9. Rooting of regenerated shoot

Cotyledon derived-shoots were used as sources to obtain rooting conditions by grafting into the root media as given in the above experiments. The results shown in Figure. 20, 21 and 22 revealed that after 7 days in rooting medium, the cut end surface of shoot began to swell unevenly to form a new callus in all rooting media

Figure. 19 Plantlets regenerated from *Carthamus tinctorius* Linn. cotyledon calli after cultured on hormone free full-strength MS medium.

Figure. 20 Characteristics of regenerated shoot from *Carthamus tinctorius* Linn. cotyledon callus cultured on MS medium supplemented with 0.5 mg/l NAA, 0.5 mg/l BA and 1.65 g/l NH_4NO_3 for 60 days and transferred to media for root induction:

(A) half strength MS medium supplemented with 0.5 mg/l NAA,0.1 mg/l BA and 6.0 %(w/v) sucrose.

(B) half strength MS medium supplemented with 0.5 mg/l NAA,0.5 mg/l GA_3 and 1.0 g/l charcoal.

Table. 14 Effect of silver nitrate and cobalt chloride on the external morphology of *Carthamus tinctorius* Linn. cotyledon callus.

$AgNO_3$ (mg/l)	$CoCl_2$ (mg/l)	Size of callus*	Morphogenetic response**
1.0	0	xx	pale green, wet, friable
3.0	0	xxxx	pale green, wet, friable
5.0	0	x	pale green, wet, friable
10.0	0	x	yellowish, wet, friable
0	1.0	xx	yellowish, wet, friable
0	3.0	xx	pale green, wet, friable
0	5.0	xxx	yellow-green, wet, friable
0	7.0	x	yellow-green, wet, friable

Size of callus* x - small (1-5 mm.)
xx - medium (5-10 mm.)
xxx - large (10-15 mm.)
xxxx - very large (>15 mm.)

**MS medium supplemented with 0.5 mg/l NAA and BA and various concentration of $AgNO_3$ and $CoCl_2$.

Table. 15 Effect of silver nitrate and cobalt chloride on callus induction and shoot regeneration of *Carthamus tinctorius* Linn. cotyledon callus.

$AgNO_3$ (mg/l)	$CoCl_2$ (mg/l)	Callusing* (%)	Regeneration* (%)	Shoot formation* (%)
1.0	0	94	0	0
3.0	0	96	32	28
5.0	0	98	18[n]	0
10.0	0	93	9[n]	0
0	1.0	96	30	0
0	3.0	98	31	0
0	5.0	93	37[n]	35
0	7.0	91	35[n]	0

*MS medium supplemented with 0.5 mg/l NAA and 0.5 mg/l BA and results observed within 4 weeks

n - necrosis callus

Table. 16 Effect of silver nitrate on the external morphology of callus regenerated from *Carthamus tinctorius* Linn. cotyledons.

Callus no.	Morphogenetic response*
1,2	1.0 cm. in length of shoot elongation was produced on callus surface in group of leaf-like structure, with 4 opposite green leaves, and a spike was occurred around the edge of leaves. Callus was greenish, wet and mix friable and compact.
3	The two groups of leaf-like structures with no petiole were proliferated on callus surface with 3-4 leaves of each group. The regenerated leaves were watery and translucent with 0.3-0.5 cm. in length but no shoot formation.
4	Leaf-like structure with no petiole, root-like structure and bud primordia was occurred on callus surface with 2 - 3 translucent green leaves at 0.3 cm. in length. Callus was pale green, wet and friable.

*MS medium supplemented with 0.5 mg/l NAA 0.5 mg/l BA and 3.0 mg/l $AgNO_3$ after 6 weeks of culture.

Table. 17 Effect of cobalt chloride on the external morphology of callus regenerated from *Carthamus tinctorius* Linn. cotyledons.

Callus no.	Morphogenetic response*
1,2	two shoot were formed with 0.7-1.0 cm. in length on callus surface, with 3 and 4 leaves respectively. The leaves were greenish with 0.3-0.5 cm. in length and formed a spike at the edge of leaves like a normal seedlings. Another group of 2-3 leaf-liked structure was observed on the surface of greenish, wet and friable callus.
3,4,5	2-4 groups of leaf-liked structure were differentiated on pale green and friable callus, with 2-3 leaves of each group, 0.2-0.4 cm. in length, watery and translucent leaves.
6	leaf-liked structure with bud primordia was differentiated on pale green and friable callus. It was consisted of 2-3 leaves with 0.1-0.3 cm. in length, and two root-liked structures were formed on callus surface with 0.5 and 0.7 cm. in length of whitish roots. The root was occurred in the air and did not grow into the medium.

*MS medium supplemented with 0.5 mg/l NAA 0.5 mg/l BA and 5.0 mg/l $CoCl_2$ after 6 weeks of culture.

(A) (B)

Figure. 21 Characteristics of regenerated shoots from *Carthamus tinctorius* Linn. cotyledon callus cultured on MS medium supplemented with 0.5 mg/l NAA, 0.5 mg/l BA and 3.0 mg/l $AgNO_3$ for 60 days and transferred to media for root induction:

(A) half strength MS medium supplemented with 0.5 mg/l NAA and 0.5 mg/l GA_3

(B) immersion an excised shoots in 1.0 mg/l IBA solution for 2 hrs. before transferred to hormone free full-strength MS medium.

Figure. 22 The characteristics of regenerated shoot from *Carthamus tinctorius* Linn. cotyledon callus cultured on MS medium supplemented with 0.5 mg/l NAA, 0.5 mg/l BA and 5.0 mg/l $CoCl_2$ for 60 days and transferred to half strength MS medium contained with 0.5 mg/l NAA and 0.5 mg/l GA_3 for root induction.

which were 1) 1/2 MS containing 0.5 mg/l NAA, 0.5 mg/l GA_3 and 1.0 g/l charcoal and 2) Immersion of excised shoots in 1.0 mg/l IBA solution for 2 hrs. prior to transferring to MS medium without plant growth regulators. Roots were observed initially from the cut surface within 4 days in full-strength MS medium without plant growth regulators. After 70 days of cultivation, average 6-8 roots were consistently produced from callus at the cut end of excised shoot with whitish in color and 4-6 cm. in length. The regenerated plants were then transfered to grow in sterile ash in comparison to whole plants cultivated from normal seedings.

10. Sucrose concentration

The influence of sucrose concentration on callus induction efficiency was also studied. Various concentrations of sucrose gave different callus induction efficiency. Callus was produced from the cut ends of surface of cotyledons after 7 days of culturing. The characteristics of callus were shown in Table 18 and Figure 23. Sucrose was necessary as a carbon source for growth of callus. No callus initiation was observed from the explants cultured on the medium without sucrose. High efficiency of callus induction was obtained at sucrose concentrations of ranging from 10 to 30 g/l. (Table 19 and Figure 24). Growth index and callus induction efficiency were reduced when sucrose concentration was increased (40 and 50 g/l). Maximum growth and callus induction was found at 20 g/l of sucrose. (Table 20 and Figure 24). After 16 days of culturing, callus regeneration was detected in all treatments except for that with 50 g/l of sucrose. Therefore, the suitable medium composition giving highest percentage of regeneration consisted of 0.5 mg/l NAA, 0.5 mg/l BA and 20 g/l sucrose (53%) (Table 19).

Table. 18 Effect of sucrose concentration on the external morphology of *Carthamus tinctorius* Linn. cotyledon callus.

Sucrose conc. (g/l)	Size of callus*	Characteristic of callus**
0	-	-
5	x	green, wet, compact
10	xx	pale green, wet, friable
20	xxxx	pale green, wet, compact
30	xxx	pale green, wet, friable
40	xxx	yellowish, wet, friable
50	xxx	yellowish, wet, friable

size of callus* x - small (1-5 mm.)
xx - medium (5-10 mm.)
xxx - large (10-15 mm.)
xxxx - very large (>15 mm.)

** Culturing on MS medium supplemented with 0.5 mg/l NAA and 0.5 mg/l BA for the periods of 4 weeks.

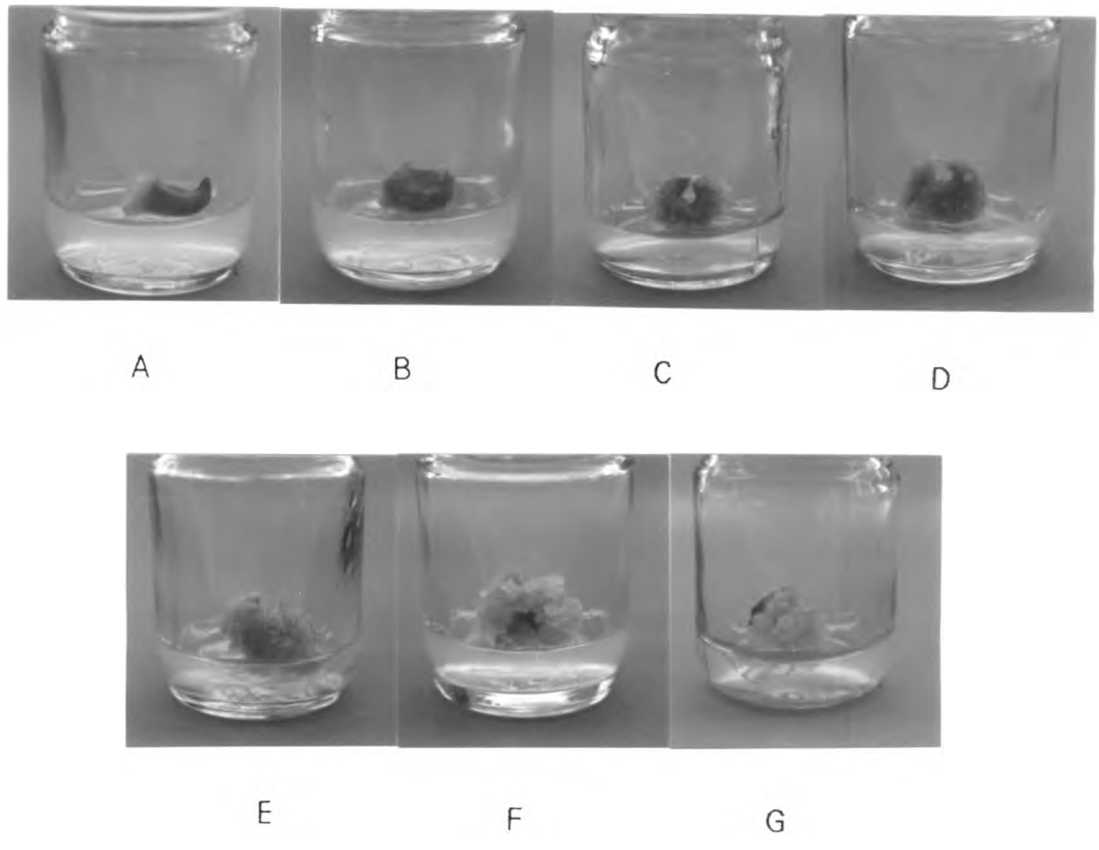


Figure. 23 Characteristics of cotyledon callus cultured on MS medium supplemented with 0.5 mg/l NAA, 0.5 mg/l BA and various concentration of sucrose for 4 weeks.

sucrose concentration in MS culture medium

A - 0 g/l B - 5 g/l

C - 10 g/l D - 20 g/l

E - 30 g/l F - 30 g/l

G - 50 g/l

Table. 19 Effect of sucrose concentration on callus induction efficiency from *Carthamus tinctorius* Linn. cotyledon callus, cultured on MS medium containing 0.5 mg/l NAA and 0.5 mg/l BA at the 4th week after culturing.

Sucrose conc. (g/l)	Callus induction efficiency*(%)	Regeneration (%)
0	0 (0/48)	0 (0/0)
5	86 (42/49)	7 (3/42)
10	96 (48/50)	15 (6/48)
20	98 (45/46)	53 (24/45)
30	94 (47/50)	45 (21/47)
40	80 (39/49)	5 (2/39)
50	78 (36/46)	0 (0/36)

*Number of explants showing callus initiation/total number of explants tested.

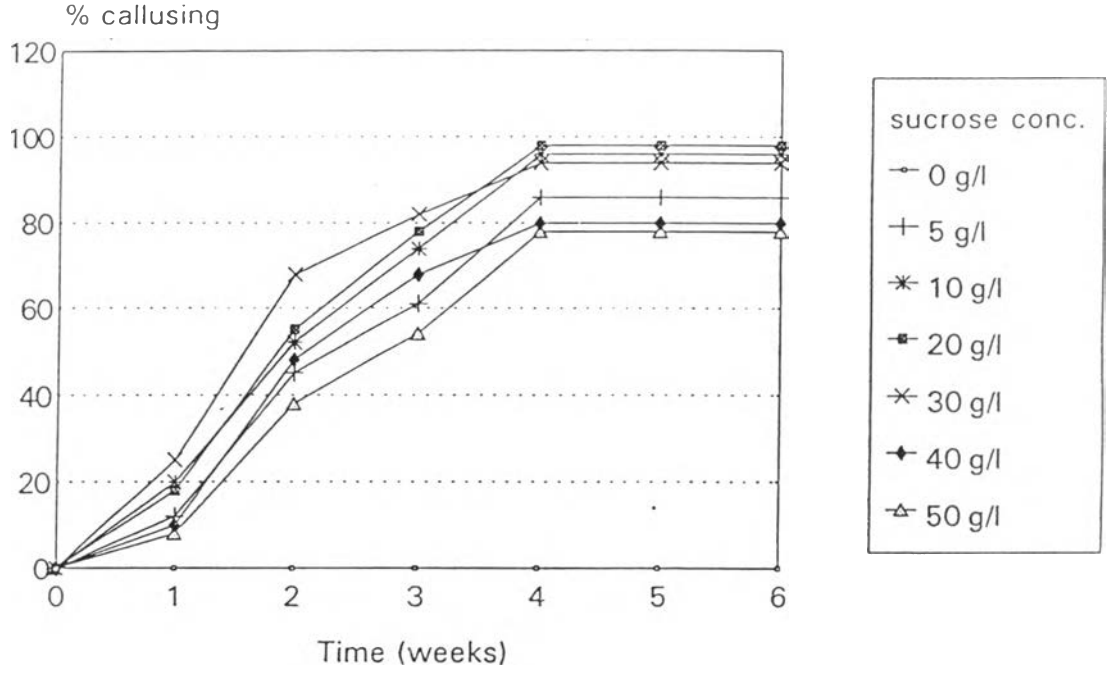


Figure. 24 Effect of sucrose concentration on callus induction efficiency of *Carthamus tinctorius* Linn. cotyledon callus at the period of 6 weeks interval.

Table. 20 Influence of sucrose concentration on growth index of the cotyledon callus of *Carthamus tinctorius* Linn.

Sucrose conc. (g/l)	Initial fresh wt. (Mean±SD)	Final fresh wt. (Mean±SD)	GI*
0	-	-	-
5	0.1288 ± 0.024	0.8614 ± 0.108	5.23
10	0.1804 ± 0.032	1.1070 ± 0.165	6.13
20	0.1659 ± 0.029	0.8690 ± 0.092	6.68
30	0.1886 ± 0.038	1.1115 ± 0.135	5.89
40	0.1671 ± 0.065	0.5902 ± 0.063	5.92
50	0.2019 ± 0.059	0.6917 ± 0.088	3.42

The medium contained 0.5 mg/l NAA and 0.5 mg/l BA. The light intensity was 5000 lux cool white fluorescent of 16 hrs. photoperiod at 25±2°C.

$$GI^* = \frac{\text{Final fresh wt.}}{\text{Initial fresh wt.}}$$

Quantitative analysis of total lipids and fatty acids in *Carthamus tinctorius* Linn.

1. Fatty acid compositions of explants during growth stages

Analysis of total lipid content and fatty acid components of developmental stages of *Carthamus tinctorius* Linn. explants and calli were carried out. The results shown in Table 21 and Figure 25-34 obviously demonstrated the rapid decrease in total lipid content starting from seeds, cotyledons at developmental stages from seed (7 and 14 days) 14 and 28 days cultured cotyledons. The lipid content was quite stable during the callus stages with 5 continuous subculturing for over 5 months. Palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2) were detected as the main fatty acids in all of the seed developmental and callus stages. In cotyledon at different stages of seedling development, the quantity of unsaturated fatty acids (C18:1 and C18:2) was higher than saturated fatty acids (C16:0 and C18:0) with linoleic acid (C18:2) as the major component. There were no significantly levels of myristic acid (C14:0), palmitoleic acid (C16:1), arachidic acid (C20:0) and behenic acid (C22:0) detected in natural seeds (Fig. 25). Fatty acid contents tended to decrease simultaneously during the development of cotyledons (7 and 14 days old seedlings) and of the excised cotyledon segment (14 and 28 days-culture). The content of saturated fatty acids (C16:0 and C18:0) in callus tissue being continuously subculturing for 5 cycles were still higher than those of unsaturated fatty acids (C18:1 and C18:2). Palmitoleic acid (C16:1) was not detected in the stage of callus development. The presence of a small amount of behenic acid (C22:0) was detected constantly in every developmental stages of explants and callus (Fig. 30-34).

Fatty acid components*

A - myristic acid (C14:0)

B - palmitic acid (C16:0)

C - palmitoleic acid (C16:1)

D - stearic acid (C18:0)

E - oleic acid (C18:1)

F - linoleic acid (C18:2)

G - arachidic acid (C20:0)

H - behenic acid (C22:0)

Table. 21 Total lipid contents (%w/w) of seed developmental and callus stages of *Carthamus tinctorius* Linn.

Stages of explant and callus development	Total lipid contents (%w/w)
Soaked seeds	28.75±0.38
Cotyledons:	
- 7 days old seedlings	10.90±0.71
- 14 days old seedlings	6.43±0.46
excised cotyledons	
- 14 days after culturing	5.43±0.45
- 28 days after culturing	1.26±0.27
Callus subculturing	
I	1.05±0.15
II	1.07±0.32
III	1.16±0.33
IV	1.11±0.27
V	1.15±0.14

Fatty acid components*

A - myristic acid (C14:0)

B - palmitic acid (C16:0)

C - palmitoleic acid (C16:1)

D - stearic acid (C18:0)

E - oleic acid (C18:1)

F - linoleic acid (C18:2)

G - arachidic acid (C20:0)

H - behenic acid (C22:0)

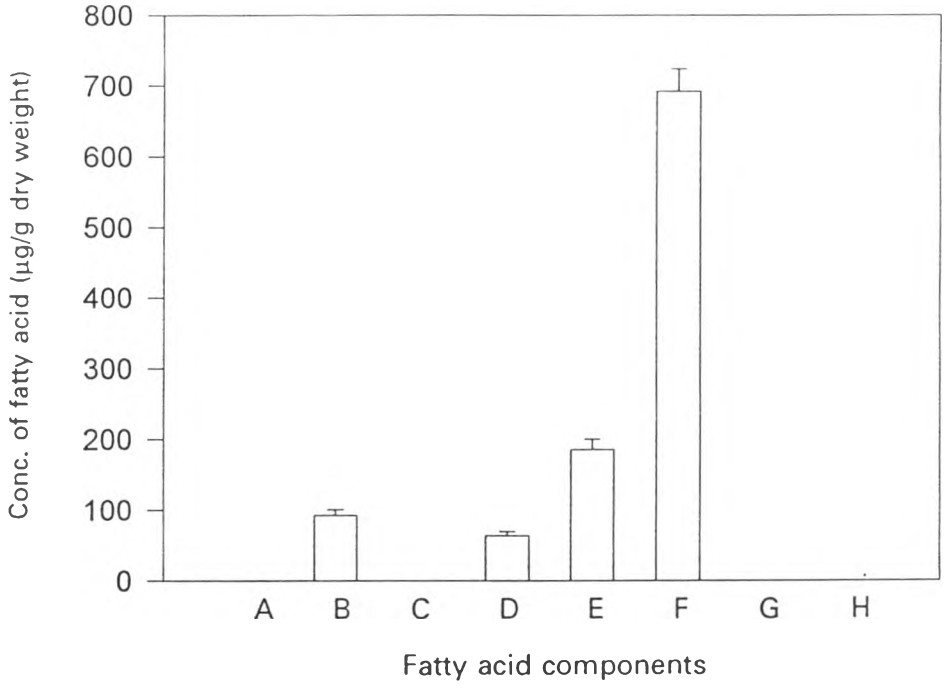


Figure. 25 Changing in fatty acid components at soaked seeds stage.

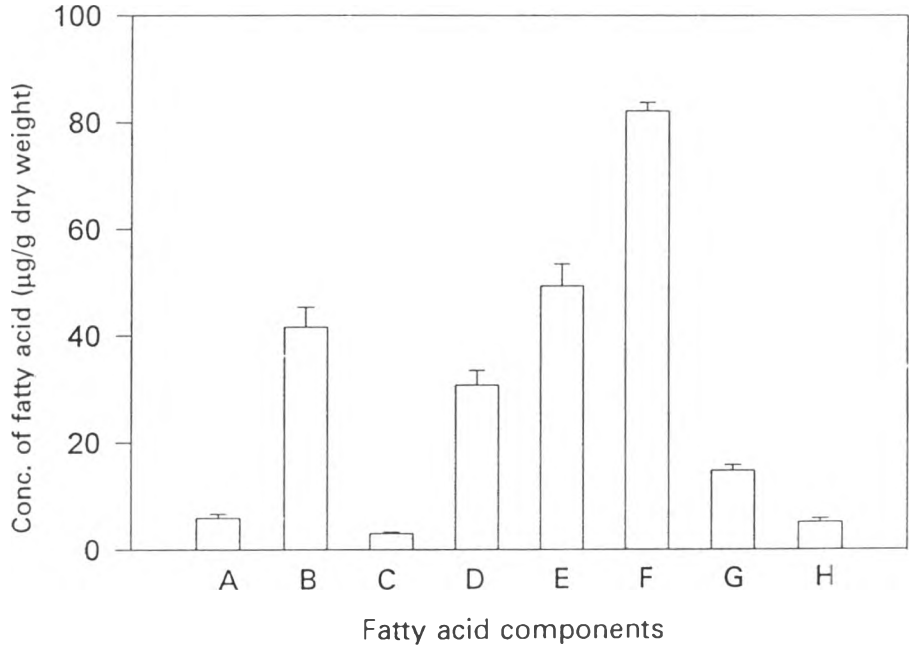


Figure. 26 Changing in fatty acid components at cotyledon 7 days old seedlings stage.

Fatty acid components*

A - myristic acid (C14:0)

B - palmitic acid (C16:0)

C - palmitoleic acid (C16:1)

D - stearic acid (C18:0)

E - oleic acid (C18:1)

F - linoleic acid (C18:2)

G - arachidic acid (C20:0)

H - behenic acid (C22:0)

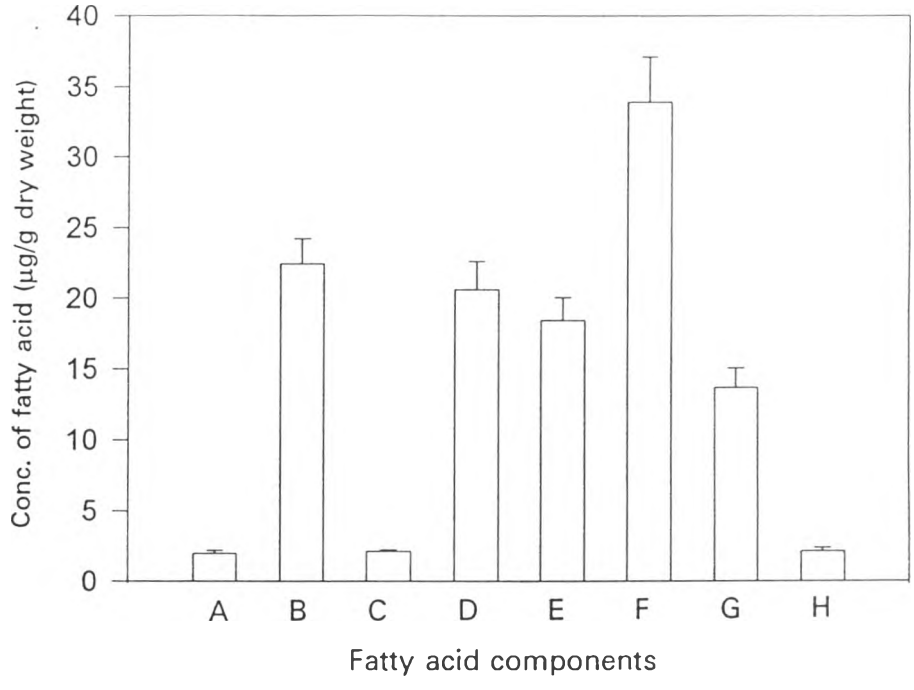


Figure. 27 Changing in fatty acid components at cotyledon 14 days old seedlings stage.

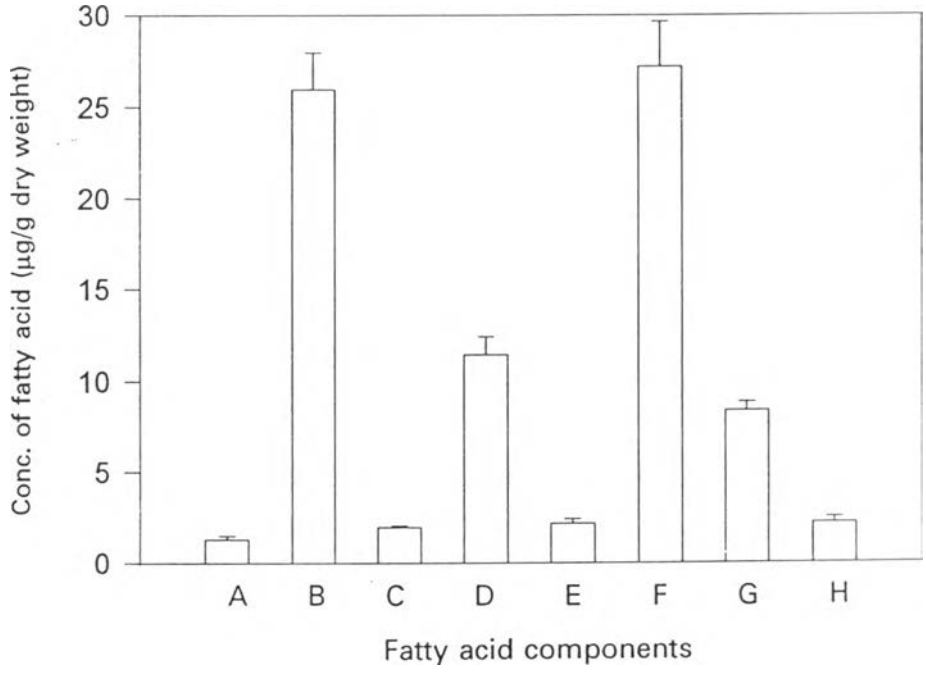


Figure. 28 Changing in fatty acid components at excised cotyledon 14 days after culturing stage.

Fatty acid components*

A - myristic acid (C14:0)

B - palmitic acid (C16:0)

C - palmitoleic acid (C16:1)

D - stearic acid (C18:0)

E - oleic acid (C18:1)

F - linoleic acid (C18:2)

G - arachidic acid (C20:0)

H - behenic acid (C22:0)

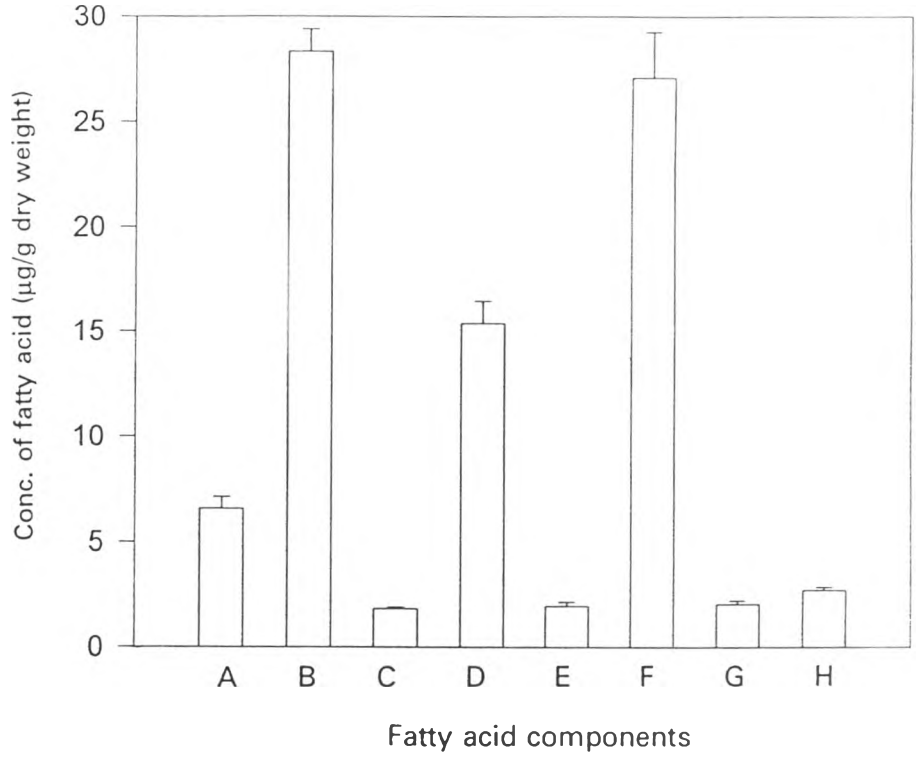


Figure. 29 Changing in fatty acid components at excised cotyledon 28 days after culturing stage.

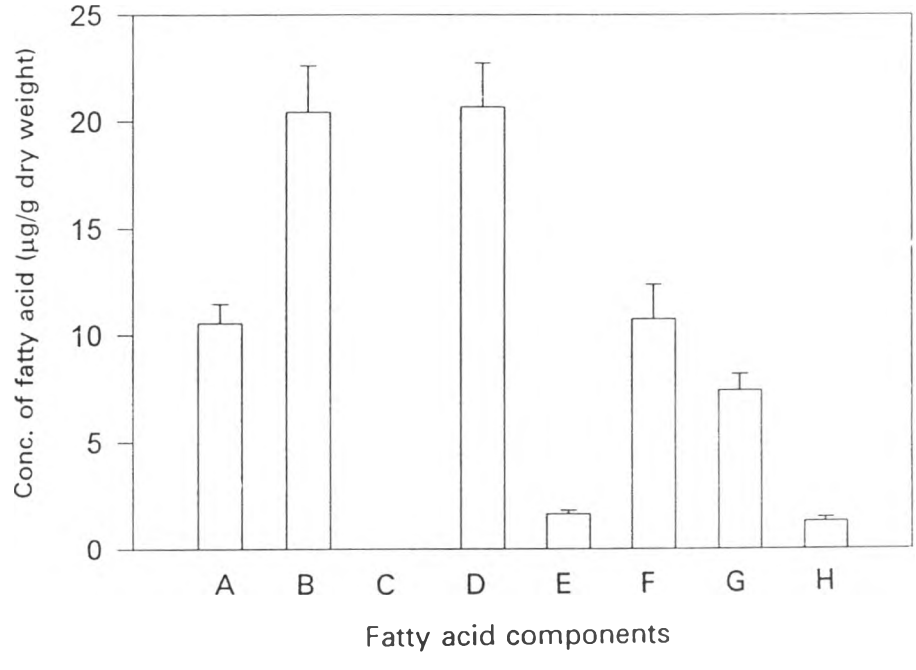


Figure. 30 Changing in fatty acid components at callus subculturing I stage.

Fatty acid components*

A - myristic acid (C14:0)

B - palmitic acid (C16:0)

C - palmitoleic acid (C16:1)

D - stearic acid (C18:0)

E - oleic acid (C18:1)

F - linoleic acid (C18:2)

G - arachidic acid (C20:0)

H - behenic acid (C22:0)

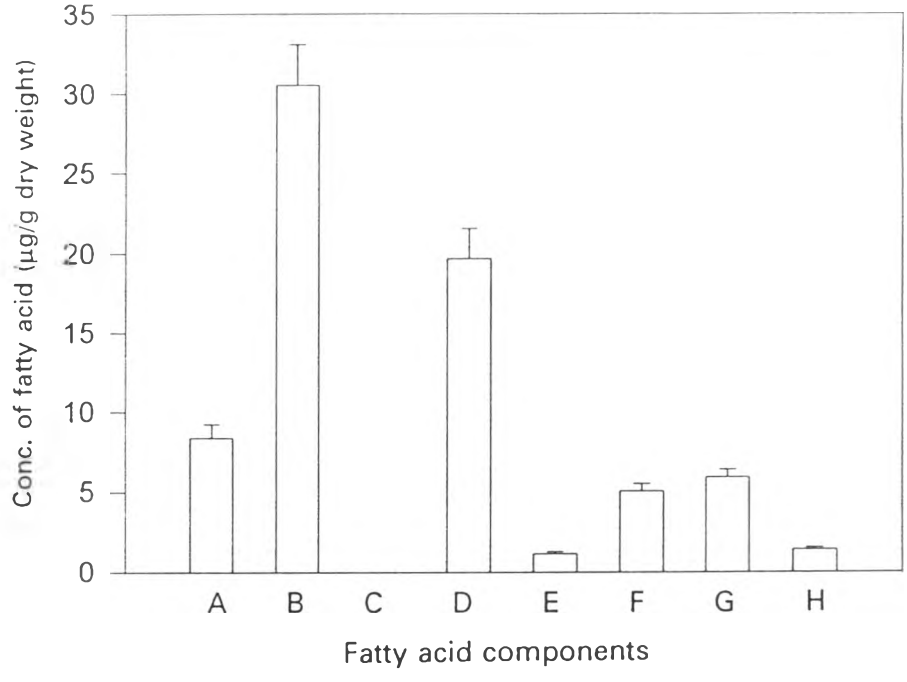


Figure. 31 Changing in fatty acid components at callus subculturing II stage.

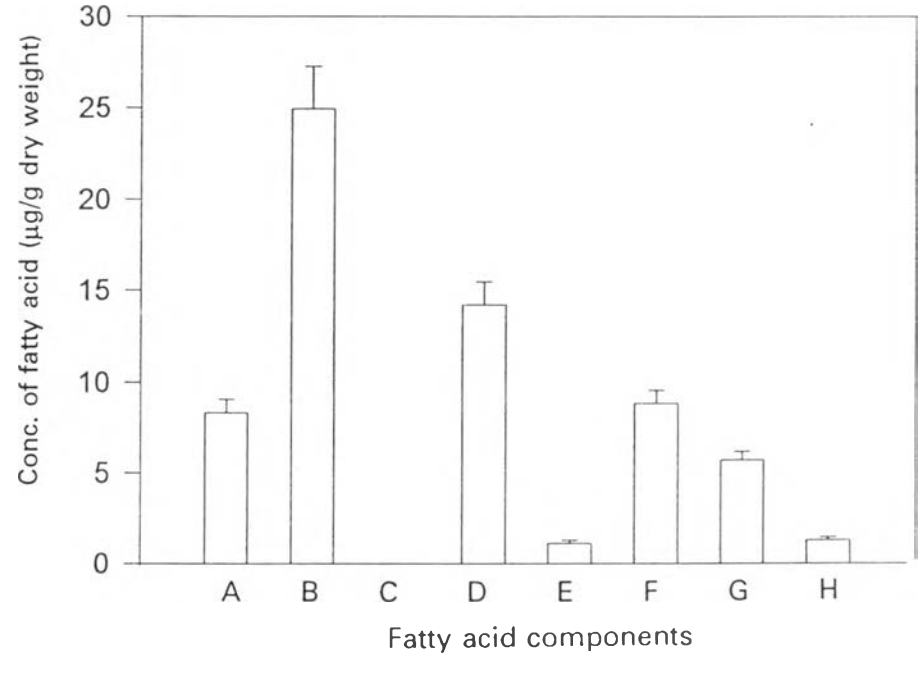


Figure. 32 Changing in fatty acid components at callus subculturing III stage.

Fatty acid components*

A - myristic acid (C14:0)

B - palmitic acid (C16:0)

C - palmitoleic acid (C16:1)

D - stearic acid (C18:0)

E - oleic acid (C18:1)

F - linoleic acid (C18:2)

G - arachidic acid (C20:0)

H - behenic acid (C22:0)

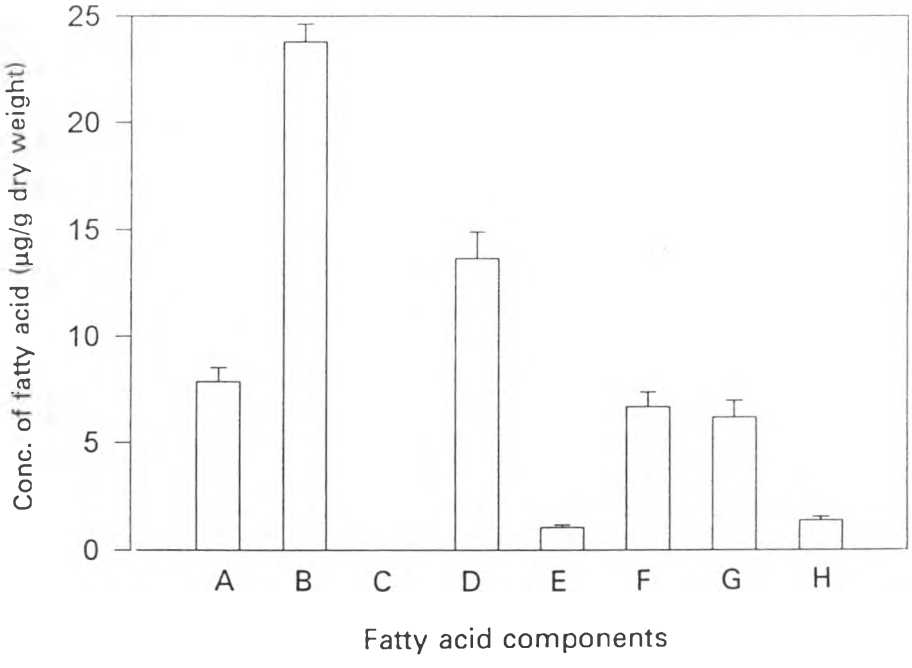


Figure. 33 Changing in fatty acid components at callus subculturing IV stage.

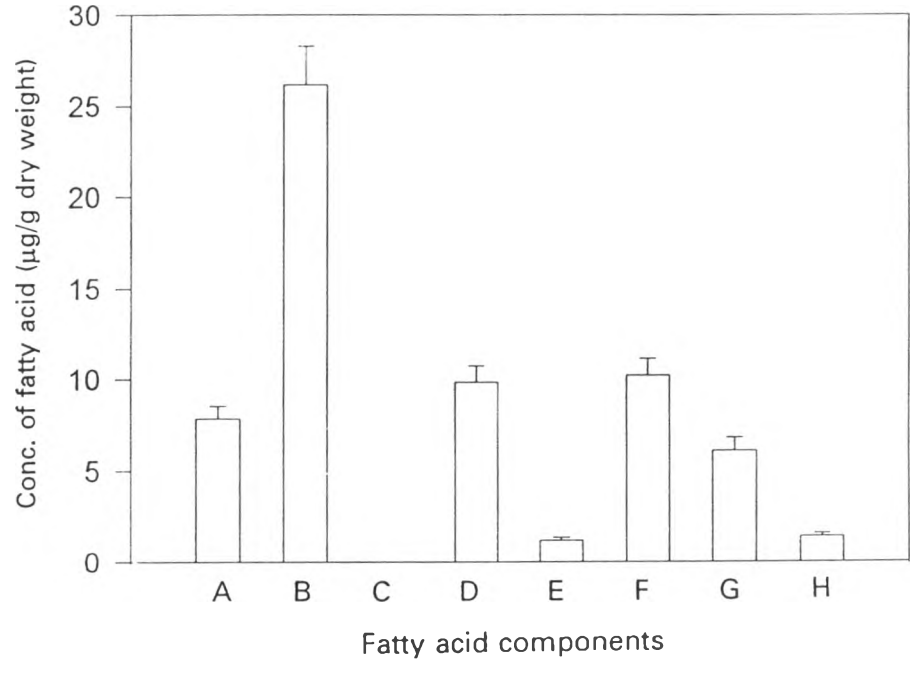


Figure. 34 Changing in fatty acid components at callus subculturing V stage.

2. Lipid content during growth and development of cotyledon-derived callus

Growth of *Carthamus tinctorius* Linn. cotyledon callus cultured on MS medium containing 0.5 mg/l NAA and BA in the presence of light or dark for 90 days was observed from the increase of dry weight. The growth rate of dark grown callus was obviously higher than that of light grown callus.

It was shown in Figure 35 that the biosynthesis of lipids was in reverse proportion to the growth rate but it was directly influenced by the presence or absence of light. Maximum total lipid contents of 2.169% (w/w) and 1.449% (w/w) were detected in dark and light grown calli, respectively. Total lipid content of dark grown callus was higher than that of light grown callus at all the detected growth stages. The total lipid content was rather constant after culturing for 3-4 weeks.

The main saturated fatty acids found in calli of both light and dark grown were palmitic acid (C16:0) and stearic acid (C18:0). The highest amounts of these two fatty acids were detected in both conditions at the first week of growth period (Table. 22 and 23) but they were later drastically decreased both in the presence and absence of light. Linoleic acid (C18:2), the main content of unsaturated fatty acid found in callus stage was highly increased in light grown callus especially at the highest growth stages (40% w/w) on the fifth week. Almost no response in linoleic acid (C18:2) in callus culture grown under the dark conditions. The content of palmitoleic acid (C16:1), the unsaturated fatty acid in natural seed slightly increased in light grown callus after the third week of growth period. The biosynthetic patterns of myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0) and linoleic acid (C18:2) were similar both in light and dark grown calli. The highest content of arachidic acid (C20:0) was detected in light grown callus at the second week of growth period but the content was varied in dark grown callus.

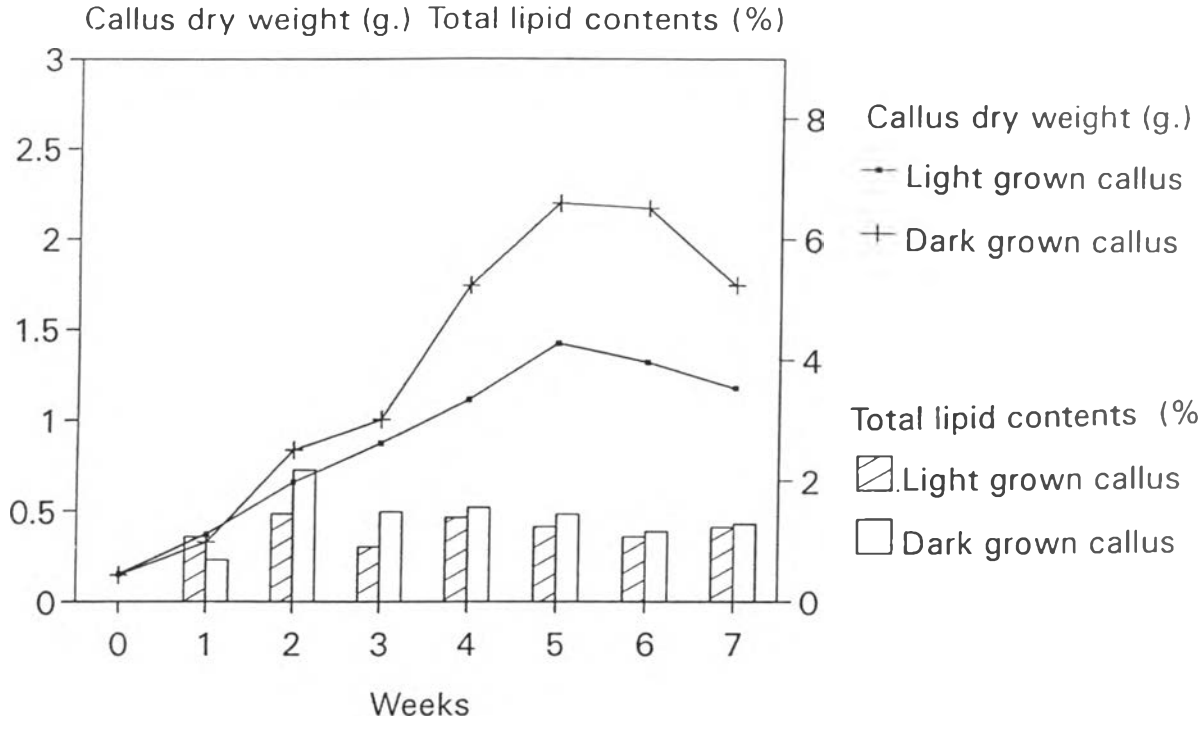


Figure. 35 Growth patterns and total lipid synthesis of cotyledon-derived callus. Culture carried out in MS basal media under 2000 lux of fluorescent for 16/8 hrs. photoperiod at 25±2°C.

Table. 22 Changing in fatty acid composition of total lipid of dark grown callus at the growth period (each value is expressed in μg/g dry weight).

Fatty acids	Weeks of culture						
compositions	I	II	III	IV	V	VI	VII
14:0	12.2±0.62	7.8±0.44	5.9±0.22	3.8±0.30	4.5±0.41	9.2±0.25	5.9±0.04
16:0	29.2±1.16	27.7±0.95	20.5±0.69	17.1±0.42	17.4±0.14	24.3±0.90	17.7±0.70
16:1	1.6±0.07	1.5±0.04	1.1±0.07	1.2±0.03	1.2±0.04	1.1±0.03	1.1±0.03
18:0	37.5±2.01	22.2±1.93	18.0±0.40	11.7±0.19	6.2±0.09	6.3±0.08	6.8±0.09
18:1	6.1±0.06	4.8±0.07	2.6±0.12	3.1±0.13	3.1±0.06	3.2±0.08	3.8±0.03
18:2	9.0±0.06	10.6±0.07	4.5±0.04	13.5±0.03	14.4±0.05	14.0±0.10	13.5±0.04
20:0	4.5±0.08	3.1±0.05	6.2±0.08	2.7±0.05	5.5±0.08	5.2±0.06	6.1±0.04
22:0	3.9±0.04	2.7±0.02	1.1±0.05	0.9±0.07	1.1±0.08	1.4±0.05	0.7±0.02

Table. 23 Changing in fatty acid composition of total lipid of light grown callus at the growth period (each value is expressed in μg/g dry weight).

Fatty acids	Weeks of culture						
compositions	I	II	III	IV	V	VI	VII
14:0	12.8±0.39	2.3±0.47	1.4±0.24	4.4±0.73	4.2±0.41	6.2±0.21	8.9±0.27
16:0	44.7±3.42	22.9±2.21	17.4±1.78	21.7±2.01	29.1±1.38	21.8±1.25	26.6±0.90
16:1	tr	tr	tr	1.7±0.41	2.1±0.17	1.7±0.07	1.7±0.04
18:0	48.6±2.87	15.1±1.22	9.7±0.48	9.8±0.49	9.6±0.76	13.7±1.01	11.6±0.93
18:1	2.6±0.09	1.1±0.06	0.8±0.03	4.6±0.21	5.6±0.42	4.5±0.23	3.9±0.15
18:2	4.3±0.62	2.7±0.05	1.4±0.05	22.6±1.27	40.7±1.96	25.8±0.98	22.6±0.74
20:0	8.2±0.06	10.1±0.37	6.3±0.05	7.0±0.05	5.3±0.07	5.4±0.04	5.4±0.09
22:0	2.3±0.07	2.3±0.04	1.0±0.03	2.1±0.07	2.2±0.06	1.2±0.03	1.3±0.02

tr - trace (< 0.1 μg)

3. Effect of plant growth regulators on fatty acid compositions

Table 24 demonstrated the effect of BA or Kn in combination with various types of auxins on total lipid content of Safflower cotyledon callus cultured on MS basal medium. The results showed that 2,4-D and NAA in combination with BA or Kn gave higher stimulation of biosynthesis than the other combinations. Palmitic acid (C16:0), stearic acid (C18:0), linoleic acid (C18:2), arachidic acid (C20:0) and behenic acid (C22:0) were detected in all combinations of both groups of growth regulators (Fig. 37, 39, 41, 42 and 43). The relative proportion of total saturated fatty acids (C14:0, C16:0, C18:0, C20:0 and C22:0) to total unsaturated fatty acids (C16:1, C18:1 and C18:2) was high. The main components of fatty acids found in cotyledon calli of *Carthamus tinctorius* Linn., cultured in the presence of eight combinations of growth regulators were palmitic acid (C16:0) and linoleic acid (C18:2) (Fig. 37 and 41). The highest amount of palmitic acid (C16:0) was detected in callus grown in the presence of NAA plus BA while the highest amount of linoleic acid (C18:2) was detected in the presence of 2,4-D plus Kn. Myristic acid (C14:0) was only detected in callus grown in the presence of BA in combination with auxins (NAA, IAA, IBA and 2,4-D) (Fig. 36). Palmitoleic acid (C16:1) (Fig. 38) and oleic acid (C18:1) (Fig. 40) were only detected in callus cultivated in the medium comprised of BA in combination with NAA & IAA while myristic acid (C14:0), palmitoleic acid (C16:1) and oleic acid (C18:1) were not detected in callus cultured on the medium comprised of Kn in combination with auxins (NAA, IAA, IBA and 2,4-D). Variation in external morphology, callus texture, color and size were demonstrated in Table 10. The overall data observed (Fig. 36 - 43) also revealed that BA was likely to give the higher efficiency in stimulation of lipid synthesis when supplemented in combination with any auxins.

Table. 24 Total lipid content of *Carthamus tinctorius* Linn. cotyledon callus cultured on MS medium supplemented with fixed concentration (0.5 mg/l) of various combination (cytokinins/auxins).

Type of hormones		Total lipid content*
Cytokinins	Auxins	%(w/w)
BA	NAA	1.441±0.316
	IAA	1.327±0.238
	IBA	1.377±0.264
	2,4-D	1.568±0.223
Kn	NAA	1.712±0.286
	IAA	1.308±0.166
	IBA	1.223±0.158
	2,4-D	1.727±0.208

*data showed as %(w/w) of 3 replications.

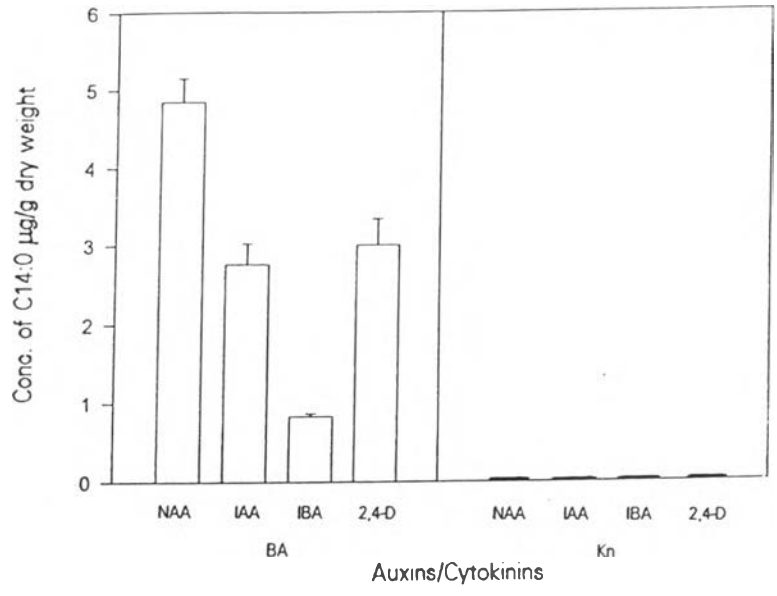


Figure. 36 Myristic acid (C14:0) content of *Carthamus tinctorius* Linn. callus cultured on MS medium supplemented with fixed concentration (0.5 mg/l) of various kinds of plant growth regulators.

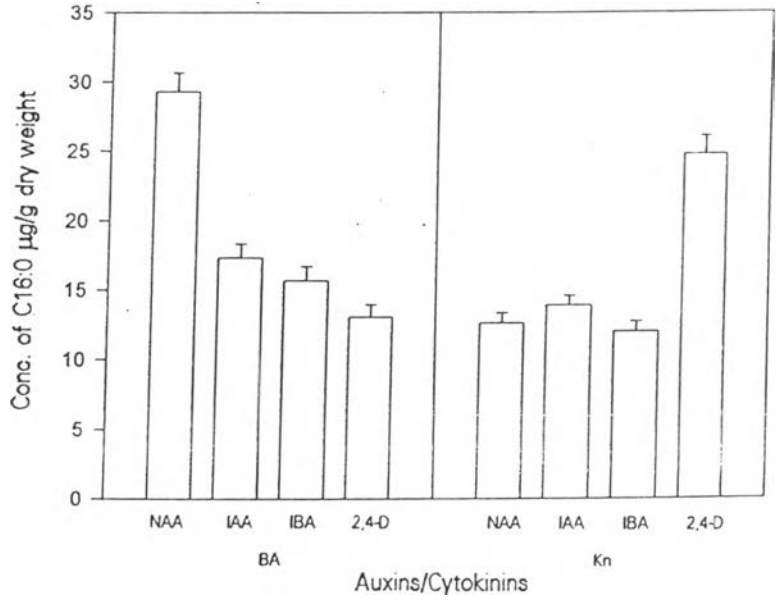


Figure. 37 Palmitic acid (C16:0) content of *Carthamus tinctorius* Linn. callus cultured on MS medium supplemented with fixed concentration (0.5 mg/l) of various kinds of plant growth regulators.

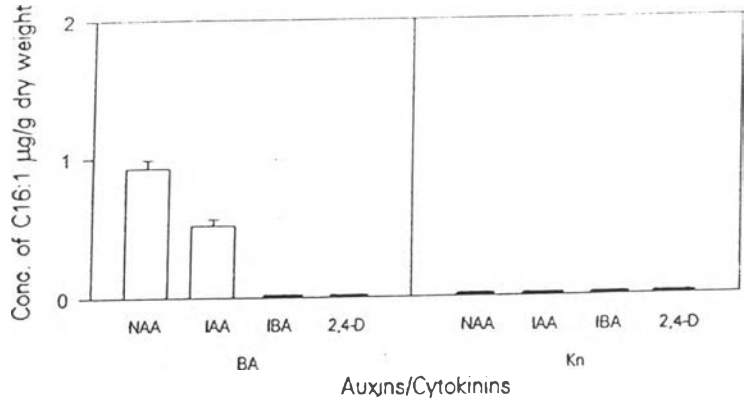


Figure. 38 Palmitoleic acid (C16:1) content of *Carthamus tinctorius* Linn. callus cultured on MS medium supplemented with fixed concentration (0.5 mg/l) of various kinds of plant growth regulators.

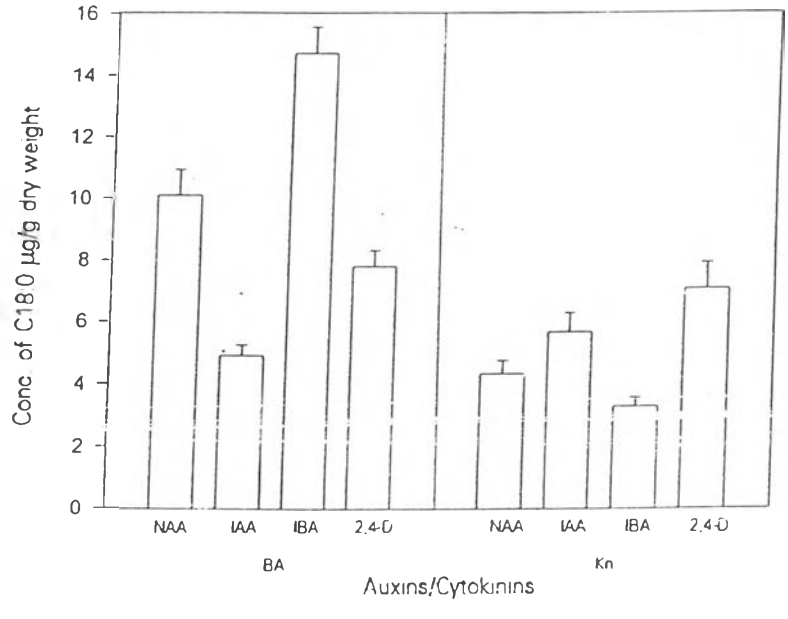


Figure. 39 Stearic acid (C18:0) content of *Carthamus tinctorius* Linn. callus cultured on MS medium supplemented with fixed concentration (0.5 mg/l) of various kinds of plant growth regulators.

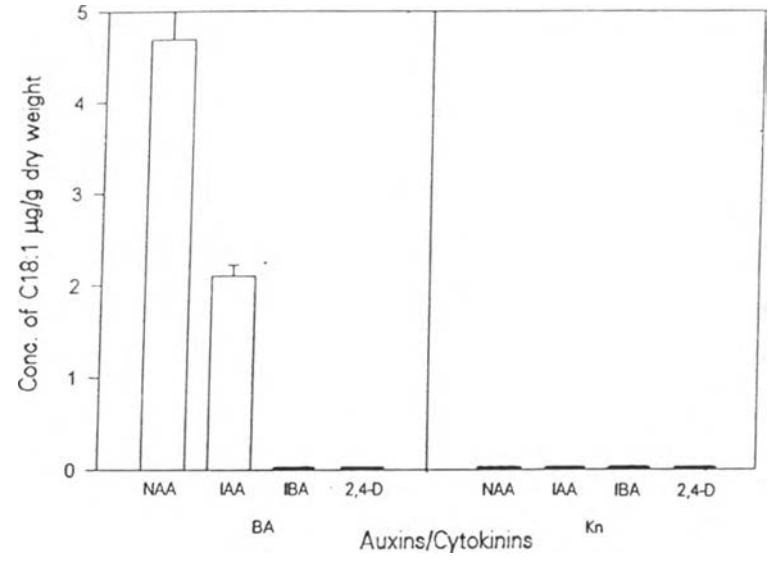


Figure. 40 Oleic acid (C18:1) content of *Carthamus tinctorius* Linn. callus cultured on MS medium supplemented with fixed concentration (0.5 mg/l) of various kinds of plant growth regulators.

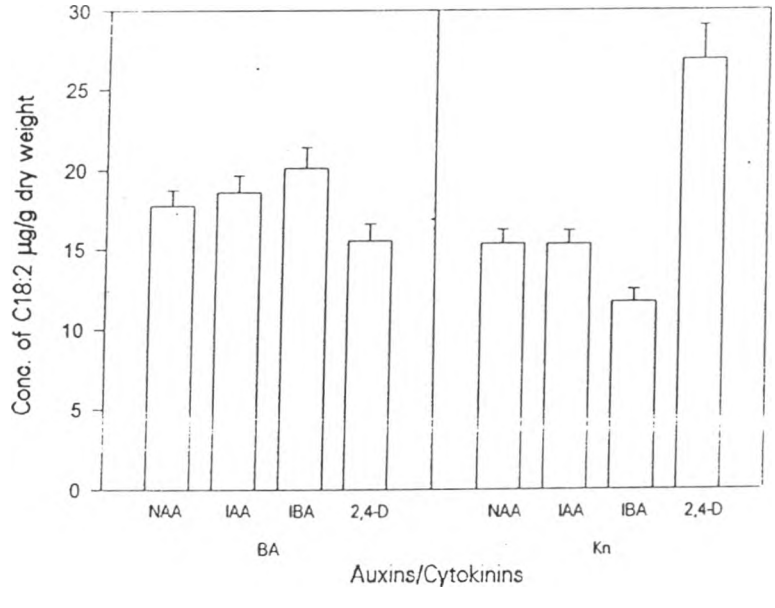


Figure. 41 Linoleic acid (C18:2) content of *Carthamus tinctorius* Linn. callus cultured on MS medium supplemented with fixed concentration (0.5 mg/l) of various kinds of plant growth regulators.

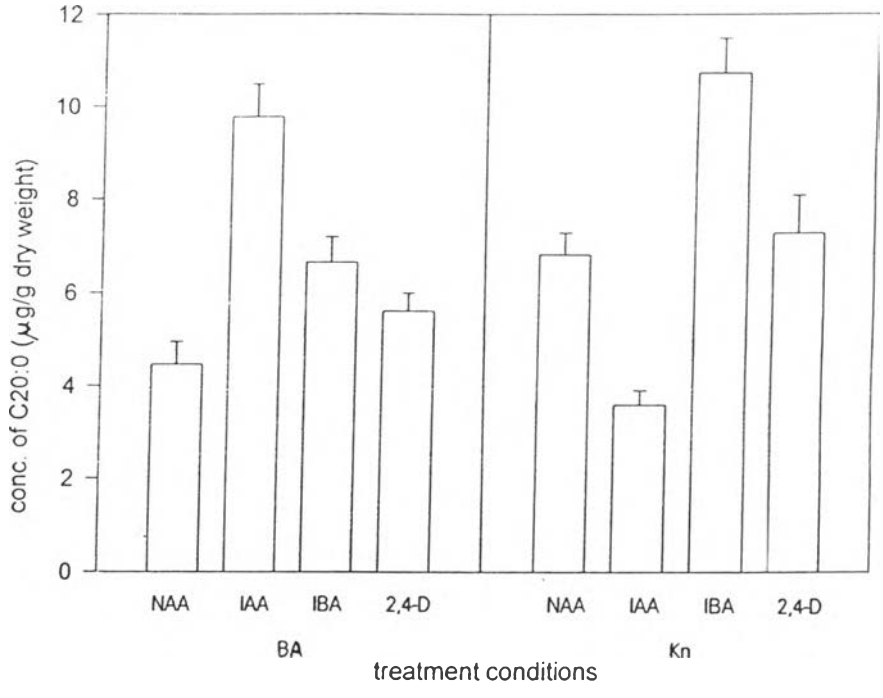


Figure. 42 Arachidic acid (C20:0) content of *Carthamus tinctorius* Linn. callus cultured on MS medium supplemented with fixed concentration (0.5 mg/l) of various kinds of plant growth regulators.

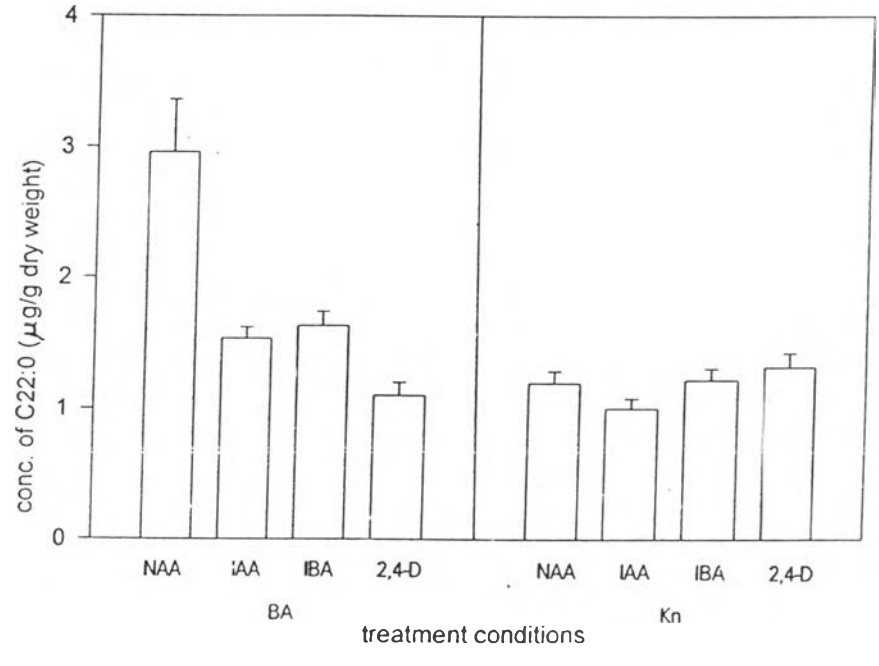


Figure. 43 Behenic acid (C22:0) content of *Carthamus tinctorius* Linn. callus cultured on MS medium supplemented with fixed concentration (0.5 mg/l) of various kinds of plant growth regulators.

4. Effect of sucrose on fatty acid composition

Sucrose as a carbon source was reported to be a possible agent to regulate the level of total lipid content in cotyledon callus of *Carthamus tinctorius* Linn. (Fig. 44). Cotyledon callus cultured on the medium containing 2.0%(w/v) sucrose synthesized the highest content of total lipid. Higher concentration of sucrose could reduce the yield of lipids in cultured calli.

Palmitic acid (C16:0), stearic acid (C18:0) and arachidic acid (C20:0) were only three saturated fatty acids detected in callus cultured on MS media at all tested of sucrose concentrations (Fig. 45, 46 and 48). Increasing sucrose concentration in the medium, palmitic acid (C16:0) content was also increased while arachidic acid (C20:0) content was decreased. Stearic acid content was highly varied with various sucrose concentrations. The percentages of saturated fatty acids were higher than that of unsaturated fatty acids. Linoleic acid (C18:2) was the only unsaturated fatty acid detected eventhough the content was quite low and its biosynthesis was not significantly affected by sucrose concentration. (Fig. 47).

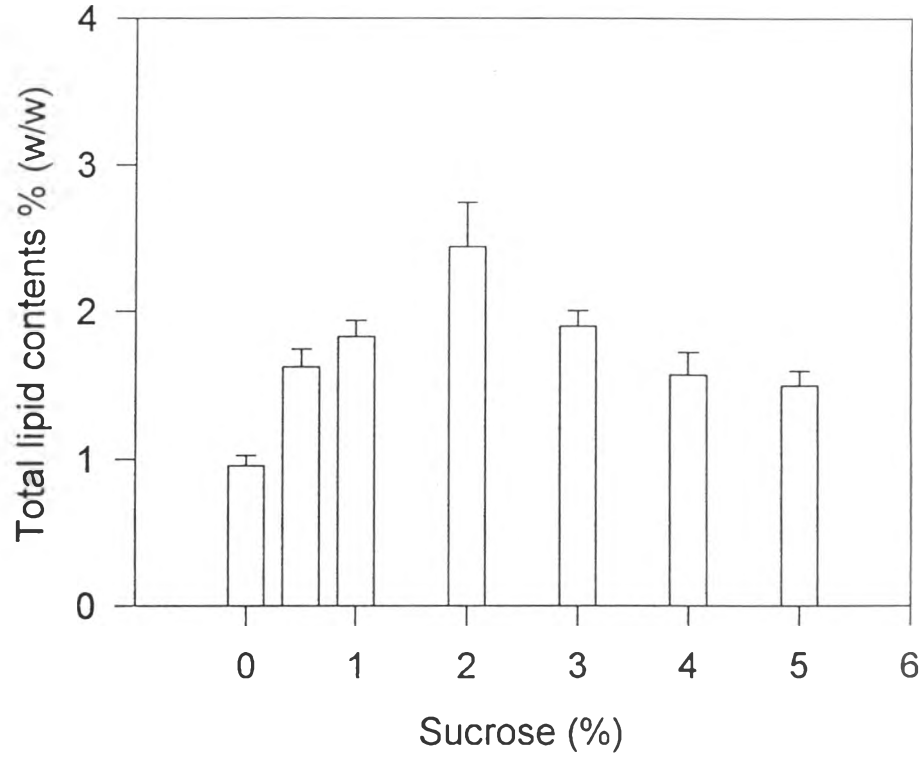


Figure. 44 Total lipid content in *Carthamus tinctorius* Linn. cotyledon callus as affected by sucrose concentration.

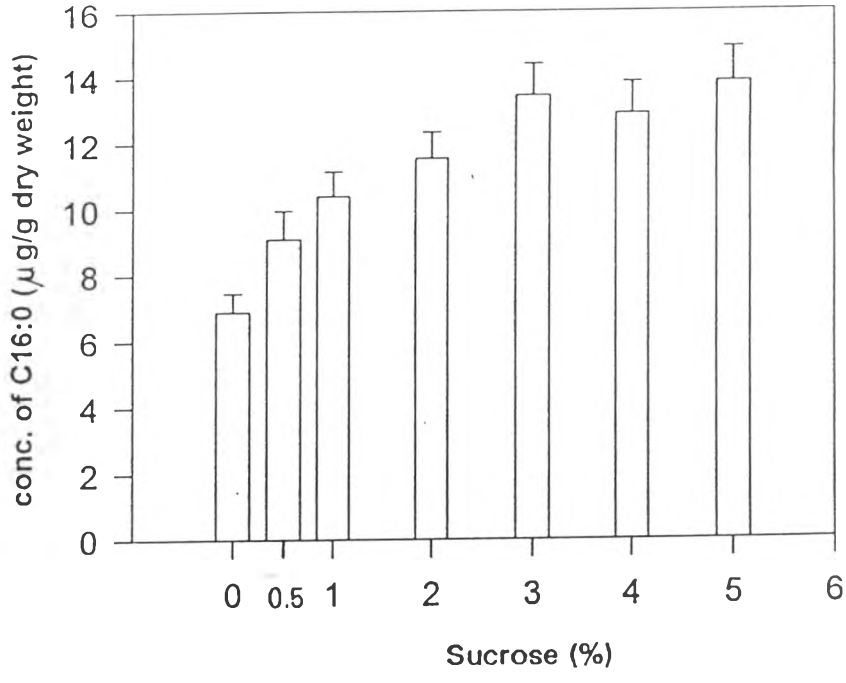


Figure. 45 Palmitic acid (C16:0) content of cotyledon callus, cultured on MS medium supplemented with various sucrose concentration.

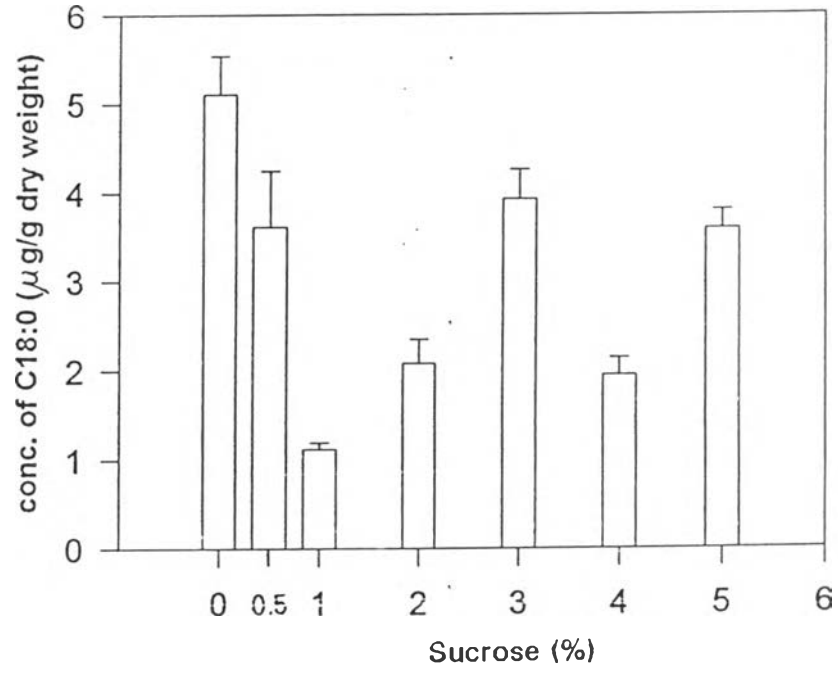


Figure. 46 Stearic acid (C18:0) content of cotyledon callus, cultured on MS medium supplemented with various sucrose concentration.

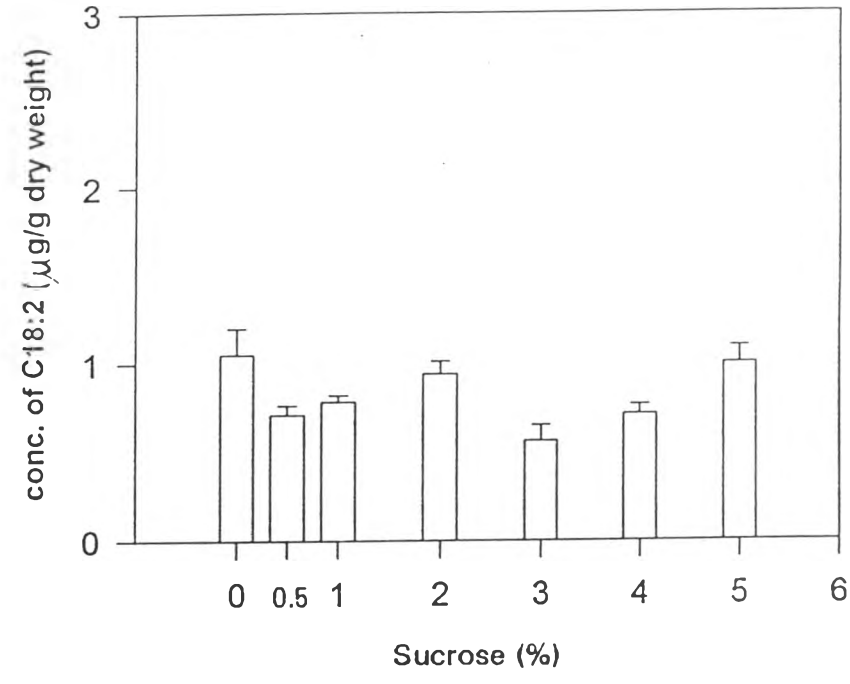


Figure. 47 Linoleic acid (C18:2) content of cotyledon callus, cultured on MS medium supplemented with various sucrose concentration.

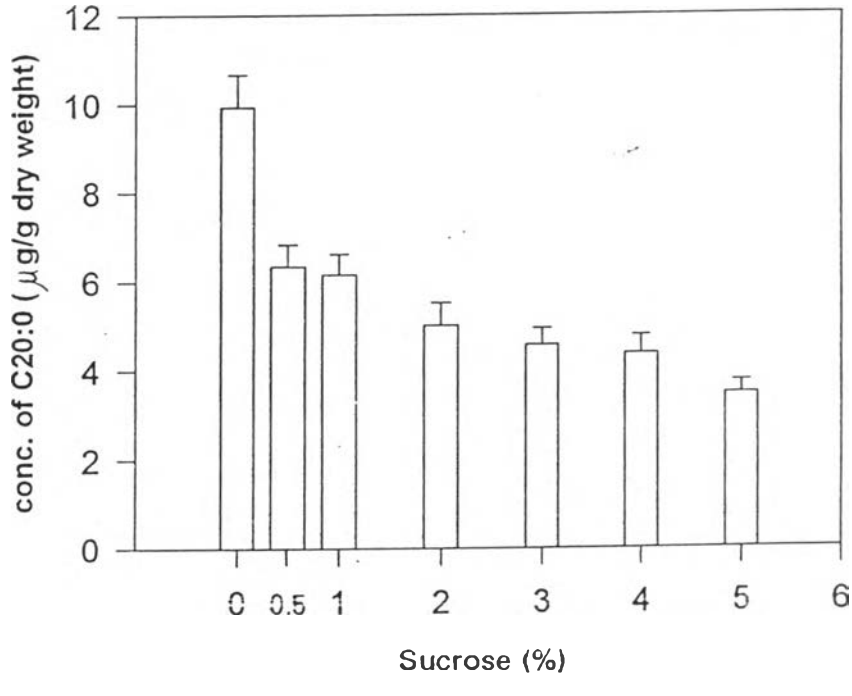


Figure. 48 Arachidic acid (C20:0) content of cotyledon callus, cultured on MS medium supplemented with various sucrose concentration.