Callus Induction of Beet Root for Speed up Economical Plant Production

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บทคัดย่อ

การชักนำให้เกิดแคลลัสเป็นเทคนิคหนึ่งของการเพาะเลี้ยงเนื้อเยื่อ เป็นวิธีที่มีประสิทธิภาพสำหรับเร่งการผลิตและปรับปรุงหรือแก้ปัญหาในกระบวนการทางชีวภาพของพืช การทดลองครั้งนี้ได้ใช้ทำการชักนำบีทรูทให้เกิดแคลลัสเพื่อเพิ่มผลิตผลที่สามารถนำไปใช้ประโยชน์ในด้านอุตสาหกรรม โดยได้ศึกษาสภาวะที่เหมาะสมของการฟอกฆ่าเชื้อและอัตราส่วนของสารควบคุมการเจริญเติบโต Naphthalene acetic acid (NAA) ร่วมกับ 6-benzylaminopurine (BAP) ที่เหมาะสมต่อการชักนำให้เกิดเป็นแคลลัส ผลการทดลองพบว่าในการฟอกฆ่าเชื้อเนื้อเยื่อรากบีทรูทโดยใช้สารละลายคลอรอกซ์เข้มข้น 15% และเขย่าเป็นเวลา 15 นาที เป็นสภาวะของการฟอกฆ่าเชื้อที่ดีที่สุด มีประสิทธิภาพสามารถฟอกฆ่าเชื้อได้สูงสุดถึง 55% จากนั้นนำเนื้อเยื่อบีทรูทลงเพาะเลี้ยงบนอาหารสังเคราะห์สูตร MS ที่เติมสารควบคุมการเจริญเติบโต NAA ร่วมกับ BAP ในอัตราส่วนความเข้มข้นต่างๆ จากการทดลองพบว่าในสูตรอาหารที่เติม NAA ความเข้มข้น 2 mg/L ร่วมกับ BAP ความเข้มข้น 0.5 mg/L ทำให้เนื้อเยื่อบีทรูทเกิดเป็นแคลลัสและมีน้ำหนักสดมากที่สุด คือ 3.87 กรัม และเมื่อทดสอบความมีชีวิตของเนื้อเยื่อจากสูตรอาหารดังกล่าวด้วยวิธี Triphenyl tetrazolium chloride (TTC) test พบว่าเนื้อเยื่อมีชีวิตทั้งหมด 100 %

คำสำคัญ: บีทรูท แคลลัส แนฟทาลีนอะซิติกแอซิด 6 - เบนซิลลามิโนพิวลีน

Abstract

Callus induction is one technique in plant tissue culture and become powerful method for speeding up plant production and improving or solving problems in plant biotechnology. Callus induction of beet root was experimented in order to increasing the plant production to apply for industrial advantages. Appropriate sterilization condition and suitable concentration of naphthaleneacetic acid (NAA)/6-benzylaminopurine (BAP) for inducing callus was done. Sterilization with 15% Clorox for 15 minute was the best condition and fifty-five% of beet root survivals were reached. This condition was applied to the beet root tissue before it was cultured on a Murashige and Skoog (MS) Medium supplemented with combination of NAA and BAP to induce callus formation. The highest fresh weight (3.87 g) of callus occurred with 2 mg/L NAA and 0.5 mg/L BAP and showed 100% viability after tested with Triphenyl tetrezolium chloride (TTC).

Keywords: Beet Root, callus, naphthaleneacetic acid (NAA), 6- benzylaminopurine (BAP)

1. Introduction

Beta valgais, known as sugar beet, garden beet; beet root, spinach beet, leaf beet and Swiss chard, is

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an edible root vegetable. Most beet root cultivars have red roots which due to pigments called betalains [1]. The betalains are a main class of natural plant pigments subdividing into two structure groups: the violet betacyanins and the yellow betaxanthins. Unlike the other plant pigments, betalains have a limited biological distribution, finding only in plant species confined to the order *Caryophylalles*, notably the beet root (*Chenopodaceae*). Although beet root contains a mixture of betalain pigments, the characteristic red-violet color of beet root is derived from a betacyanin namely betanin. Recently, the betanin have gained interest because of the potential role as food colorants in food industry [2] and an antioxidant reagent in medical advantages [3]. Most Thailand areas have warm to hot weather conditions. Those weathers are unsuitable to grow beet roots because the beet root develops its deepest red color in cool temperature range. Beet roots also grow in tropical climate but with considerable loss of color intensity [4].

To increase beet root production in Thailand, micropropagation of this economic plant through callus culture techniques has been developed. The objectives of this study were to establish an efficient sterilization protocol and condition for beet root callus induction from root explants, which up to now, the information remains limited.

2. Materials and Methods

2.1 Plant Material

A root of beet root (*Beta vulgaris*) was soaked with soap and washed with tap water to remove soil. The outer part of root was pared off and sectioned into square size (1x1 cm^2) and washed again with running tap water for 45 minutes and soaked again with 70% (v/v) alcohol for 10 minutes.

2.2 Optimization of Tissue Sterilization Condition

All of beet root tissues (1x1 cm^2) were sterilized with various concentrations (5%, 10%, 15%, and 20% v/v) of Clorox containing 1 ml Tween 20 per 100 ml for 10 and 15 minutes, then rinsed three times in sterile distilled water. The beet root tissues were sectioned into 3x3 mm size and cultured on MS medium [5]. The pH was adjusted to 5.6 with 0.1 M NaOH before adding agar and autoclaving at 121 ˚C for 15 min. Beet root expl ants were cultured at 25 ˚C under 1000 lux of light intensity and 12 hours photoperiod for 10 days. Experiment was repeated two times with 10 replications. Efficiency (percentage) of tissue survival was determined.

2.3 Callus Induction

Beet root explants were cultured on MS medium supplemented with combination of (0, 1, 2 or 5 mg/L) NAA and (0.01, 0.50, 1.00, 5.00 or 10.00 mg/L) BAP. All explants were incubated at 25 ˚C un der 1000 lux of light intensity and 12 hours photoperiod. The Experiment was repeated two times with 5 replications. Callus formation, average fresh weight and cell viability were recorded after 30 days

2.4 Cell Viability Test

The 2, 3, 5-triphenyltetrazolium chloride (TTC) assay [6] was used for viability of callus.

2.5 Chemicals

All chemicals and solvents (analytical grade) were purchased from Fluka, Switzerland; Sigma, America; Carlo Erba, France; Umivar, America; Merck, German.

3. Results

3.1 Optimal Condition for Beet Root Explants Sterilization

After incubated initial explants on MS basal medium for 10 days, the efficiency of survival tissue was shown in Table 1. The result showed that the optimal condition with 15% Clorox for 15 minute was the best condition for sterilization. Fifty-five percent of explants survival were reached (Figure 1).

Table 1 The number of sterilized beet root explants after using sterilization conditions with vary concentration of Clorox and times.

Treatments	concentrations of Clorox (%)	Time (Minute)	average number of survival tissue (Pieces)
1	5	10	3.5±0.71
2	5	15	4.0±1.41
3	10	10	4.0±1.41
4	10	15	4.0±2.83
5	15	10	5.0±0.00
6	15	15	5.5±4.95
7	20	10	4.0±2.83
8	20	15	4.5±0.71

± represented standard deviation (S.D.)

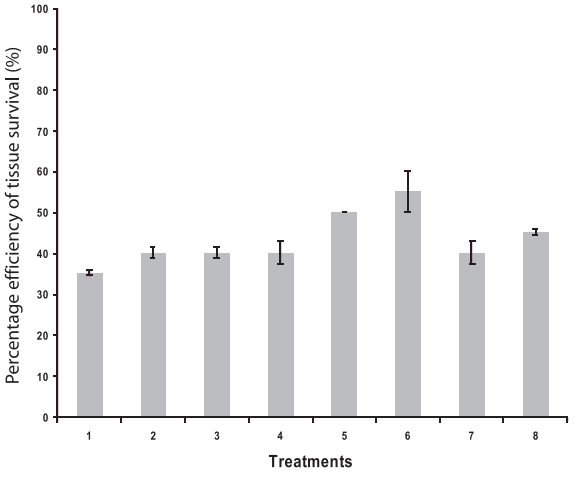

Figure 1 Percentage efficiency of tissue survival was determined after sterilization culture at 25ºC under 1000 lux of light intensity and 12 hours photoperiod.

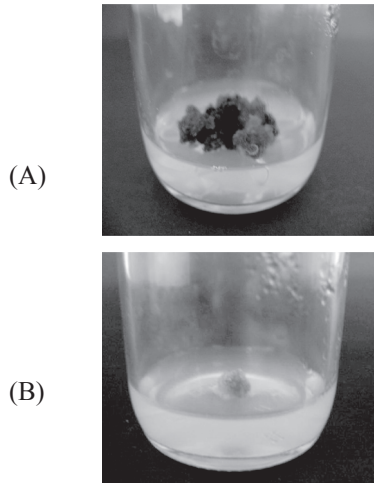

Figure 2 Two types of beet root callus were obtained. Red friable callus (A) was found in all treatments, whereas green-yellow friable callus (B) was only on MS medium containing 5 mg/L NAA with 1 mg/L, 5 mg/L or 10 mg/L BAP.

Table 2 Callus formation on MS medium with combination of NAA and BAP with in 30 days

Growth Regulators (mg/L)		Callus fresh weights (g)			
	NAA	0.00	1.00	2.00	5.00
BAP	0.01	0.35±0.04	0.66±0.19	0.68±0.25	1.49±0.07
	0.50	0.44±0.03	2.04±0.30	3.87±1.72	2.04±0.99
	1.00	0.35±0.07	0.28±0.02	2.18±1.01	1.25±0.78
	5.00	0.14±0.05	0.66±0.39	1.69±1.61	0.65±0.37
	10.00	0.17±0.00	0.98±0.35	0.85±0.13	0.82±0.12

± represented standard deviation (S.D.)

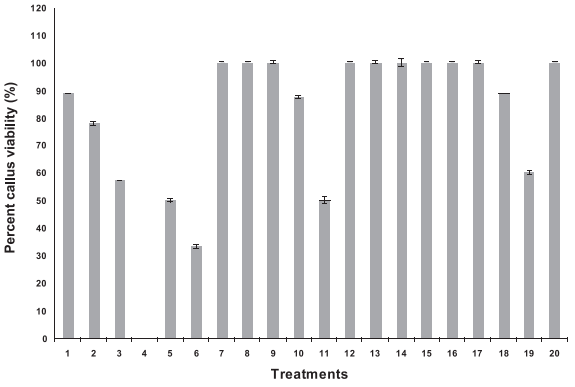

Figure 3 Percent callus viability in different concentrations and combination of NAA and BAP was investigated after beet root explants were culture at 25°C under 1000 lux of light intensity and 12 hours photoperiod for 30 days. The treatments no. 1-5 are medium containing 0 mg/L NAA supplemented with 0.01, 0.5, 1, 5 or 10 mg/L BAP. The treatments no. 6-10 are medium containing 1 mg/L NAA supplemented with 0.01, 0.5, 1, 5 or 10 mg/L BAP. The treatments no. 11-15 are medium containing 2 mg/L NAA supplemented with 0.01, 0.5, 1, 5 or 10 mg/L BAP. The treatments no. 16-20 are medium containing 5 mg/L NAA supplemented with 0.01, 0.5, 1, 5 or 10 mg/L BAP.

3.2 Callus Formation

The combinations of NAA and BAP effected on different growth callus as shown in Table 2. The highest callus fresh weight was observed on MS medium supplemented with 2 mg/L NAA and 0.5 mg/L BAP. Two color types of callus were obtained; red friable callus (Figure 2A) and green-yellow friable callus (Figure 2B) which referred to as Type I and Type II, respectively. Type I callus was found mostly in the experiment whereas Type II was only on MS medium supplemented with 5 mg/L NAA with 1 mg/L, 5 mg/L and 10 mg/L BAP.

3.3 Cell Viability (TTC assay) of Callus

After using tetrazolium assay, the efficiency of callus viability was showed in Table 3. The result showed that more than 50% of viability callus was observed as in Figure 3.

4. Discussion

The outer surfaces of roots generally are infested with spores and other microbial cells. To reduce the amount of contamination on explants derived from this part, root explants should be washed under running tap water for 45 minutes before treatment with a sterilized solution. This technique was successful for removing contamination from plant tissue and using in micropropagation techniques [7]. After washing, the

Table 3 The amount of viability callus formed in MS medium with combination of NAA and BAP with in 30 days

Growth Regulators (mg/L)		viability callus (Pieces)			
	NAA	0.00	1.00	2.00	5.00
BAP	0.01	4.0±0.00	1.5±0.71	2.0±1.41	4.0±0.00
	0.50	3.5±0.71	4.0±0.00	4.0±0.00	4.5±0.71
	1.00	2.0±0.00	3.0±0.00	3.5±0.71	4.0±0.00
	5.00	0.0±0.00	2.5±0.71	4.0±1.41	1.5±0.71
	10.00	0.5±0.71	3.5±0.71	4.0±0.00	5.0±0.00

± represented standard deviation (S.D.)

explants tissue is submerged into the disinfectant. A 10-50% solution of Clorox is one of the most commonly used sterilants in plant tissue culture [8]. For materials that are difficult to disinfect due to heavy pathogen contamination, mercuric chloride was used in the sterilization step associated with sodium hypochlorite [9], [10]. Investigation of appropriated concentration and exposure time of sodium hypochlorite (Clorox) on beet root explants is the way to obtain a large percentage of explants free from pathogens. This experiment, sterilization with 15% Clorox for 15 minute was the best condition.

Callus formation from many types of tissues of sugar beet on Murashige and Skoog (MS) media, which contained a combination of a cytokinin and an auxin or contained the sole plant hormone has been reported [11]-[15] Growth of explant tissue on culture medium depends on plant growth regulators and there is less information of callus induction from root explants of beet root. Therefore, the suitable combination ratio of BAP and NAA obtained from this study could be basic information for callus and pigment induction of this economically important crop species. From this study, the highest fresh weight (3.87 g) of red friable callus was observed in MS complemented with 2 mg/L NAA and 0.5 mg/L BAP.

There are a variety of factors have effect to regulation of pigment biosynthesis in betalain accumulating plants such as beet root. Growth regulators are one of the factors involved in pigment formation. As the report of Leathers in 1992 [16], changes in the ratio of auxin and cytokinin result in obtained color of calli. Red sector appeared on yellow calli transferred to media containing a reduced auxin concentration. In this research, red calli were found mostly in the treatment which have a low auxin concentration. Growth regulator composition in both combination and concentration will need to be determined which the optimal condition contributed to induce red pigment formation in callus.

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