

IN VITRO GROWTH RESPONSE OF EXPLANTS AND MICROPROPAGATION OF *Brassica oleraceae* L. var *Capitata*

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Abstract: Experiments were carried out to establish optimum culture condition and to identify the most responsive explants for regeneration of *Brassica oleraceae* L. var *Capitata* through plant tissue culture system. The experiment was induced by manipulating various growth regulators during organogenesis using stems, petioles, leaves and roots as explants. The experimental design of this study was Complete Randomized Design (CRD) method. For shoot regeneration using different concentrations of BAP and NAA, the most responsive explant was stem explants in which MS medium supplemented with 1.5 mg/L BAP and 0.5 mg/L NAA produced 8.400 ± 0.889 shoots per explant. Whereas, for root regeneration using different concentrations of BAP and NAA, the most responsive explant was leaf in which MS media supplemented with 0.5 mg/L BAP and 0.5 mg/L NAA produced 51.133 ± 2.929 roots per explant. This research showed that a morphogenesis study of *Brassica oleraceae* L. var *Capitata* through tissue culture system was successfully achieved. This study could lead to the better research and development (R&D) for vegetables in the future.

Keywords: morphogenesis, *in vitro*, Murashige and Skoog medium, callus, plantlets.

I. INTRODUCTION

Tissue culture is one of the methods to propagate plants in a short period of time. It is also known as *in vitro* propagation and micropropagation. Tissue culture is one of the methods in which small part of plants (explant) whether the leaves, stems (organ) or cells were placed into the cultured medium for growth purpose in sterile or aseptic that is in the condition that free from microorganisms such as fungus, bacteria, and viruses [1]. Besides that, plant tissue culture or micropropagation means the way of rapidly multiplying plant materials stock to ensure large number of plant progeny could be produced [2]. Plant tissue culture needs the medium to replace the role of soil and fertilizer. There are many types of plant tissue culture medium. The most commonly used is known as Murashige and Skoog medium (MS) [3].

Brassica oleraceae L. var *Capitata* or commonly known as cabbage is derived from Brassica family. Cabbage is an important vegetable plant; grown on large surfaces worldwide, occupying the third place in the world, after solanaceae and onion [4]. It contains high amounts of vitamin such as vitamin K, C, A and folic acid, flavonoids, fiber, proteins and mineral anticarcinogenic and glucosinolates [5]. For instance, antioxidant is rich in the cabbage can be used to treat many types of cancer. The risk of breast and colon cancer will be lowered by eating cabbage two to three times per week [6]. Due to its importance, *Brassica oleraceae* L. var *Capitata* has very high demand by consumers. The sustainability of

Brassica production has to be ensured. Besides propagating Brassicas through conventional methods, as an alternative, it can also be produced through plant tissue culture system. Therefore, in the present study, explants of *Brassica oleraceae* L. var Capitata will be cultured through plant tissue culture system to obtain mass propagation of this important vegetable crop.

II. MATERIAL AND METHOD

A. Source of seeds

The seeds of *Brassica oleraceae* L. var *capitata* were obtained from commercial agricultural nursery that is nearby Sultan Idris Educational University (UPSI), Tanjong Malim, Perak. The seeds must be fully sterilized to prevent from the contamination agent such like bacteria and fungus.

B. Culture Medium Preparation

MS basal medium (free hormone) was used for germination of sterilized seeds. The materials used to produce the medium were 30.00 g/L sucrose, 8.00 g/L technical agar and 4.40 g/L basal salt mixture. Then, for further micropropagation experiments, the explants culture media was enriched with various plant growth regulators and at various concentration combinations. The pH of the media was adjusted to pH 5.8 prior to autoclaving at 105 kPa and temperature at 121°C for 20 minutes.

C. Sterilization of Seeds

Sterilization of seeds is an important initial step. Firstly, the seeds were left under the running tap water for one hour. Secondly, the seeds surface were sterilized in commercial sodium hypochlorite at different concentration. The concentrations were 70 % (5 minutes), 50 % (5 minutes), 20 % (10 minutes), 10 % (10 minutes) and distilled water (10 minutes). The seeds were soaked in sodium hypochlorite and were gently agitated. Next, the seeds were transferred into the laminar air flow cabinet. After that, the seeds were dipped in 70 % of ethanol for one minutes and rinsed for about three times with sterile distilled water all over again. Lastly, the seed were dried on a sterile tissue or filter paper. The seeds that were ready to be cultured were placed into the jar containing MS (Murashige and Skoog, 1962) basal medium.

D. Explant Preparation and Culture Procedure

The explants used were obtained from the 4 -week-old aseptic seedlings. The part of the explants excised were stems, leaves, roots and petioles. Leaves explants were excised into small pieces of 10 mm X 10 mm in size. Roots, petioles and stems explant were cut into 10 mm in length. All the explants were cultured on MS medium supplemented with various concentration combination of hormone.

E. Screening for Suitable NAA and BAP for Shoot and Root Regenerations

In this research, two types of hormone were used which were Naphthalene Acetic Acid (NAA) and 6-Benzylaminopurine (BAP). Both hormones function as plant growth regulators for the formation of shoots and roots. These hormones were prepared in combinations.

III. DATA ANALYSIS

All data was statistically analysed. The analysis of variance (ANOVA) for the design were carried out in order to detect the significance differences among the treatments.

IV. RESULT AND DISCUSSION

A. Identification of Shoot Regeneration Media

Table 1 showed the results of different concentrations and combinations of BAP and NAA on stem, petiole, leaf and root explants cultured on MS media for regeneration of shoot. For stem explant, mostly all stem explant gave positive respond to all combination of NAA and BAP. The best respond was cultured on MS media supplemented with 1.5 mg/L BAP + 0.5 mg/L NAA with 8.400 ± 0.899 shoots per explant (Fig. 1a). Moreover, petiole explant showed respond to only four treatments. The best respond was on MS media supplemented with 1.0 mg/L BAP + 0.5 mg/L NAA with 5.567 ± 1.070 shoots per explant (Fig. 1b). Next, the regeneration of shoot from leaf and root explant showed the lowest respond towards NAA and BAP. Only three treatments showed respond for leaf explant. MS media with 1.0 mg/L BAP + 0.5

mg/L NAA gave the highest shoot regeneration with 2.533 ± 0.540 (Fig. 1c). However, root explants showed respond to only two treatments only. The highest was MS media supplemented with 1.0 mg/L BAP + 0.5 mg/L NAA with 1.267 ± 0.544 shoots per explant (Fig. 1d).

In the regeneration of shoot using NAA and BAP, stem was the best explant from all four explant. This was supported by Yaacob et al. (2014) in the research of *in vitro* regeneration in *Citrus assamensis* [7]. While the least responsive explant was root because it only responded to two treatments. The optimum result of stem explant was when the explant was cultured on MS media supplemented with 1.5 mg / L BAP + 0.5 mg / L NAA.

Table 1: The Effect of Different Concentrations and Combinations of BAP and NAA on Stem, Petiole, Leaf and Root Explants Cultured on MS Media for Regeneration of Shoot at $23 \pm 1^\circ\text{C}$ with 16 Hours Light and Eight Hours Dark.

No	Treatments	Explants (Mean $\pm$ SE)			
		Stem	Petiole	Leaf	Root
1	0.5 mg/L BAP + 0.5 mg/L NAA	$0.000 \pm 0.000_f$	$2.333 \pm 0.410_c$	$1.733 \pm 0.271_c$	$0.000 \pm 0.000_b$
2	1.0 mg/L BAP + 0.5 mg/L NAA	$6.967 \pm 1.149_b$	$5.567 \pm 1.070_a$	$2.533 \pm 0.540_a$	$1.267 \pm 0.544_a$
3	1.5 mg/L BAP + 0.5 mg/L NAA	$8.400 \pm 0.889_a$	$0.000 \pm 0.000_d$	$0.000 \pm 0.000_d$	$0.000 \pm 0.000_b$
4	2.0 mg/L BAP + 0.5 mg/L NAA	$4.033 \pm 0.809_{bc}$	$3.633 \pm 0.628_b$	$2.267 \pm 0.452_b$	$0.000 \pm 0.000_b$
5	0.5 mg/L BAP + 1.0 mg/L NAA	$3.300 \pm 0.766_{bcd}$	$0.567 \pm 0.092_d$	$0.000 \pm 0.000_d$	$0.000 \pm 0.000_b$
6	1.0 mg/L BAP + 1.0 mg/L NAA	$3.400 \pm 0.433_{bc}$	$0.000 \pm 0.000_d$	$0.000 \pm 0.000_d$	$0.000 \pm 0.000_b$
7	1.5 mg/L BAP + 1.0 mg/L NAA	$4.700 \pm 0.897_b$	$0.000 \pm 0.000_d$	$0.000 \pm 0.000_d$	$0.000 \pm 0.000_b$
8	2.0 mg/L BAP + 1.0 mg/L NAA	$2.200 \pm 0.399_{cde}$	$0.000 \pm 0.000_d$	$0.000 \pm 0.000_d$	$0.000 \pm 0.000_b$
9	0.5 mg/L BAP + 1.5 mg/L NAA	$2.200 \pm 0.550_{cde}$	$0.000 \pm 0.000_d$	$0.000 \pm 0.000_d$	$0.000 \pm 0.000_b$
10	1.0 mg/L BAP + 1.5 mg/L NAA	$1.500 \pm 0.287_{def}$	$0.000 \pm 0.000_d$	$0.000 \pm 0.000_d$	$0.000 \pm 0.000_b$
11	1.5 mg/L BAP + 1.5 mg/L NAA	$1.433 \pm 0.257_{def}$	$0.000 \pm 0.000_d$	$0.000 \pm 0.000_d$	$0.000 \pm 0.000_b$
12	2.0 mg/L BAP + 1.5 mg/L NAA	$0.000 \pm .000_f$	$0.000 \pm 0.000_d$	$0.000 \pm 0.000_d$	$0.000 \pm 0.000_b$
13	0.5 mg/L BAP + 2.0 mg/L NAA	$0.000 \pm 0.000_f$	$0.000 \pm 0.000_d$	$0.000 \pm 0.000_d$	$0.000 \pm 0.000_b$
14	1.0 mg/L BAP + 2.0 mg/L NAA	$2.400 \pm 0.518_{cde}$	$0.000 \pm 0.000_d$	$0.000 \pm 0.000_d$	$0.000 \pm 0.000_b$
15	1.5 mg/L BAP + 2.0 mg/L NAA	$3.467 \pm 0.602_{bc}$	$0.000 \pm 0.000_d$	$0.000 \pm 0.000_d$	$0.000 \pm 0.000_b$
16	2.0 mg/L BAP + 2.0 mg/L NAA	$0.867 \pm 0.234_{ef}$	$0.000 \pm 0.000_d$	$0.000 \pm 0.000_d$	$0.000 \pm 0.000_b$
17	0.0 mg/L BAP + 0.0 mg/L NAA	$2.800 \pm 0.574_{cd}$	$0.000 \pm 0.000_d$	$0.800 \pm 0.297_c$	$0.233 \pm 0.092_b$

Mean $\pm$ SE, n=30. Mean with Different Letters in the Same Column Differ Significantly at $p=0.05$

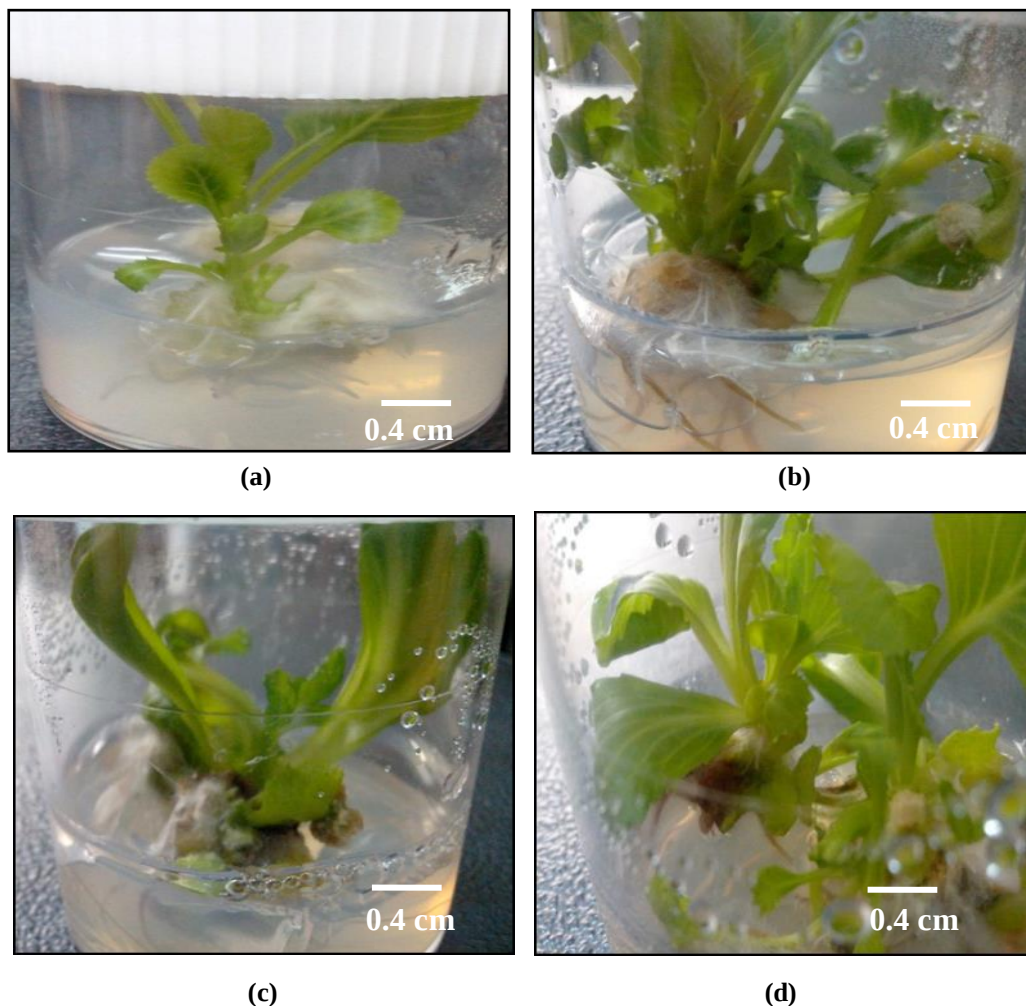


Fig. 1. Identification of Shoot Regeneration Media of *Brassica oleraceae* L. var *Capitata*. (a) Regeneration of shoot from stem explant cultured on MS media supplemented with 1.0 mg/L BAP + 0.5 mg/L NAA; (b) Regeneration of shoot from petiole explant cultured on MS media supplemented with 1.0 mg/L BAP + 0.5 mg/L NAA; (c) Regeneration of shoot from leaf explant cultured on MS Media supplemented with 1.0 mg/L BAP + 0.5 mg/L NAA; (d) Regeneration of shoot from root explant cultured on MS media supplemented with 1.0 mg/L BAP + 0.5 mg/L

B. Identification of Root Induction Media

Response and development of rooting of *Brassica oleraceae* L.var *Capitata* explant when subcultured on root induction media with different concentration and combination of BAP and NAA is shown in Table 2. For stem explant, the best respond was MS media with 1.0 mg/L BAP + 0.5 mg/L NAA with 30.167 ± 2.769 roots per explant (Fig. 2a). Meanwhile, petiole explant showed the highest respond MS media supplemented with 0.5 mg/L BAP + 0.5 mg/L NAA with 29.800 ± 1.783 roots per explant (Fig. 2b). Leaf explant showed best respond for regeneration of root on MS media added with 0.5 mg/L BAP + 0.5 mg/L NAA with 51.133 ± 2.929 roots per explant (Fig. 2c). Besides, root explant gave optimum respond when MS media supplemented with 0.5 mg/L BAP + 1.0 mg/L NAA with 23.500 ± 1.049 roots per explant (Fig. 2d).

Regeneration of root on NAA and BAP showed that all four types of explants were responsive. However, the highest respond was on leaf explant when cultured in the combination treatment of 0.5 mg/L BAP + 0.5 mg/L NAA. This was line with Hussein (2012) that stated that auxin such as Naphtalene acetic acid (NAA) have been reported to promote plant rooting in vitro culture condition [8]. In addition, the use of BAP was also agreed by Ngomuo, Mneney and Ndakidemi (2013) stating that N6-benzylaminopurine (BAP) is the most commonly preferred cytokinin on growth and development [9].

Table 2: The Effect of Different Concentrations and Combinations of BAP and NAA on Stem, Petiole, Leaf and Root Explants Cultured on MS Media for Regeneration of Root at 23±1°C with 16 Hours Light and Eight Hours Dark.

No	Treatments	Explants (Mean ± SE)			
		Stem	Petiole	Leaf	Root
1	0.5 mg/L BAP + 0.5 mg/L NAA	9.333 ± 0.828 _{cde}	29.800 ± 1.783 _a	51.133 ± 2.929 _a	15.933 ± 1.208 _{bc}
2	1.0 mg/L BAP + 0.5 mg/L NAA	30.167 ± 2.769 _a	25.267 ± 2.744 _b	36.100 ± 2.944 _b	21.967 ± 2.091 _b
3	1.5 mg/L BAP + 0.5 mg/L NAA	13.067 ± 1.262 _{bcd}	1.000 ± 0.311 _{gh}	0.000 ± 0.000 _g	0.000 ± 0.000 _h
4	2.0 mg/L BAP + 0.5 mg/L NAA	2.200 ± 0.466 _{gh}	9.667 ± 1.387 _{de}	7.700 ± 0.738 _{ef}	2.267 ± 0.518 _{gh}
5	0.5 mg/L BAP + 1.0 mg/L NAA	11.233 ± 4.181 _{cde}	29.267 ± 3.913 _b	10.500 ± 3.064 _{de}	23.500 ± 1.049 _a
6	1.0 mg/L BAP + 1.0 mg/L NAA	17.467 ± 1.817 _b	0.000 ± 0.000 _h	18.033 ± 2.643 _c	17.033 ± 1.863 _b
7	1.5 mg/L BAP + 1.0 mg/L NAA	11.300 ± 0.934 _{cde}	11.167 ± 0.685 _{cd}	3.067 ± 0.711 _{fg}	5.500 ± 0.352 _f
8	2.0 mg/L BAP + 1.0 mg/L NAA	17.167 ± 1.527 _b	8.433 ± 1.276 _{def}	3.033 ± 0.905 _{fg}	9.900 ± 1.034 _{de}
9	0.5 mg/L BAP + 1.5 mg/L NAA	8.500 ± 1.494 _{def}	15.233 ± 1.236 _c	7.967 ± 1.228 _{def}	7.033 ± 0.962 _{ef}
10	1.0 mg/L BAP + 1.5 mg/L NAA	14.700 ± 1.127 _{bc}	12.833 ± 1.233 _{cd}	9.933 ± 1.719 _{de}	6.567 ± 0.926 _f
11	1.5 mg/L BAP + 1.5 mg/L NAA	6.067 ± 0.975 _{efg}	3.433 ± 0.891 _{fgh}	0.000 ± 0.000 _g	0.000 ± 0.000 _h
13	0.5 mg/L BAP + 2.0 mg/L NAA	0.000 ± 0.000 _h	8.133 ± 1.762 _{def}	0.000 ± 0.000 _g	14.300 ± 1.149 _{bc}
14	1.0 mg/L BAP + 2.0 mg/L NAA	9.500 ± 1.402 _{cde}	22.900 ± 1.479 _b	8.500 ± 1.341 _{de}	13.767 ± 1.125 _c
15	1.5 mg/L BAP + 2.0 mg/L NAA	13.633 ± 1.686 _{bcd}	11.433 ± 1.146 _{cd}	13.167 ± 1.063 _d	10.800 ± 1.183 _d
16	2.0 mg/L BAP + 2.0 mg/L NAA	6.667 ± 1.453 _{efg}	5.633 ± 1.362 _{efg}	12.200 ± 1.821 _{de}	17.300 ± 1.176 _b
17	0.0 mg/L BAP + 0.0 mg/L NAA	6.700 ± 1.336 _{efg}	1.267 ± 0.166 _{gh}	1.300 ± 0.329 _g	4.767 ± 0.243 _{fg}

Mean ± SE, n=30. Mean with Different Letters in the Same Column Differ Significantly at p=0.05

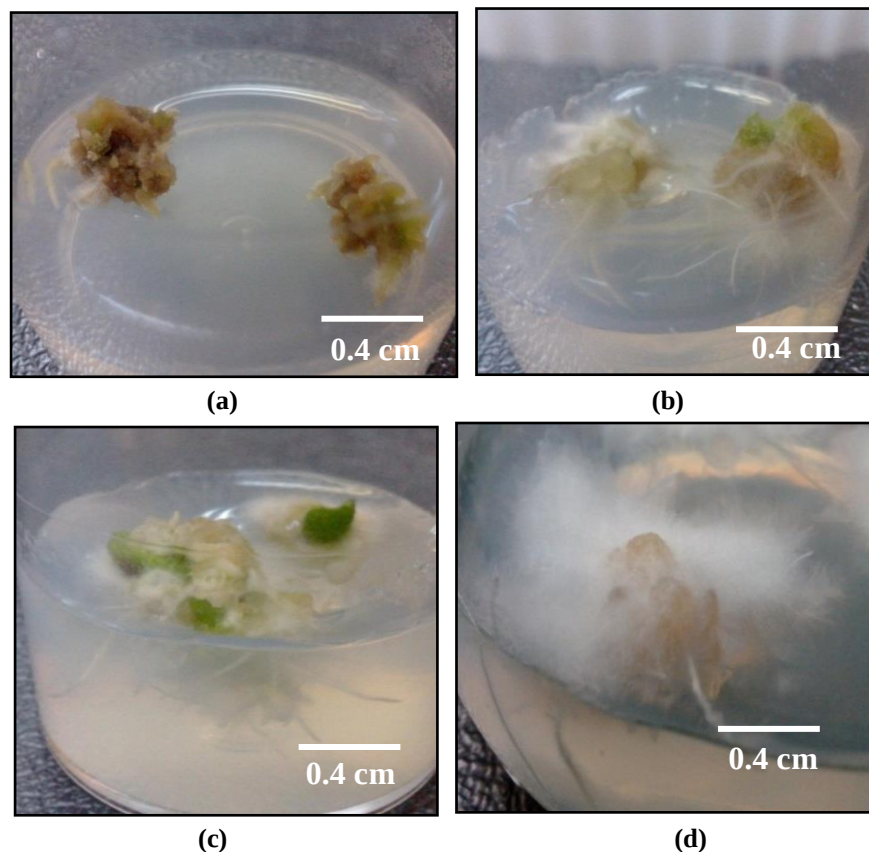


Fig. 2. Identification of Root Regeneration Media of *Brassica oleraceae* L. var Capitata. (a) Regeneration of root from stem explant cultured on MS Media supplemented with MS 1.0 mg/L BAP + 0.5 mg/L IAA; (b) Regeneration of root from petiole explant cultured on MS Media supplemented with 0.5 mg/L BAP + 0.5 mg/L NA; (c) Regeneration of root from leaf explant cultured on MS media supplemented with 0.5 mg/L BAP + 0.5 mg/L NAA; (d) Regeneration of root from root explant cultured on MS media supplemented with 0.5 mg/L BAP + 1.0 mg/L NAA

V. CONCLUSION AND RECOMMENDATION

As a conclusion, morphogenesis studies of *Brassica oleraceae* L. var *Capitata* through tissue culture system were successfully achieved. Studies on *Brassica oleraceae* L. var *Capitata* needs to be expanded as the number of studies is still limited. Therefore, this study provide information and knowledge on micropropagation of *Brassica oleraceae* L. var *Capitata*. This research is hoped to be able to contribute and benefit the agricultural industry in worldwide.

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