

In Vitro Regeneration of *Brassica Oleraceae* L. Var *Capitata* through Stems, Roots, Leaves and Petioles Culture

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Abstract— Experiments were carried out to establish optimum culture condition and to identify the most responsive explants to regenerate *Brassica oleraceae* L. var *Capitata* through plant tissue culture system. The explants were induced with manipulation of growth regulators during organogenesis. Plant growth regulators that were used are α -Naphthalene acetic acid (NAA) and 6-Benzylaminopurine (BAP). Different combinations and concentrations of plant growth regulators were added into the Murashige and Skoog, [1] (MS) medium. The formations of callus were initiated after two weeks of subculture. The new plantlets were raised in a short period of time when stem and root explants were cultured on MS medium containing 1.0mg/l BAP and 0.5 mg/l NAA. The explants of petiole and leaves showed that there were slower growths of callus formation for most of the concentration. The well rooted in vitro raised plantlets were successfully transferred to soil and their survival rate under natural environment was 90%.

Keywords— Regeneration, in vitro, Murashige and Skoog, callus, plantlets, growth regulators.

I. INTRODUCTION

CABBAGE or its scientific name *Brassica oleraceae* L. var. *Capitata* is from the family of Brassicaceae (Cruciferae). *Brassica oleraceae* L. (cabbage), *Brassica nigra* L. Koch. (mustard) and *Sinapis alba* L.(radish) are the most familiar in this family [2]. The *Brassica oleraceae* L. var. *Capitata* also known as cabbage, green cabbage, white cabbage, pau choy, kole chai, bao cai, patha gobi, muttakose, band gobi, kubis and etc. [3]. Besides, it has a lot of nutrients that give benefits for human health. For example, antioxidant in the cabbage can be used to treat cancer. Eating cabbage two to three times per week will lower the risk of breast and colon cancer [4]. The presence of glucosinolates will help to treat cancers of the colon, lung, skin, and stomach. Moreover, it also will lower the rates of cataracts, heart disease, and stroke.

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Then, folic acid and iron also needed to prevent and correct anaemia. Folic acid will help to lower the risk of spina bifida in babies. Moreover, if the cabbages are eaten raw or the juice is taken it will be useful to treat ulcers. Records show that the ancient Greeks, Romans, Indians and Chinese used brassica vegetables greatly. This research will contribute to the developing of an effective protocol for *in vitro* regeneration of *Brassica oleraceae* L. var. *Capitata* by adding various concentrations of NAA and BAP as this vegetable has high demand in the market.

II. MATERIALS AND METHOD

The hybrid seeds of *Brassica oleraceae* L.var *Capitata* were obtained from commercial agricultural nursery that is near Sultan Idris Educational University (UPSI), Tanjong Malim, Perak, Malaysia. The seeds are left under running tap water for 60 minutes. Then, the seed were soaked in the sodium hypochloride at different concentration which are 70% (5 minutes), 50% (5 minutes), 30% (10 minutes), 20% (10 minutes) [5] and distilled water (5 minutes). This was done due to remove the seed coating and the dust that surround the seeds.

After that, the seed were washed with 70% ethanol for 60 seconds and rinsed three times with sterile distilled water. Ready clean seeds were put into the jem jar containing agar solidified with MS medium. Cultures were kept in the culture room incubated in dark photoperiod at $25 \pm 1^\circ\text{C}$ with 16 hours light and 8 hours dark photoperiod with 1000 lux of light intensity [6]. Seeds of *Brassica oleraceae* L.var *Capitata* were germinated after 7 days of culture.

The stems, leaves, roots and petioles explants obtained from *in vitro* of plantlets were then placed on MS medium containing 30 g/L sucrose and 8.0 g/L technical agar supplemented with various concentration of NAA (0.5-2.0 mg/L) and BAP (0.5-2.0 mg/L). Media were autoclaved at 121°C for 21 minutes. The pH of the medium employed in this experiment was adjusted to pH 5.8. Regeneration of the plantlets was obtained and the explants of roots, stems, leaves and petioles were isolated. After two weeks of subculture, callus initiation were occurred. The observation was recorded.

III. RESULTS AND DISCUSSION

TABLE I
PERCENTAGE OF CALLUS FORMATION FROM PETIOLES, STEMS, LEAVES,
AND ROOTS EXPLANT CULTURED ON MS MEDIUM FORTIFIED WITH VARIOUS
CONCENTRATION OF NAA AND BAP.

Hormone concentration (mg/L) NAA + BAP	Explants	% Callus
CONTROL	Petioles	3.86 ± 0.40
	Leaves	2.40 ± 0.40
	Stems	NR
	Roots	NR
0.5mg/L + 0.5mg/L	Petioles	59.00 ± 4.33
	Leaves	34.17 ± 5.15
	Stems	NR
	Roots	14.79 ± 1.52
0.5mg/L + 1.0mg/L	Petioles	46.45 ± 3.95
	Leaves	24.87 ± 2.80
	Stems	67.50 ± 2.80
	Roots	33.28 ± 4.01
0.5mg/L + 1.5mg/L	Petioles	44.00 ± 9.91
	Leaves	NR
	Stems	75.83 ± 7.12
	Roots	NR
0.5mg/L + 2.0mg/L	Petioles	48.93 ± 5.02
	Leaves	21.67 ± 3.07
	Stems	51.79 ± 5.66
	Roots	NR
1.0mg/L + 0.5mg/L	Petioles	63.52 ± 3.16
	Leaves	16.29 ± 2.15
	Stems	50.00 ± 6.02
	Roots	67.05 ± 1.02
1.0mg/L + 1.0mg/L	Petioles	66.00 ± 2.45
	Leaves	36.52 ± 4.00
	Stems	85.00 ± 1.46
	Roots	84.62 ± 1.14
1.0mg/L + 1.5mg/L	Petioles	82.35 ± 3.21
	Leaves	5.19 ± 0.92
	Stems	93.18 ± 0.77
	Roots	91.40 ± 0.46

Observation and data was recorded along the culture and subculture process. Data analyzed to its mean of callus percentages. After 24 days of culture, the aseptic seedlings of *Brassica oleraceae* L. var *capitata* was produced. According to [7], the age of the explant can be very important, as physiologically younger tissue is generally much more responsive *in vitro*. The explants were taken from the young age to avoid non-responsive that might give the problem soon. The subculture also were did in control condition but with the supplemented of various hormone concentration of NAA and BAP. Referred to [8], the application combination of NAA and BAP has been used for shoot regeneration in many species.

In this study, the suitable concentration combination and explant that gave the best response to the combination of NAA and BAP was observed. The percentage rate of callus formation were recorded. Callus is formed from a wounded plant that produces a proliferation of undifferentiated cells at the wound site [9].

By observing the data of callus percentage that had been recorded in the table 1, the most responsive explant was stem. The mean result for stem was 93.18 ± 0.77 in which it responded in MS medium fortified with 1.0mg/L + 1.5mg/L. While the second responsive explant was shown by root. The

mean of callus percentage was 91.40 ± 0.46 in MS medium fortified with 1.0mg/L + 1.5mg/L. Analysis of the callus percentage showed leaf explant is the least responsive followed by petiole explant. The lowest value recorded was by leaf explant (5.19 ± 0.92). Although the callus percentage were low, the explant show the responsiveness to the plant growth regulators of NAA and BAP. For instance, the leaf explant become swelled after react with the hormone and sometimes produced the root as the responsiveness from the auxine hormone. The differentiation of the tissues *in vitro* is controlled by various growth regulators such as auxins, cytokinins, gibberelins, ethylene, abscisic acid and etc. [10].

As the conclusion, the higher the concentration of NAA and BAP, the higher the responsiveness rate of explants and the ability of plantlets formed. The plantlet that produced *in vitro* method is safe and free from diseases and pests. A well-adapted regeneration protocol for cabbage and cauliflower should be developed using the locally cultivated varieties [11]. According to this, the suitable and efficient protocol in this study will be used to make sure this species can give the benefit for the society in the next generation.

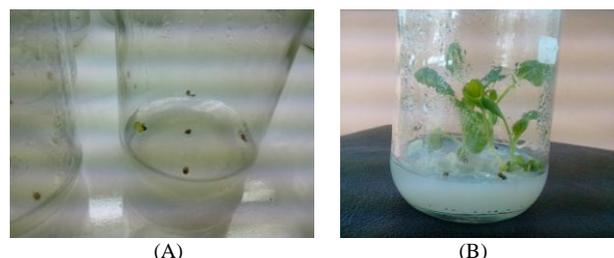


Fig. 1 (A) *In Vitro* Seedling, (B) 42 Days Old Of *Brassica Oleraceae* L. Var *Capitata*

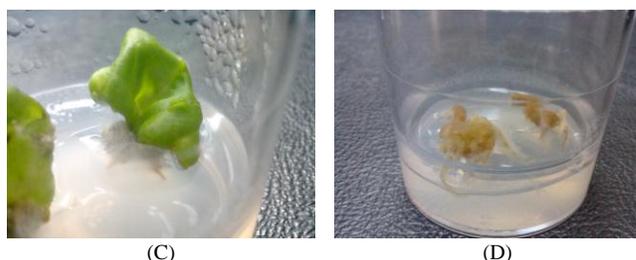
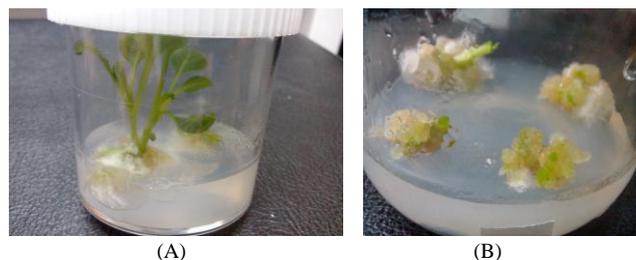


Fig. 2 (A) Shoots regenerated from stem, (B) Callus from petioles, (C) Callus from leaves, (D) Callus from roots

IV. CONCLUSION

The effectiveness *in vitro* plant regeneration and acclimatization protocol is very useful in breeding process of *Brassica oleraceae* L. var. Capitata in a short period of time and in genetic improvement by using biotechnological technique.

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