ISSN 2348-1218 (print)
International Journal of Interdisciplinary Research and Innovations ISSN 2348-1226 (online)
Vol. 11, Issue 4, pp: (60-64), Month: October 2023 - December 2023, Available at: www.researchpublish.com

Effect of Explants and Growth Regulators on Callus Induction and Shoot Regeneration of *Ocimum basilicum* L. (Sweet Basil)

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DOI: https://doi.org/10.5281/zenodo.10394290

Published Date: 16-December-2023

Abstract: **The purpose of this study was to examine the shoot regeneration and propagation ability of *Ocimum basilicum* L. through plant tissue culture technique. Callus formation was also observed in this study. Shoots, stems and roots of 8-week-old aseptic seedlings were used in this study. All cultures were incubated at a temperature of 25 ± 1ºC and illuminated with fluorescent light for 16 hours of light and 8 hours dark. Shoot and stem explants were found to be very responsive to produce maximum callus weight when cultured on Murashige and Skoog (MS) medium added with 2.0 mg/L Benzylaminopurine (BAP) and 1.0 mg/L Naphthalene Acetic Acid (NAA) with 2.20 ± 0.24 g and 2.01 ± 014 g respectively. Meanwhile, shoot regeneration was observed to be optimum when stem explant was cultured on MS medium supplemented with 2.0 mg/L and 0.5 mg/L. Tissue culture technique is an effective method in propagating *Ocimum basilicum* L. and its features are preserved. Due to the demand of this plant species by the consumers, this study suggests that *in vitro* propagation could be a suitable technique to promote mass supply of *Ocimum basilicum* L. in future.**

Keywords: ***Ocimum basilicum*** **L., tissue culture, explants, callus, regeneration, propagation.**

I. INTRODUCTION

The wild stock of some important plant species has been reduced slowly due to over exploitation and the lack of efforts for its replenishment. In Malaysia, there are more than 2000 plant species that have healing qualities and highly potential to be commercialized [1]. Malaysian Agricultural Research and Development Institute (MARDI) recorded gross profits of more than RM5.4 billion a year from herbal-related products. Therefore, it is important to preserve the value of the medicinal plants as it brings economic importance and vast valuable indigenous knowledge such as preparation methods, plant part uses and other important traditional knowledge [2].

Continuous plant propagation activities will help to ensure the supply of these valuable plants. There are various plant propagation techniques. One of the prominent plant propagation techniques today is through plant cell, tissue and organ culture. Tissue culture is a way to produce complete plantlets from a small plant part called explants such as shoots, stems or cells, cultured in sterile or aseptic media for growth. Tissue culture is one of the ways that have been used by horticulturist and scientists to increase their crops and produce high quality crops [3]. It involves biological approach in which the explants of animal or plant tissues are transferred to an artificial environment where they can adapt and continue to survive on its own. Tissue culture is also recognized as a technique to maintain a cell or a bit of animal tissue and plants *in vitro*. Meanwhile, *in vitro* refers to something that is executed outside the body of the organism, usually in a beaker or a test tube.

There are few factors that contribute to the success of the tissue culture process. One of the factors is having a complete material and apparatus. Another important factor is to have good and suitable laboratory to carry out this experiment [4]. To date, tissue culture technique has been an alternative for plant propagation especially plants with demand and commercial values.

One of the plant family that is usually being propagated using plant tissue culture technique is the Lamiaceae family. *Ocimum basilicum* also known as sweet basil is one of the important edibles, aromatic plants from the Lamiaceae family [5]. *Ocimum basilicum* is an annual herb and usually planted in the tropical region [6] and possesses many neutraceuticals properties [7]. The plant is usually propagated using seed. However, germination by seeds shows a high degree of variability because of cross-pollinated nature of the plant. Other than that, the seeds of *Ocimum basilicum* take time to germinate by using traditional method [8]. Thus, it is not an attractive approach for producing a large number of elite plants within a short period of time. Besides, the percentage of seed germination through conventional method is lower compared to tissue culture technique. This is due to the seed is surrounded by jelly, making the lifetime of the seed to survive is short during the germination period. This problem gives effect to the wild stock of this important plant species that has been reduced slowly due to over exploitation and no efforts for its replenishment has been undertaken. Therefore, plant tissue culture technique is a good alternative to overcome these issues. Mass propagation of *Ocimum basilicum* could be achieved and therefore the supply and need of *Ocimum basilicum* could be sustained. In the present study, *in vitro* response from explants of *Ocimum basilicum* L. through tissue culture techniques was investigated and achieved.

II. METHODOLOGY

Ocimum basilicum L. seeds were washed thoroughly under running tap water for two hours followed by treatment with three drops of Tween 20 for 30 min and then washed three to five times with distilled water. Subsequently, seeds were rinsed with 70% ethanol for a minute, then surface sterilized with 10% concentration of sodium hypochlorite solution for 10 min and rinsed thoroughly with distilled water. Then, the seeds were left soaked in sterile distilled water overnight and placed in the laminar flow. Next, the seeds were rinsed three times with sterile distilled water and let dried on sterile filter paper in the laminar flow. The sterilized seeds were then cultured onto the basal medium supplemented with 3.0 % (w/v) sucrose and solidified with 0.8% (w/v) technical agar. The aseptic seedlings of *Ocimum basilicum* L. that were germinated were used as the sources of the explants. Stems, roots and shoots from 4-week-old plantlet were used as explants. The explants were excised into 5x5 mm and cultured onto MS basal medium that was supplemented with various combinations and concentrations of Benzylaminopurine (BA) and Napthalene Acetic Acid (NAA). Explants were placed horizontally on the surface of the medium in culture jar. Several combinations of growth regulators were used in this study. MS Medium with different hormone combinations were prepared. All cultures were maintained in the culture room at 25°C ± 1°C and illuminated with fluorescent lights of 16 hours of light and 8 hours in dark photoperiod. Observations were done every week and data were collected after week 8 of culture. The formation of roots and callus on explants were also observed. From the results obtained, the most responsive explant and the most optimum medium for regeneration of shoots and formation of roots were identified. Analysis of Variance (ANNOVA) using Duncan's Multiple Range Test (DMRT) was used in this study. Mean with different letters in the same column differ significantly at $p < 0.05$.

III. RESULTS AND DISCUSSION

The main purpose of this study was to determine the type of explants and hormone concentrations that were optimum callus induction and shoots regeneration. Thus, evaluation was made based on the explant and hormone concentration that generated the highest percentage of shoot regeneration and number of shoots per explant in the shortest period of time. Observation was made on the percentage of shoot regeneration and callus formation, number of regenerated shoots per explants and percentage of rooting.

Based on the result, most explant responded 3 weeks after being cultured on MS medium supplemented with various growth regulator concentrations (Table 1). For each explants used, the callus formation was observed when root explants (1.16 ± 0.08 g) cultured on MS medium supplemented with 1.0 mg/l BAP and 0.5 mg/l NAA (Fig. 1). Meanwhile, for shoot explants (2.20 ± 0.2 g) when cultured on MS medium supplemented with 2.0 mg/l BAP and 1.0 mg/l NAA (Fig. 2). Whereas, for stem explants (2.01 ± 0.14 g) when cultured on MS medium supplemented with 2.0 mg/l BAP and 1.0 mg/l NAA (Fig. 3).

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TABLE 1: *In vitro* micropropagation of shoots from leaf and stem explants of *Punica granatum* L. cultured on MS medium supplemented with various concentration of BAP and NAA

MS Medium + Growth Regulators	Callus (g) (Min±SE)			Shoots Per Explants (Min±SE)
	Shoot	Stem	Root	
MS Basal	0.00 ± 0.00g	0.00 ± 0.00f	0.00 ± 0.00e	0.00 ± 0.00f
MS + 0.5 mg/l BAP + 2.0 mg/l NAA	0.57 ± 0.08f	0.70 ± 0.06d	0.79 ± 0.10c	3.00 ± 0.04d
MS + 1.0 mg/l BAP + 2.0 mg/l NAA	0.78 ± 0.06e	0.62 ± 0.06de	0.72 ± 0.07c	3.00 ± 0.07d
MS + 1.5 mg/l BAP + 2.0 mg/l NAA	0.60 ± 0.07f	0.85 ± 0.07d	0.70 ± 0.06c	4.00 ± 0.15b
MS + 2.0 mg/l BAP + 2.0 mg/l NAA	0.96 ± 0.09e	0.87 ± 0.10d	0.68 ± 0.08c	4.00 ± 0.12b
MS + 0.5 mg/l BAP + 1.0 mg/l NAA	0.56 ± 0.04f	0.70 ± 0.05d	0.39 ± 0.04d	2.90 ± 0.23d
MS + 1.0 mg/l BAP + 1.0 mg/l NAA	0.59 ± 0.03f	0.91 ± 0.09d	0.79 ± 0.10c	3.77 ± 0.14c
MS + 1.5 mg/l BAP + 1.0 mg/l NAA	1.43 ± 0.00c	1.85 ± 0.08b	0.93 ± 0.08b	3.87 ± 0.10c
MS + 2.0 mg/l BAP + 1.0 mg/l NAA	2.20 ± 0.24a	2.01 ± 0.14a	1.13 ± 0.13a	4.00 ± 0.08b
MS + 0.5 mg/l BAP + 0.5 mg/l NAA	1.51 ± 0.16b	0.86 ± 0.07d	0.81 ± 0.06bc	1.57 ± 0.04e
MS + 1.0 mg/l BAP + 0.5 mg/l NAA	1.11 ± 0.10d	1.17 ± 0.05bc	1.16 ± 0.08a	3.97 ± 0.03c
MS + 1.5 mg/l BAP + 0.5 mg/l NAA	1.19 ± 0.10d	0.91 ± 0.07d	0.99 ± 0.07b	4.00 ± 0.18b
MS + 2.0 mg/l BAP + 0.5 mg/l NAA	0.93 ± 0.11e	1.23 ± 0.08bc	0.95 ± 0.11b	4.23 ± 0.06a

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

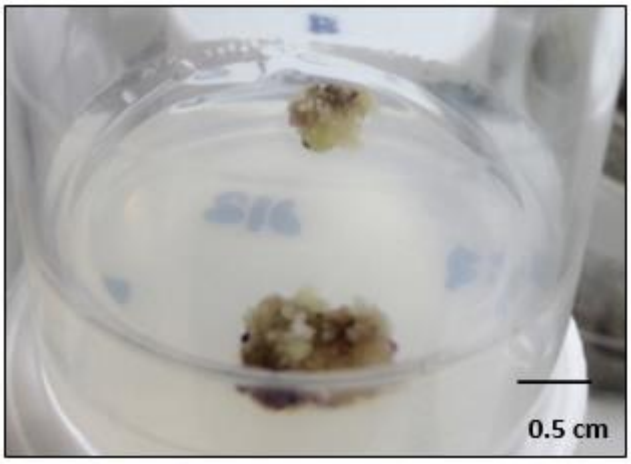

Fig 1: Callus formation of *Ocimum basillicum* L. from root explants cultured on MS medium supplemented with 1.0 mg/L BAP and 0.5 g/L NAA.

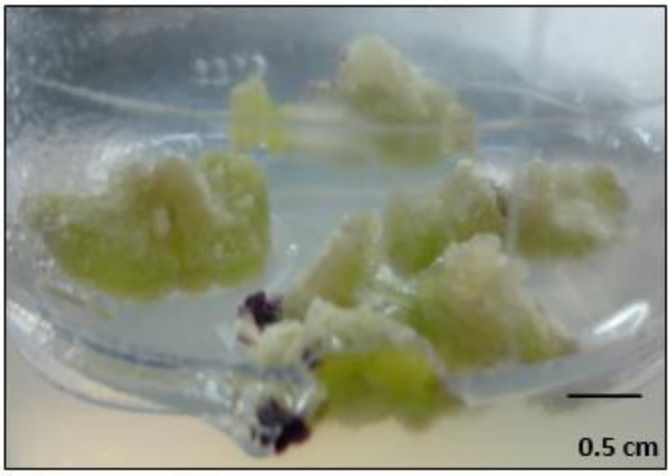

Fig. 2: Callus formation of *Ocimum basilicum* L. from shoot explants cultured on MS medium supplemented with 2.0 mg/L BAP and 1.0 mg/L NAA.

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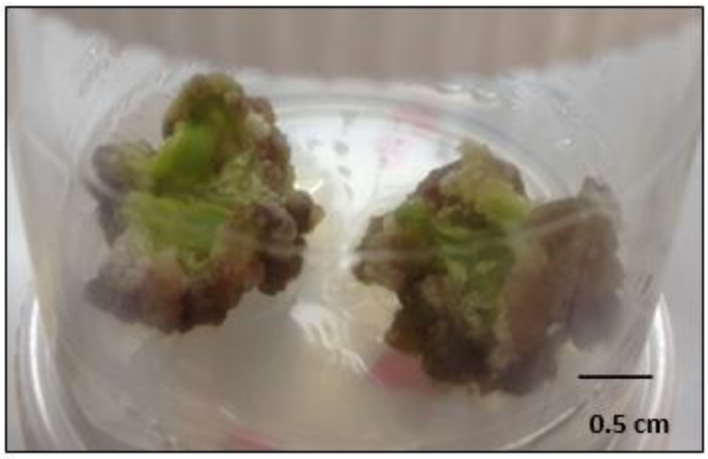

Fig. 3: Callus formation of *Ocimum basilicum* L. from stem explants cultured on MS Medium supplemented with 2.0 mg/L BAP and 1.0 mg/L NAA.

In the meantime, stem explants also produced regeneration of shoots when cultured on MS medium supplemented with different concentration of BAP and NAA. However, there was no response in formation of shoot when cultured from root and shoot explants. Indirect shoot regeneration was optimum (Fig. 4) when stem explant was cultured on MS medium added with 2.0 mg/l BAP with 0.5 mg/l NAA with 4.23 ± 0.5 shoots per explant.

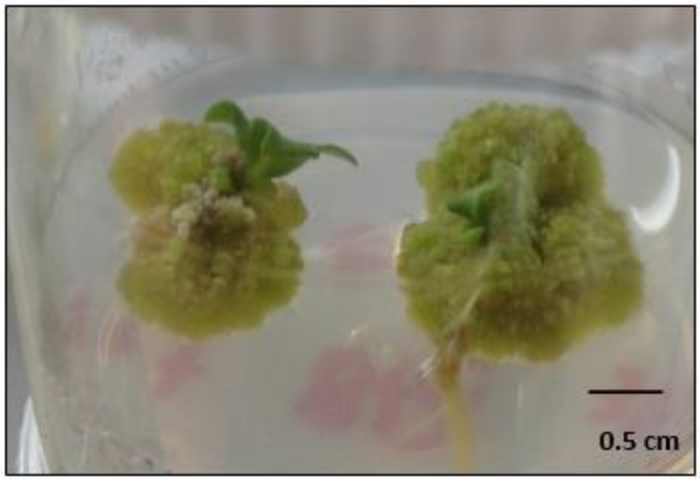

Fig. 4: Callus and shoot formation of *Ocimum basilicum* L. from stem explants cultured on MS Medium supplemented with 2.0 mg/L BAP and 0.5 mg/L NAA

Tissue culture of *Ocimum basilicum* L. showed that *in vitro* technique could be applied to these herbaceous plants, which is non-woody flowering plant as well. *In vitro* techniques are considered as an easy and reliable methods for rapid propagation of plants, especially medicinally important plants [9]. In this study, the initiation of culture was carried out using aseptic seedlings. One of the advantages of using aseptic seedlings is, they consist of young or juvenile tissues which are actively dividing (meristematic cells) and they are usually more responsive in culture.

Murashige and Skoog medium were used in this study due to the effectiveness for the response of the explants during culture [10]. The highest efficiency of shoot formation using cotyledonary explant, obtained in MS medium containing 5.0 mg/L BAP and 0.2 mg/L NAA [11]. In a different study, among the different concentrations and combinations of growth regulators, the highest percentage of shoot formation (90%) and the highest average number of shoots (5.88%) were observed in 0.2 mg/L BAP from shoot tip explants [12]. This study was aimed at identifying the best type of explants obtained from *in vitro* grown seedlings of sweet basil as well as the most efficient growth regulator concentration and combinations for shoot formation and regeneration. The highest efficiency of shoot formation using cotyledonary explant was obtained in MS medium containing 5.0 mg/l BAP and 0.2 mg/l NAA [11]. In a different study, among the different concentrations and combinations of growth regulators, the highest percentage of shoot formation (90%) and the highest average number of shoots (5.88%) were observed in 0.2mg/L BAP from shoot tip explants [12].

IV. CONCLUSION

In this study, in vitro response of the explants of *Ocimum basilicum* L. was observed. Callus induction and shoot regeneration were obtained when explants were cultured on MS medium supplemented with various concentrations of BAP and NAA. Plant tissue culture technique is proven to be one of the best alternatives for the propagation of plants. It is suggested, the studies of secondary metabolites contents in callus, direct shoot and root regeneration of *Ocimum basilicum* L. could be further investigated in the future.

ACKNOWLEDGEMENT

Authors would like to express their appreciation to Universiti Pendidikan Sultan Idris (UPSI), Institute of Postgraduate Studies (IPS) of UPSI and Department of Agricultural Science, Faculty of Technical and Vocational (FTV), Universiti Pendidikan Sultan Idris, Tanjong Malim, Perak Darul Ridzuan, Malaysia, for supporting and providing all research facilities.

REFERENCES

[1] MARDI (2015) Retrieved Februari, 2015 from http://www.mardi.gov.my/web/guest/skop-penyelidikan15.

[2] Sahri Z, Nordin, S and Harun, H (2012) Malaysia Indigenous Herbs Knowledge Representation. Knowledge Management International Conference 2012, Johor Bahru, Malaysia. Article in a conference proceedings.

[3] El-Fadl A, Reda E., Ahmad ME and Alhady MRAA (2022) *In Vitro* Propagation of Avocado (*Persea Americana* Mill). Egyptian J. Desert Res. 1: 78-93.

[4] Yancheva SD and Kondakova V (2016) Plant Tissue Culture Technology : Present and Future Development.

[5] Mosadegh H, Trivellini A, Maggini R, Ferrante A, Incrocci, L et al (2021). *In vivo, in vitro* screening of *Ocimum basilicum* L. ecotypes with differential UV-B radiation sensitivity. Horticulturae. 7(5):

[6] Zulfiqar F, Chen J, Finnegan PM, Younis A et al (2021). Application of trehalose and salicylic acid mitigates drought stress in sweet basil and improves plant growth. Plants. 10(6): 1078.

[7] Corrado G, Chiaiese P, Lucini L, Miras Moreno B et al (2020). Successive harvests affect yield, quality and metabolic profile of sweet basil (*Ocimum basilicum* L.). Agronomy. 10(6): 830.

[8] Saha S, Monroe A and Day MR (2016) Growth, yield, plant quality and nutrition of basil (*Ocimum basilicum* L.) under soilless agricultural systems. Annals of Agricultural Science. 61: 181-186.

[9] Thomas, D.T and Philip B. (2005) Thidiazuron-induced high frequency shoot organogenesis from leaf-derived callus of a medicinal climber, *Tylophora indica* (Burm.F) Merrill. In Vitro Cell. Dev.Biol- Plant. 41:124-128.

[10] Murashige T and Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant. 15:473–497

[11] Bicca Dode L, Bobrowski VL, Braga, EJB, Seixas, FK et al (2003) *In vitro* propagation of *Ocimum basilicum* L. (Lamiaceae). Acta Sci. 2:435- 437.

[12] Banu LA and Bari MMA (2007) Protocol establishment for multiplication and regeneration of *Ocimum sanctum* Linn. An important medicinal plant with high religious value in Bangladesh. Journal of Plant Sciences. 2(5): 530-537.