IN VITRO MORPHOGENIC RESPONSE FROM DIFFERENT EXPLANTS OF SWEET BASILS, *Ocimum basillicum* L.

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ABSTRACT

The purpose of this study was to examine the ability of seed germination *Ocimum basilicum* L. to germinate through tissue culture, the rate of time difference in seed germination in traditional or modern technology. In addition, callus formation was also carried out in the study to see the rate of cell differentiation at the exploratory part. At the end of the study, it was also tested by acclimatization of the explant to green house. The complete regeneration of the *Ocimum basilicum* L. plant and callus production was successfully produced through the tissue culture technique. The part of leaves, stems and roots of 8-week aseptic seedlings were used in this experiment. All cultures are stored at a temperature of 25 ± 1ºC and illuminated with fluorescent light for 16 hours of light, 8 hours dark. Plant acclimatization is done on three different soil types, namely garden soil, coconut powder and vermiculation. After completion, all data was recorded and analyzed using ANNOVA. Using MS medium (Murashige and Skoog, 1962) root explants were found to be very responsive for 0.5 mg / l BAP and 1.0 mg / l NAA concentration to produce maximum callus weight, and The stem explant for a concentration of 0.5 mg / l BAP with 1.5 mg / l of NAA will produce double shoots. Growth of seedlings are most optimum when regenerated from stem explants are acclimated in a garden with the survival rate of 88 ± 0.8%. As the conclusion, this method of tissue culture is the best method in producing new generation of *Ocimum basilicum* L. and its features are preserved. The implications of this study suggest that this tissue culture method can help to regenerate this species from extinction and the benefits of its use can continue to apply.
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TINDAK BALAS MORFOGENIK *IN VITRO* DARIPADA PELBAGAI EKSPLAN POKOK SELASIH, *Ocimum basilicum* L

ABSTRAK

Tujuan kajian ini dijalankan adalah untuk menguji kemampuan biji benih *Ocimum basilicum* L. bercambah melalui tisu kultur, kadar masa perbezaan percambahan biji benih secara tradisional atau teknologi moden. Selain itu juga pembentukan kalus juga dilaksanakan dalam kajian untuk melihat kadar pembentukan sel pada bahagian eksplan yang dikaji. Pada akhir kajian ini juga diuji dengan pengujian aklimitasi eksplan terhadap persekitaran sebenar. Regenerasi lengkap bagi tumbuhan *Ocimum basilicum* L. dan penghasilan kalus telah berjaya dihasilkan melalui teknik kultur tisu Eksplan yang digunakan dalam kajian ini ialah bahagian daun, batang dan akar anak benih aseptik yang berumur 8 minggu. Semua kultur disimpan pada suhu 25 ± 1ºC dan kala cahaya 16 jam cahaya, 8 jam gelap. Aklimitasi tumbuhan dilakukan pada tiga jenis tanah yang berbeza iaitu tanah kebun, serbuk kelapa dan vermikulasi. Setelah selesai, semua data direkodkan dan dianalisis menggunakan ANNOVA.. Dengan menggunakan medium MS (Murashige and Skoog, 1962) eksplan akar didapati sangat responsif bagi kepekatan 0.5 mg/l BAP dan 1.0 mg/l NAA bagi menghasilkan berat kalus yang maksima, dan eksplan batang bagi kepekatan 0.5 mg/l BAP bersama 1.5 mg/l NAA akan menghasilkan pucuk berganda. Pertumbuhan dan perkembangan anak pokok adalah paling optimum apabila anak pokok yang diregenerasikan daripada eksplan batang diaklimatisasikan di dalam tanah kebun dengan kadar keterushidupan sebanyak 88 ± 0.8%. Kesimpulannya, kaedah tisu kultur ini merupakan kaedah yang terbaik dalam penghasilan generasi baru *Ocimum basilicum* L. dan ciri-cirinya adalah terjaga. Implikasi daripada kajian ini menunjukkan bahawa kaedah tisu kultur ini boleh membantu untuk memperbanyakkan spesis ini daripada pupus dan faedah penggunannya boleh terus diguna pakai
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LIST OF ABBREVIATION

2,4,5-T	2,4,5-Tricholoro-phenoxyacetic acid
2,4-D	2,4-Dichlorophenoxy acetic acid
2iP	6-dimethylamino purine
4-CPA	4-chlorophenoxy acetic acid
ANOVA	Analysis of Variance
B	Boron
BAP	Benzylaminopurine
C	Carbon
Ca	Calcium
$CaCl_2.2H_20$	Calcium chloride dehydrate
Cl	Chlorine
cm	Centimeter
Co	Cobalt
Cu	Copper
dicamba	Dicholoro-2-methoxy-benzoic acid
DMRT	Duncan Multiple Range Test
DNA	Deoxyribonuclic acid
EDTA	Ethylene Diaminetethraacetic Acid
Fe	Ferum

FeEDTA	Iron chelate
H	Hydrogen
I	Iodine
IAA	Indole-3-acetic acid
IBA	Indole-3-butric acid
K	Potassium
Kinetin	N-2-furanilmethyl-1H-purine-6-amine
kPa	kilopascal
Mg	Magnesium
ml	Mililitre
mm	Milimetre
mM	Milimolar
Mn	Mangan
Mo	Molybdenum
MS	Murashige and Skoog
N	Nitrogen
Na	Sodium
NAA	Naphthelena acetic acid
$NaC_6H_7O_6$	Sodium Alginate Solution
NaOH	Sodium hydroxide
O	Oxygen
P	Phosphorus
pCPA	p-chlorophenoxy acetic acid

pH	Percentage hydrogen
picloram	4-amino-3,5,6-tricholoro-picolinic acid
S	Sulphur
SE	Standard Error
SEM	Scanning Electron Microscopy
SSOc	Synthetic seed *Ocimum basilicum*
TDZ	Thiazuron-N-phenyl-N-1,2,3 thiadiazol-5ylurea
Zeatin	6-4-hydroxy-3-methyl-trans-2-butenylaminoporine
Zn	Zinc

CHAPTER 1

INTRODUCTION

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1.1 Introduction

Humans need oxygen to breathe. Oxygen comes from carbon dioxide during photosynthesis process by plants. At the same time, this process also helps plants to contribute to the stability of earth. Plants give benefits for humans to survive. For example, plants supply oxygen for breathing, food crops, shelter for human, insects, microorganisms and so on. There are about 250,000-380,000 currently-known plant species, or organism under the kingdom of *Plantae* that lives on Earth (Encyclopedia of life, 2009). At least 6500 medicinal plant species were reported in Asia, one of the largest biodiversity regions of the world. In Malaysia, more than 1200 species have been identified as medicinal plants (Chang *et al*., 2000).

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Angiosperm is one of the characteristics of the plant. Sometimes, it is also known as Anthophyta. This word derives from two different words, which is ‘anthos’, which means flower and ‘phyta’, which means plants. The term ‘angiosperm’ is derived from two Greek words; ‘angio’, which means ‘vessels’ and ‘sperm’, which means ‘seed’. Normally, humans differentiate the type of plants between angiosperm and gymnosperm by observing the presence of flower on the plant. If the flower exists, it is identified as angiosperm plant. But if it is absent, then the plant is identified as gymnosperm.

Ocimum basilicum L. is one of the plants that are grouped under angiosperm. Angiosperm is divided into two groups that are monocotyledon and dicotyledon. *Ocimum basilicum* L. is categorized under dicotyledon group. It is because; *Ocimum basilicum* L. has a seed that contains two nutrient storage areas. Based on the leaves, the characteristic of dicotyledon leaves is different from the monocotyledon leaves. Dicotyledon leaflets have net or branching veins, while monocotyledon has parallel veins. Every plant should have roots to hold the ground and as a base of the plant to maintain the growth. Both of them have different types of root. The root of dicotyledon plants is tap root, and monocotyledon plants have fibrous root. Every type of the root has a different function.

Herbs can be defined from their own characteristics. In the botanical field, the term "herb" is defined differently. Any non-woody flowering plant, flavor, scent or other properties, including grass plants and forbs are considered as ‘herbs’. In Malaysia, ‘traditional vegetables’ are defined as indigenous plants either in the daily diet or for medicinal purposes (Saidin, 2000). Usually, people call it as “ulam” and normally it is

used to tell apart between traditional medicine and modern medicine. They can easily be found anywhere especially in villages. For example, they are *Cosmos caundatus* (Ulam Raja), *Morinda citrifolia* (Mengkudu), *Centella asiatica* (Pegaga), *Psophocarpos tetragonolobus* (Kacang Kelisa) and so on.

Herbal medicines are used as remedies for illness such as fever, diarrhea, sore throats, sinus problems, respiratory problems and skin condition (Sujaidi, 2009). Herbs also have been recognized as alternative medicines and economical resources. Nearly 80% of the world populations rely on the use of traditional medicines to meet their health care needs (Sandhya *et al.,* 2006) and up to 90% of developing countries rely on the use of medicinal plants (WHO, 2002). *Ocimum basilicum* L. is also an example of ulam. Normally, people called it as "Selasih". Typically, most people use *Selasih* leaves as their side dish or daily diet. At the same time, people use *Ocimum basilicum* L. seed as an extra flavor in beverages.

Ocimum basilicum L. is one example of herbaceous plant. Sometimes, it is called as shrub. This plant grows similarly to *Ortosiphon aristatus*; it has a main stem and is divided into several branches. Its height is around 30cm to 60cm, but it can reach up to 1 meter. Its stem looks like a square, has a downy surface around the stem and is yellowish-green in color.

The shape of the *Ocimum basilicum* L. leaf is oval at the center and cuneate at the base part. The measurement is 2.5cm to 5cm in length and 1cm to 2.5cm in width approximately. The end of the leaf is tip. The margin around the leaf is serrulate, and the arrangement of the leaves is opposite to each other.

Ocimum basilicum L. comes from Magnoliophyta division. Thus, *Ocimum basilicum* L. is an angiosperm plant, which is the plant that produce flower. *Ocimum basilicum* L. flower has nice petals. The petals are white and purple in color. The white color is at the outer region and purple color is at the middle region. The arrangement of the petals is in the form of rosemoss, where three roses of flower are combined around the stem of the flower. The length of the flower line is around 15cm.

Ocimum basilicum L. is a dicotyledon plant. Therefore, it must have seeds. The seed's shape is like a nut and black in colour. The size is small like mustard seeds. *Ocimum basilicum* has the same physiology to other plants. *Ocimum basilicum* L. carries out photosynthesis, respiration, transpiration and absorption of water and nutrient process.

Photosynthesis is a process of making food for its own life. Besides, this process also is very important for humans to continue their life. The photosynthesis process occurs in the chlorophyll that only present in the plant cell. This process shows how products are made, which is through the absorption of carbon dioxide and water from its surrounding with the presence of light and chlorophyll, then all of these will convert to form simple sugar, oxygen and water. The simple sugar is in the form of glucose and contains the building blocks of other nutrients. The photosynthesis process rate depends on the light intensity, temperature and concentration of carbon dioxide.

Living things have their own life cycle. This includes plants. Plants have their own life cycle. A complete life cycle includes the organism's growth and development. There are two types of plant reproduction, which are propagation and

micropropagation. The propagation process is divided into two types, the sexual and asexual process.

Sexual reproduction process is a normal reproduction process of mating between two gametes that are male and female gamete and fertilization process occur in the flower. Finally, the result of the fertilization process will form a new offspring. The offspring will be genetically different from their parents. Generally, this sexual reproduction process includes flowering and non-flowering plant. Both of them must have male and female gamete. Asexual reproduction process is mostly done manually by humans. Sometimes, it is also called as assisted reproduction. Mostly, there are several types of asexual reproduction process, also known as cutting process. The cutting process has several types, which are stem cutting, stem tip cutting, cane cutting, leaf cutting, leaf petiole cutting and root cutting. Vegetative parts of plants are used for cutting. Layering has several types too. There are simple layering, tip layering and air layering. Layering also helps plants to generate new production. Other than that, another reproduction process is micropropagation. Micropropagation involves tissue culture process. Mostly, these processes are used by horticulturist to multiply the number of the plants or to produce a new plant that have same genetic value and high quality plants.

Tissue culture is a way to produce complete plantlets from a small plant part called explants such as leaves, 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. Tissue culture is one of the ways of biological research 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. 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.

Every experiment has its own advantages and disadvantages. Nevertheless, it is similar to tissue culture experiment. The advantages of doing this experiment are the breeding rate is high and the culture process is done in sterile environment, so that the plant will grow uniformly and free from any microorganism that may contribute to any disease. Other advantages of doing this experiment are the plant may reproduce uniformly and have same physical and genetic characteristics and properties as the mother plant; it also can help propagate plants that are hard to be reproduced by using sexual or other asexual production methods. However, the tissue culture process has disadvantages during and after handling. Experiments must be done in a controlled environment. High maintenance is needed to make sure experiments are carried out smoothly, so that the micropropagated plants are successfully produced and have high quality.

The wild stock of some important plant species has been reduced slowly due to over exploitation and no efforts for its replenishment has been undertaken. In Malaysia, there are more than 2000 plant species that have healing qualities and highly potential to be commercialized (MARDI, 2010). 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 traditional knowledge (Sahri *et al.,* 2012).

Ocimum basilicum L. can be germinated through traditional method that is by using the seeds. But, the conventional method is not an attractive approach for producing a large number of elite plants within short period of time. To fulfill the request of this potential medicinal plant, *in vitro* culture is one of the alternative methods to conserve the diminishing plant population. *In vitro* techniques are considered as an easy and reliable method for the rapid propagation of plants, especially medicinally important plants (Thomas and Philip, 2005; Thomas and Jacob, 2004). Large number of aseptic plants could be produced within a short time using plant tissue culture protocols.

Besides the production of plantlets through *in vitro* culture processes, artificial plant seeds could also be produced. Nowadays, artificial seed technology is one of the most important tools to germinate plants and scientists of plant tissue culture. It offers powerful advantages for a large scale mass propagation of this plant species. In general, synthetic seeds sometimes known as artificially covered somatic embryos, shoot tips, axillary buds or any parts of meristematic tissue that can be used to germinate a seed and have the ability to form a whole new plant under *in vitro* and *in vivo* also can acclimatize and remain its possible until after storage (Capuano *et al*., 1998). The somatic embryo can be encapsulated, handled and used like a natural seed, was first

suggested by Murashige (1977) and efforts to engineer them into synthetic seed have been ongoing ever since Kitto and Janick (1982), Gray (1987).

Bapat *et al.* (1987) proposed the encapsulation of shoot tip in *Morus indica*, has made the concept of synthetic seed release from its bonds to somatic embryos and broaden the technology to the encapsulation of various *in vitro* derived propagules. In addition, an implementation of artificial seed technology to somatic embryogenesis of embryos that comes from the somatic tissues is an efficient technique that allocate for seed germination in a large scale production of selected genotype (Ara *et al*., 2000).

The purpose of switching towards artificial seed technology was for the fact that the cost-effective mass propagation of plant genotypes will be promoted. It can also be a technique for a new transgenic plants established through biotechnological techniques to be transferred directly to the environment.

Plant tissue culture processes play a very important role in sustaining plant propagation and supplies. Therefore, it is very important for the people to be aware of such alternative propagation methods in order to ensure important plants and crops production could be continuously produced in future.

1.2 Problem Statement

Ocimum basilicum is an economically important species of the tropical and subtropical locales of the world because of its scrumptious natural products, and pharmaceutical and fancy use. It is to a great extent utilized as a treat.

The usual propagation method of *Ocimum basilicum* was by 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. Thus, it is not 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 than the culture tissue technique. It is because of the seed is surrounded by jelly, making the lifetime of the seed to survive is short in 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.

1.3 Research Objectives

Ocimum basilicum L. is the plant species that will be use in this research. *Ocimum basilicum* L. was chosen because of the percentage of this species to grow and germinate in large amount in a short period through traditional method is quite difficult.

It is in contrast with the culture tissue method; the growth percentage is better and mostly will survive very well. Therefore, this research is to study the morphogenesis potential of *Ocimum basilicum* L. through the tissue culture system.

The objectives for this research are:

1. To establish an efficient regeneration protocol of *Ocimum basilicum* L. through tissue culture techniques.

2. To investigate callus induction of *Ocimum basilicum* L. through tissue culture techniques.
3. To acclimatize *in vitro* plantlet of *Ocimum basilicum* L.

1.4 Scope and Limitations of Study

1.4.1 Scope of Study

This experiment is conducted to establish organogenesis of *Ocimum basilicum* L. through tissue culture technique because it has high percentage of seed germination than conventional method. Thus, the problem of hardening the germination of the seed can be solved, and at the same time, the limitation number of this species of herbs can be solved too. The aspects looked into are the quality of seed and breeders. Through conventional method, the chances of having variety of characteristic to the plants are high because of the cross-pollinated nature of the plant.

1.4.2 Limitation of Study

There are few problems that need to solve and try other method to handle this experiment. One of the limitation studies is obtaining of quality seeds. Besides, the laboratory equipment's also are incomplete, thus it takes time to prepare and handling the experiment.