Faculty of Resource Science and Technology

ESTABLISHMENT OF AXENIC CULTURE AND CALLUS INDUCTION OF *PHALERIA MACROCARPA* (SCHEFF.) BOERL, (MAHKOTA KOTA)

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Bachelor of Science with Honours
(Plant Resource Science and Management)
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Establishment of Axenic Culture and Callus Induction of *Phaleria macrocarpa* (Scheff.) Boerl, (Mahkota Dewa)

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This report is submitted in partial fulfilment of the requirement for degree of Bachelor of Science with Honours in Plant Resource Science and Management

Plant Resource Science and Management

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I declare that this thesis entitle “Establishment of Axenic Culture and Callus Induction of *Phaleria macrocarpa* (Scheff.) Boerl, (Mahkota Dewa)” is the best result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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LIST OF ABBREVIATIONS

2,4-D	2,4-Dichlorophenoxyacetic acid
BAP	6-Benzylaminopurine
KOH	Potassium hydroxide
HCl	Hydrochloric acid
Mg/l	miligram per litre
MS	Murashige and Skoog medium
PGR	Plant growth regulator

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Establishment of Axenic Culture and Callus Induction of *Phaleria macrocarpa* (Scheff.) Boerl, (Mahkota Dewa)

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ABSTRACT

Phaleria macrocarpa (Scheff.) Boerl or Mahkota Dewa is originally from the Papua New Guinea (Irian Jaya), Indonesia. All parts of the plants could be used for medical treatments but could not be consumed directly because it may cause numb and swollen. The objectives of this study were to determine the best method for axenic culture of young leaf of *P. macrocarpa* and to induce callus of *P. macrocarpa* from leaf explants by using different combination of 2,4-D and BAP. Result showed that the best method to produce axenic leaf explant was agitated the leaf explants in 15% of Clorox concentration for 10 minutes which the percentage of axenic explants was 86.67%. Every treatment treated with washing the leaf explants under running tap water and soaked in 70% of Ethanol for 30 seconds. Result showed that the best treatment to induce callus from leaf explants was culturing in media that contained 7.5 mg/ml 2,4-D + 1.0 mg/ml BAP which the percentage of explants produced callus was 86.67%. For recommendation, the leaf should be washed with detergent for pre-treatment while for inducing callus, use different type of PGR and types of explants.

Key words: *Phaleria macrocarpa*, axenic culture, surface sterilization, callus induction.

ABSTRAK

Phaleria macrocarpa (Scheff.) Boerl atau Mahkota Dewa berasal dari Papua New Guinea (Irian Jaya), Indonesia. Kesemua bahagian pada pokok ini boleh digunakan untuk kegunaan perubatan tetapi bahagian-bahagian ini tidak boleh di gunakan secara terus kerana boleh menyebabkan kebas dan bengkak. Objektif kajian ini adalah untuk mewujudkan kaedah yang terbaik untuk menghasilkan ekplans daun yang bersih dari penyakit dan kulat. Objektif kajian yang seterusnya adalah untuk menghasilkan prosedur yang terbaik untuk induksi kalus daripada ekplan daun dengan meggunakan pelbagai kombinasi daripada konsentrasi 2,4-D dan BAP. Keputusan menunjukkan, kaedah terbaik untuk menghasilkan ekplan daun yang bersih dari kontaminasi adalah dengan mengagitasi ekplans daun di dalam 15% kepekatan Clorox selama 10 minit dengan peratusan eksplans yang bersih adalah 86.67%. Setiap kaedah akan dimulakan dengan mencuci ekplan daun di bawah air paip yang berterusan selama 30 minit dan kemudian di rendam di dalam 70% Ethanol selama 30 saat. Keputusan kajian juga menunjukkan prosedur terbaik untuk menghasilkan induksi kalus daripada ekplan daun adalah kombinasi 7.5 mg/ml 2,4-D + 1.0 mg/ml BAP dengan peratusan eksplans yang berjaya mengeluarkan kalus adalah 86.67%. Bagi cadangan untuk kajian akan datang, mencuci ekplan daun menggunakan bahan pencuci untuk pra-rawatan dan untuk penghasilan kalus, pelbagai jenis PGR yang lain dan jenis ekplan boleh digunakan.

Kata Kunci: *Phaleria macrocarpa*, kultur bersih, permukaan pensterilan, induksi kalus.

1.0 INTRODUCTION

1.1 Research Background

Phaleria macrocarpa (Scheff.) Boerl, also known as God's Crown or Mahkota Dewa, is a medicinal plant belongs to the Thymelaeaceae family. It is a native plant from the island of Papua New Guinea (Irian Jaya), Indonesia. This plant grows to 5 - 18 m tall and can be found up to 1,200 m above sea level (Burkill, 1966). Almost all parts of this plant including fruits, seeds, stems and leave can be used in treatment. However, Mahkota Dewa cannot be consumed directly as it may cause swollen, numb and unconsciousness (Harmanto, 2003).

Recent studies also showed that *P. Macrocarpa* exhibited numerous different bioactivity. A study showed that this plant exhibited anti-oxidative, anti-inflammatory and cytotoxic activity (Hendra *et al.,* 2011). A wide variety of bioactive compounds can be found in *P. Macrocarpa* such as flavanoids, alkanoids, polyphenols and saponins (Tambunan and Simanjuntak, 2006). Moreover, fruits, leaves and stems of this plant which contain phenolic compounds show antioxidant activity (Irianti *et al.,* 2009).

Increasing of using traditional medicine is the cause to increase of using the medicinal plants. *P. Macrocarpa* is still not been planted very well and have a low quality. Therefore, *P. Macrocarpa* need to be produced in a large quantity continuously. Nowadays, *P. Macrocarpa* is propagated more by seeds and also cuttings (Mentary, 2006).

There are two ways to propagate this plant for such the conventional method which are by seeds and cuttings or through *in vitro,* by tissue culture. Propagating Mahkota Dewa by seeds are done within less or more 3 months. Therefore, seeds cannot be used as the medicinal parts as they are used to propagate the plant itself. The other problem is, propagate through seeds can cause the chemical properties in the plants change (Griffith *et al,* 1996). Then, propagating through cutting have its own disadvantages for such, the growth depends on the seasons, easy to be attacked by diseases and takes a very long time to grow (Mentary, 2006).

Propagating Mahkota Dewa by using *in vitro* can be a way to overcome the disadvantages of using the conventional methods (seeds and cuttings). Source of plant is very important for it will affect the growth and product of the plants. The using of *in vitro* with tissue culture method can help to overcome the problems that occur when using the conventional ways. These days, not many of *P. Macrocarpa* is propagated by using tissue culture. Tissue culture can produce more of good plant *P. Macrocarpa* from a small quantity of any part of the plant itself. It will produced a good quality of plantlet as long as the tissue culture method, the explant used and the combination of plant growth regulator (PGR) are good and suitable (Mentary, 2006).

Because of the knowledge of chemistry is being updated every single day, people get to know the molecule of plant's hormone that known as plant growth regulator (PGR). Those hormones can be useful in tissue culture from small explant or undifferentiated callus (Mentary, 2006). PGR plays a crucial role in growth of culture. The concentrations of PGR, ways to use them and period of induction are the main factors said Gunawan (1995).

Regarding to the concept made by George and Sherrington (1984), induction of callus need a high concentration of auxin while in the same time need a very low concentration of cytokinin. Applying *in vitro* technique to Mahkota Dewa will really useful because the growth is fast, free from disease and continuous (Mentary, 2006).

1.2 Problem Statement

Phaleria macrocarpa is a popular medicinal plant in Indonesia and has introduced in Malaysia because of its commercial potential as pharmaceutical uses (Ahmed Asrity, 2014). Nature is crucial as it provides basic needs like production of foods, shelters, clothing, fertilizers, flavours and fragrances, and most important is medicines for diseases (Gordaliza, 2007). Herbal medicine plays a key role in the development of pharmaceuticals and therefore there is high demand in the production of natural medicine the international market (Lay *et al.,* 2014). Mahkota Dewa is commonly used as herb based medicine as a cure for few disease such as cancer, diabetes, allergies, high blood pressure and stroke (Lay *et al*., 2014).

In order to produce a good herbal medicine, a good quality of motherplant need to be produced, therefore tissue culture is the promising way to produce whole plant of Mahkota dewa that is free disease (Mentary, 2006). However, result from previous study of Mentary (2006) is way far from convincing, hence more study should be tried to obtain a more reliable working protocol in callus induction of this species. Mentary (2006) even suggested that another experiment on induction of callus should be done on half strength media. Hence, in this experiment the media that will be used will be half-strength media.

1.3 Objective

The objectives of this study are as follow:

1. To determine the best method for axenic culture of young leaves of *P. Macrocarpa.*
2. To induce callus of *P. Macrocarpa* from leaf explants using different concentration of different combination PGR.

2.0 LITERATURE REVIEW

2.1 Botanical studies

Classification of these botanical studies are based on Depkes (1991) (as cited in Mentary, 2006) :

Division : Spermatophyte

Sub-division : Angiosperm

Class : Dicotyledon

Order : Thymelaeales

Family : Thymelaeaceae

Genus : *Phaleria*

Species : *Phaleria macrocarpa* (Scheff.) Boerl.

Phaleria macrocarpa (Scheff.) Boerl. (Mahkota Dewa) is a medicinal plant from the family of Thymelaeaceae. The common name of *P. macrocarpa* is Simalakama (Melayu). Makutadewa, Makuto Mewo, Makuto Rojo (Jawa). Another name for this mahkota dewa is *pau* (from chinese word) and *Crown of God* (English).

P. macrocarpa thrives in loose, fertile soil at an altitude of 10-1200 m above sea level.. This plant can grow up to 1-2.5 m but some also can reach to more than 2.5 m.The characteristic of this plant is it has a round trunk, the surface is rough, brown, woody and simpodial. It has single leaf, the arrangement is opposite and short stemmed. The leaf also has lanceolate or elliptip shape, the lamina is flat, the surface is smooth and dark green in colour.

This mahkota dewa flowers over the whole year and the flowers scattered on the stem. The colour of the flowers are white and have fragrant. The fruit is rounded in shape and has a diameter of 3-5 cm. It also has a smooth surface on the outside and when it is ripen the colour is red while it is green in colour when still young. The fruit has white flesh and it is watery. The seeds of this fruits are round to oval shape, they are hard and light brown in colour.

2.2 Propagation of *Phaleria macrocarpa*

2.2.1 Conventional method to propagate *P. macrocarpa*

In propagating *P. macrocarpa*, there are two conventional ways that can be practiced which is by generative phase (seeds) and vegetative phase (cuttings) stated by Cayani (2012). *P. macrocarpa* is not depend on season but there is modification needs to be taken when planting it. When this plant is propagated by seeds, the seeds must come from matured fruits. After 10-14 days of seeding, there will be growing of leaf (Tijar, 2011).

The seeding then will be moved to another media when its height reach 10-15 cm or already aged 2 months. This plants need lot of water for its growth. This mahkota dewa itself will flowering when it is already 10-12 months and start to fruit 2 months later. Same goes to the method that is using cuttings only the fruiting season in cuttings is much faster (Tijar, 2011). Both of methods, whether using seeds or cuttings,each of them has their own advantages and disadvantages (Mentary, 2006).

As known that mahkota dewa's seeds are been used for medicinal use,such as external medicine, it cannot to be used again to propagate a new plant because the seeds are needed for medicine. Propagation through seeds can cause changes in the chemical contents while propagation through cuttings, one of the disadvantages is, it can easily affected by diseases that are occurred in parent material (Mentary, 2006).

2.2.2 *In Vitro* technique

Besides the conventional methods, there is one other way to propagate this plant which is through *in vitro* by using tissue culture. Tissue culture technique is one of methods to isolate and take any part of the parts (explant) then grow them in aseptic condition (free from disease). The explants can be produced in a mass and becoming into a perfect plant (Nugroho & Sugito, 1996).

Tissue culture also defined as a method to isolate part of plant like protoplasm, cell, a group of cells, tissue and plant's organ, and grow them in an axenic condition with high nutrition, including PGR (Gunawan, 1995). In the first place, this tissue culture is done to prove the truth of totipotency of cell theory but then is used in experimenting the physiology of plant and the biochemistry (Mentary, 2006).

Tissue culture is one of the techniques to grow up the organ, tissue and growth cell (Gamborg, 1992). The biology principle of organ culture and tissue culture are first introduced by Haberlandt , one of the physiologists in 1902, by culturing the cell on the media, but the cell failed to grow. Then, in 1934, White from AS succeed to grow the root of tomatoes through *in vitro* by using adding extract of yeast and thiamine. In 1939, White, Goutheret, and Noubecort reported their result of the research that

succeed in growing callus in synthetic media (Hartman & Kester, 1983).

Tissue culture is using the theory that introduced by Schwann and Schleiden, that cell has the potency to grow by itself, even has the ability of totipotency. Totipotency is an ability for each cell, from any part of plant the cell is taken, whenever the cells are put inside a suitable environment, it will grow into a perfect plant (Nugroho & Sugito, 1996). Mantell *et al.* (1985) said that totipotency is defined as ability of cells to grow and develop when there is suitable outdoor environment. Nowadays, tissue culture is just not to produce mass production of new plant, but also to produce secondary metabolites, free disease plant and organelles for a particular trait (Mentary, 2006).

There are few methods in propagation through *in vitro* which are multiplication of shoots from axillary buds and adventitious or somatic embryo formation that include direct morphogenesis and indirect morphogenesis (Wattimena, 1992). Direct morphogenesis occur directly from the tissue of the explant while indirect morphogenesis occurs as the formation happens after going through the formation of callus (Mentary, 2006).

Utilization culture technique *in vitro* in agronomy is to help in vegetative propagation of plant in order to supply seeds from good parent material, clean the plant materials or seeds from any diseases or virus that occured in parent material, assist in the conservation and preservation of germplasm and production of chemical compounds for pharmaceutical, dyes for food industry and cosmetics (Gunawan, 1995).

Organogenesis of the explants formed shoots via callus or without callus depends on the PGR, type of explants, type of plants and media used. The type and the concentration of PGR also affect the PGR content in the tissue and then will formed the organogenesis from the explants. Regenarating plants can be done directly or through stages which are callus induction media and adventitious shoots induction media (Mentary, 2006).

2.3 Explant

Explant is defined as part of the plant to be used as an initiation of a culture (Gunawan, 1988). Explant can be regenerated through process of organogenesis. Choosing the best explant is very important by choosing the right part of the plant before it is being cultured (Gunawan, 1992).

Choosing the right explant can influenced the success of any plant tissue culture. Type of explant that will be used is crucial for it depends on the species, cultivar, and the objectives to be achieved (Mentary, 2006). In general, all species of plants can be propagated in tisue culture if the nutrient needs, plant hormone and ways of propagating known (Hartmann & Kester, 1983).

Source of explant affects the growth and ways of morphogenetic development. Choosing explant, the purpose of its culture needs to be considered. In order to get callus or organogenesis, it is better to use the leaf or together with the midrib is used (Gunawan, 1992). The season when the explants are taken , quality of the explant, media and explant aseptic condition, the size of explants do influence the success of regeneration (Murashige, 1974).