IN VITRO GROWTH RESPONSE OF *Brassica oleracea* var. *italica* THROUGH TISSUE CULTURE SYSTEM

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SULTAN IDRIS EDUCATION UNIVERSITY

2019

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05-4506832 pustaka.upsi.edu.my Perpustakaan Tuanku Bainun Kampus Sultan Abdul Jalil Shah PustakaTBainun ptbupsi

DISSERTATION SUBMITTED IN FULFILLMENT OF THE REQUIREMENT FOR MASTER OF SCIENCE (AGRICULTURE SCIENCE)

(MASTER BY RESEARCH)

FACULTY OF TECHNIQUE AND VOCATIONAL

SULTAN IDRIS EDUCATION UNIVERSITY

2019

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ACKNOWLEDGEMENT

In the name of Allah, the Most Gracious and Most Merfciful.

Alhamdulillah all praises to Allah for the strength and His blessing in completing this thesis. Special appreciation goes to my supervisor, Dr Nor Azlina Binti Hasbullah, for her supervision and constant support. Her invaluable help throughout the experimental and thesis work have contributed to the success of this research.

My deepest appreciation goes to my beloved parent, Azis Bin Abd Rahman and Norashimah Binti Husain for the endless love, pray, and support me for keep going and never giving up. There is no word in this world that can describe how much I love both of you.

My gratitude goes to my beloved brother and sister in law, Muhamad Asyraf and Ainur Yusra Nabila for every support in any form especially for letting me stay at your house. To my sister Nurul Auni, thank you for being there whenever I need you. To Haniey Syafiyah, you are forever my sweetheart.

To my beloved husband, Azmil Hakim who always been so understanding during my pursuit of study that made the completion of thesis possible. Thank you for your endless love and support.

I owe my thanks to my lab mates, Fatimah bt Mat Rasad and Fara' Ain bt Daud for the stimulating discussion, help and support, for the sleepless nights we were working together. Thank you for the memories and the friendship.

I gratefully thank to En, Azizi for allowing me to conduct my research in his lab. I could never have finished this without your great guidance. To those who indirectly contributed in this research, your kindness means a lot to me. Thank you very much.

ABSTRACT

Brassica oleracea var. *italica* is an important vegetable crops mostly grown in Cameron Highland, Malaysia. These vegetables are highly vulnerable to high temperature and grow best in cool climate. The purpose of this research was to examine *in vitro* growth response of *B. oleracea* var. *italica* through tissue culture system. Complete plant regeneration was successfully achieved when root, stem and leaf aseptic explant was cultured on MS medium supplemented with various combination of plant growth regulators. The experimental design used in this study was Completely Randomized Design (CRD). MS medium supplemented with 1.0 mg/L IBA + 0.5 mg/L BAP was found to be the optimum medium for shoot regeneration with 5.367±0.768 shoots per explant. Meanwhile 2.0 mg/L NAA was the optimum medium for leaf and root explant for root induction producing 7.567±0.498 and 8.767±0.707 roots per explant. Optimum callus induction was obtained when leaf explants were cultured on MS medium supplemented with 1.0 mg/L NAA ± 1.5 mg/L BAP. Friable cream callus were produced. Meanwhile synthetic seeds of *B. oleracea* var. *italica* were produced when micro shoots were encapsulated with 4.0% sodium alginate solution added with 1.5 mg/L BAP + 1.0 mg/L NAA. Synthetic seeds germination rate was 4.532±0.860 shoots per explant. Finally, acclimatization of *B. oleracea* var. *italica* was accomplished when plantlets were transferred to the combination of black and red soil (2:1) with 86.67% survival rate. In conclusion, this research showed that *in vitro* propagation of *B. oleracea* var. *italica* through tissue culture was successfully achieved. This research has provided positive implication in agriculture field which can be widely used to produce clones of a plant especially in Malaysia and also worldwide.

ABSTRAK

Brassica oleracea var. *italica* adalah sayuran penting yang kebanyakannya ditanam di Cameron Highland, Malaysia. Sayuran ini sangat mudah terjejas kepada suhu tinggi dan tumbuh dengan lebih baik dalam iklim sejuk. Tujuan kajian ini adalah untuk mengkaji tindakbalas tumbesaran *B. oleracea* var. *italica* melalui sistem tisu kultur. Pertumbuhan semula tumbuhan yang lengkap berjaya dicapai apabila eksplan akar, batang dan daun aseptik dikultur dalam medium MS yang ditambah dengan pelbagai kombinasi penggalak pertumbuhan tumbuhan. Rekabentuk kajian yang telah digunakan adalah rekabentuk rawak penuh (CRD). Medium MS yang telah ditambah dengan 1.0 mg/L IBA + 0.5 mg/L BAP didapati sebagai medium optimum kepada pertumbuhan pucuk yang menghasilkan 5.367±0.768 pucuk bagi setiap eksplan. Manakala 2.0 mg/L NAA merupakan medium optimum kepada eksplan daun dan akar untuk induksi akar yang menghasilkan 7.567±0.498 dan 8.767±0.707 akar bagi setiap eksplan. Induksi kalus yang optimum dicapai apabila eksplan daun dikultur dengan medium MS yang ditambah dengan 1.0mg/L NAA ± 1.5 mg/L BAP. Kalus krim yang rapuh telah dihasilkan. Sementara itu, biji benih tiruan *B. oleracea* var. *italica* telah dihasilkan apabila pucuk mikro dikapsulkan menggunakan larutan natrium alginat yang ditambah 1.5 mg/L BAP + 1.0 mg/L NAA. Kadar percambahan biji benih adalah 4.532±0.860 pucuk bagi setiap eksplan. Akhir sekali, aklimatisasi *B. oleracea* var. *italica* ke persekitaran luar telah berjaya dicapai apabila plantlet dipindahkan kepada campuran tanah hitam dan merah (2:1) dengan kadar keterusan hidupan sebanyak 86.67%. Kesimpulannya, kajian ini menunjukkan bahawa propagasi tanaman *B. oleracea* var. *italica* melalui sistem kultur tisu telah berjaya dicapai. Kajian ini memberikan implikasi positif dalam bidang pertanian di mana ia dapat digunakan untuk menghasilkan klon tumbuhan secara meluas khususnya di Malaysia dan juga di seluruh dunia.

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CHAPTER 1

INTRODUCTION

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1.1 Introduction

The agricultural sector involves an activity that gives various benefits to the country and the people, especially in terms of the development of the country's economy. According to Suhaimi (2017), agriculture is one of the country's revenue contributors since before independence. Agriculture is also known as food manufacturers and goods through farming, livestock breeding, and forestry.

The government currently focuses more on the agricultural sector. This is because agricultural activity is no longer a requirement for living, but also to give profits to entrepreneurs. It aims to transform Malaysia from being an industrial nation to an agricultural country. According to Austin and Amir (2012), agriculture

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contributes to about 10% of Malaysia's GDP, and at slightest one-third of the country's populace depends on the segment for its business, with a few 14% utilized on ranches and plantation. The government has made a lot of changes for the development in the field of agriculture. This situation can be seen in one of the government's efforts to improve the living standards of the people of Malaysia and help increase the revenue of the country. Among the steps that had been taken was by applying the latest farming methods through the application of modern technology as well as findings through research and development (R&D). The agricultural sector has become increasingly important as it is capable of lifting and expanding the use of biotechnology. The use of biotechnology can accommodate several types of agriculture, mainly highland agriculture.

Highland is one of the natural resources of our country. Highland is defined as land that has a height of 300 metres above sea level (Manan, 2001). Usually, at this level, the highland climate change is more pronounced and has lower temperatures. This situation affects plants and life in the area. For example, Cameron Highlands is famous for tourism activities as well as vegetables and fruits farming. Unfortunately, highlands are now increasingly threatened by developmental projects such as construction of roads, housing, hotels, and tourism activities.

To overcome the shortage in agriculture, biotechnology methods have been introduced. One of the areas that apply from the field of biotechnology was *in vitro* culture. *In vitro* culture or knowing as tissue culture breeding technique involves micro breeding process that separates a group of small tissues known as explants from animals or plants. It is then placed in the appropriate and essential nutrients for growth outside

the sterile living organisms. Seed production techniques of plant tissue using this essential nutrient medium can produce the same seed as the original tree. Furthermore, the tissue culture technique is among the best to meet this purpose compared to other techniques like cross-breeding or marcotting.

1.2 Background of Study

Malaysia is a developing country in various sectors, whether in agricultural or industrial field. Increasing population results in increasing demands in all goods. To fulfil these demands, various technologies were created and developed for this purpose. In agricultural field, various discoveries were done to increase productivity and product quality. Many studies were carried out and the result met one of the methods that are most productive and efficient in this sector known as tissue culture method, which is a technique used in the production of a plant using a small part of tissue from explants like leaf, stem or cell cultured on the media under sterile or aseptic for growth. According to Hussain, Qarshi, Nazir and Ullah (2012), controlled conditions such as appropriate stock of minerals, medium pH level, suitable conditions, and decent vaporous and liquid environment have to be provided to the culture to enhance their growth and multiplication.

According to Taha (2004), first person who involve in tissue culture was Rechinger in year 1839. He used shoot, bud, root and stem cutting. The explant was placed on the surface of soil and being moisten by water. Rechinger made a conclusion that explant cutting that more than 1.5 mm were expand. However, he did not use

nutrient or aseptic condition. Although the method that being used cannot be regarded as tissue culture technique, but the principle is equal to tissue culture technique that being practiced nowadays

The platforms that can be utilized in order to breed broccoli in low land area is plant tissue culture, which refers to *in vitro* culture (Smith, 2000), or organogenesis technique, which is a technique employed in the production of a plant using explants in sterile or aseptic media and environment; it is also a popular method used in the formation of *Brassica* crops. As indicated by Darus (2010), organogenesis is beneficial in overcoming problems such as insufficient seedling. Other than enhancing the growth of plants that are difficult to grow in normal climate and condition, plants that are free from diseases can also be produced through organogenesis.

1.3 Problem Statement

Broccoli are planted in high land area; hence, it is difficult to grow them in low land area. As stated by Warland, McKeown and McDonald (2006), the growth and physiological processes of broccoli were affected by high temperature and its quality and marketable yield were reduced. This is supported by Safaryani, Haryanti and Hastuti *(*2007), mentioned that broccoli does not withstand high temperature and it is only suitable to be planted in high land area. In other words, normal temperature hinders the germination of broccoli. Hence, this problem can be overcome by using organogenesis as the best alternative to germinate broccoli. As proven by Ravanfar,

Aziz, Kadir, Rashid and Sirchi (2009), broccoli can be grown in low land area through organogenesis and acclimatization.

The improvement of crop using conventional method has several limitations (Gana, 2010). Crop cultural techniques by using conventional methods in soil or sand medium have often faced technical, environmental and time constrains problem. For example, plant propagation through seeds usually require along time period and the results are different from its parent. Another obstacle that is faced is natural disturbance, either cause by living bodies, such as pests and diseases, and also environmental stress that can interfere with the success of plant propagation in the field. The need of plant seeds in large quantities, quality, free of pest and diseases, availability in a short time, it often cannot be met with conventional method either generative or vegetative (Triwibowo, 2006). In horticultural crops, vegetative propagation in the maintanence of genetic uniformity and preservation of cloning identity is essential, so that an efficient vegetative propagation technique is required (Mumo, Rimberia, Mamati and Kihurani, 2013).

According to Hosoki, Kigo and Shiraishi (1991), broccoli that belongs to *Brassica* family can easily be infected by cabbage moth and cabbage worm as well as by bacterial soft and black rot. This shows that broccoli is a plant that is susceptible for infection, especially by insects; hence, it is necessary for farmers to take precautionary steps as to ensure that broccoli will not be infected by bacteria or insect. In addition, Bernama (2015), mentioned that even though Cameron Highland is a cool climate place suitable for the growth of *Brassicae* plant, farmers still need to put ample poultry

manure and imported agrochemical and pesticides in order to ensure the quality of B*rassicae* family.

In sum, plant tissue culture is the best alternative in overcoming the risk of insect attack on broccoli as well as to reduce the cost that farmers have to be bear. This is because in tissue culture, the environment can be controlled and it allows no opportunity for insects to attack the plant.

1.4 Research Objective

1. To establish organogenesis of *B. oleracea* var. *italica.*
2. To investigate callus induction of *B. oleracea* var. *italica.*
3. To develop artificial seed for *B. oleracea* var. *italica.*
4. To establish acclimatization and study micromorphology of *in vivo* and *in vitro* of *B. oleracea* var. *italica.*

1.5 Importance of Research

The use of biotechnology can overcome deficiencies and problems faced while performing conventional farming methods. According to Rashid (2017), in order to solve the problem of food supply, various technologies have been created and developed for the purpose of enhancing the productivity of the agricultural sector.

Breeding technique in tissue culture is a technique in the field of biotechnology, which has been successfully developed for concerted cultivation, consequently improving the productivity or the quality of crop production.

According to Muhammad, Sriani and Rahmi (2015), plant breeding in biotechnology and molecular biology has ability to overcome some of the problems that occur in conventional breeding. Tissue culture has advantages compared with conventional plant propagation, which they do not require a large area, they are able to produce the number of seeds of plants in a relatively short period, can be executed throughout the year without depending on the season, produced healthier seeds and enabling genetic manipulation (Yusnita, 2014).

1.6 Scope and Limitation of Study

This study has been carried out using tissue culture system in *B. oleracea* var. *italica*. The type of broccoli selected is a common type of broccoli. Aseptic seedling parts such as roots, stems, and leaves were used as explants source. The experiment were limited by plant contaminations. However it could be overcome with numbers of replications. In addition, propagation of broccoli was limited by time duration, in this study broccoli does not achieved until flowering stage.