MORPHOGENESIS STUDIES OF *Brassica oleraceae* L. var *Capitata* THROUGH TISSUE CULTURE SYSTEM

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2021

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DISSERTATION TO QUALIFY FOR A MASTERS IN SCIENCE (RESEARCH MODE)

FACULTY OF TECHNICAL AND VOCATIONAL

SULTAN IDRIS EDUCATION UNIVERSITY

2021

ACKNOWLEDGEMENT

Bismillahirahmanirrahim…

Alhamdulilah..Thank you for being grateful to Allah for His permission to complete this study.

A special thanks to the Faculty of Technical and Vocational (FTV) for allowing me to do this research, especially in providing facilities, apparatus and materials in the lab.

An appreciation to the most beloved lecturer, Profesor Madya Dr. Nor Azlina Binti Hasbullah who guided and instructed me throughout my research and writing. Thank you so much Dr. Azah.

Special thanks to my beloved mom and dad, Mrs. Rohani Derahman and Mr. Daud Ahmad that have given me great moral, emotional and financial support. I love you forever ma and abah. All the good kind cannot be repaid with the money.

Thanks to my beloved husband, Mr. Mohamad Firdaus Abdul Rashid Fikri for always giving me the support and encouragement to finish our master's degree together. You've always been kind to me. Thanks to Allah for sending him to me.

Thanks to my beloved siblings, Fatihah, Faris, Fadhil, Fahmi and Faisal for always sacrifice to help me. I am nothing without all of you!

Thanks to my friends, for always encouraged me to finish this research. Thank you aufa, kak timah and azizi.

Thanks also for those involved to complete this research. Thank you very much. May Allah bless all of us. In shaa Allah..ameen.

ABSTRACT

Experiment were carried out to establish the most optimum culture condition and to identify the most responsive explants for regeneration *Brassica oleraceae* L. var *Capitata* through plant tissue culture system. Acclimatization process for established plantlets was also conducted. The experimental design of this study was Complete Randomized Design (CRD) method. For shoot regeneration using different concentrations of BAP and NAA, the most responsive explant was stem explants in which MS medium supplemented with 1.5 mg/L BAP and 0.5 mg/L NAA produced 8.400 ± 0.889 shoots per explant. Whereas, the combination auxin and cytokinin also showed stem explants was the most responsive. Subsequently, for root regeneration using different concentrations of BAP and NAA, the most responsive explant was leave in which MS media supplemented with 0.5 mg/L BAP and 0.5 mg/L NAA produced 51.133 ± 2.929 roots per explant. While the combination of auxin and cytokinin showed the most responsive was root. Next, for callus induction with combination of BAP and NAA showed that petiole was the most responsive explant. Callus was white in colour with friable structure. Meanwhile, for callus induction with combination of auxin and cytokinin showed stem was the most responsive. Callus was light brown and friable. In addition, synthetic seeds were produced when microshoots were encapsulated with 4.0% sodium alginate solution added with 1.0 mg/L BAP and 0.5 mg/L NAA. The germination rate was 6.832 ± 0.622 shoots per explant. Finally, the acclimatization of this plantlet succeeded with combination of (black soil and red soil at 2:1 ratio) with the survival rate of 86.67%. This research showed that morphogenesis studies of *Brassica oleraceae* L. var *Capitata* through tissue culture system was successfully achieved. Further studies on chemical such as secondary metabolites contained in the callus and explants extract should be carried out.

KAJIAN MORFOGENESIS TERHADAP *Brassica oleraceae* L. Var *Capitata* MELALUI SISTEM KULTUR TISU.

ABSTRAK

Eksperimen ini bertujuan mencari persekitaran kultur paling optimum dan mengenalpasti eksplan yang paling responsif bagi pertumbuhan semula tumbuhan *Brassica oleraceae* L. var *Capitata* melalui sistem kultur tisu. Proses aklimatisasi plantlet yang terhasil turut dijalankan. Kajian ini menggunakan kaedah reka bentuk kajian rawak lengkap (CRD). Bagi pertumbuhan semula pucuk menggunakan kepekatan BAP dan NAA yang berbeza, eksplan batang adalah paling responsif di mana medium MS yang ditambah dengan 1.5 mg/L BAP dan 0.5 mg/L NAA menghasilkan 8.400 ± 0.889 pucuk bagi setiap eksplan. Manakala, bagi kombinasi auksin dan sitokinin yang lain menunjukkan eksplan batang paling responsif. Kemudian, bagi pertumbuhan semula akar menggunakan kepekatan yang BAP dan NAA yang berbeza, daun merupakan eksplan yang paling responsif di mana media MS dengan 0.5 mg/L BAP dan 0.5 mg/L NAA menghasilkan 51.133 ± 2.929 akar bagi setiap eksplan. Bagi kombinasi auksin dan sitokinin yang lain pula didapati akar merupakan eksplan yang paling responsif. Kajian induksi kalus menggunakan gabungan BAP dan NAA menunjukkan eksplan petiol adalah yang terbaik. Kalusnya berwarna putih dan rapuh. Sementara itu, induksi kalus menggunakan gabungan auksin dan sitokinin yang lain menunjukkan batang adalah paling responsif. Kalusnya berwarna coklat muda dan berstruktur rapuh. Selain itu, biji benih sintetik pula dihasilkan apabila pucuk mikro dikapsulkan dengan menggunakan larutan natrium alginat 4.0% ditambah dengan 1.0 mg/L BAP dan 0.5 mg/L NAA. Kadar percambahan adalah 6.832 ± 0.622 pucuk bagi setiap eksplan. Akhir sekali, aklimatisasi plantlet tanaman ini berjaya dengan kombinasi tanah (tanah hitam dan tanah merah pada nisbah 2:1) dengan kadar kelangsungan hidup 86.67%. Penyelidikan ini telah menunjukkan bahawa kajian morfogenesis tanaman *Brassica oleraceae* L. var *Capitata* melalui sistem kultur tisu berjaya dicapai. Kajian lebih lanjut mengenai bahan kimia seperti metabolit sekunder yang terkandung dalam ekstrak kalus dan eksplan boleh dilaksanakan pada masa akan datang.

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LIST OF ABBREVIATIONS

°	Degree Symbol
2,4-D	2,4-Dichlorophenoxyacetic acid
2ip	2-isopentenylaminopurine
6CO	Carbon dioxide
$6H_2O$	Water
6O2	Oxygen
ABA	Abscisic acid
Al	Aluminium
ANNOVA	Analysis of variance
B	Boron
BAP	6-Benzylaminopurine
C	Celcius
$C_6H_{12}O_6$	Glucose
Ca	Calcium
$CaCl_2$	Calcium Chloride
$CaCl_2.2H_2O$	Calcium Chloride Dehydrate
Cl	Chlorine
Co	Cobalt
CO_2	Carbon dioxide

Cu	Cuprum
DNA	Deoxyribonucleic Acid
Fe	Iron
g	gram
GA_3	Gibberelic acid
IAA	Indole-3-Acetic Acid
IBA	Indolebutyric Acid
KPa	KiloPascal
L	Litre
M	Mol
MARDI	Malaysian Agricultural Research and Development Institute
Mg	Magnesium
mg/L	Milligram per Litre
Mm	millimetre
mm	millimeter
Mn	Manganese
Mo	Molybdenum
MS	Murashige and Skoog
N	Nitrogen
Na	Sodium
NAA	Naphthalene Acetic Acid
$NaC_6H_7O_6$	Sodium alginate
Ni	Nickel

O_2	Oxygen
P	Phosphorus
pH	potential Hydrogen
S	Sulphur
SE	Standart Error
SEM	Scanning Electron Microscope
Si	Silicon
SPSS	Statistical Package for the Social Sciences
TDZ	Thiazuron-N-phenyl-N-1,2,3 thiadiazol-5ylurea
UPSI	Sultan Idris Educational University
USDA	United States Department of Agriculture
V	Vanadium
WPM	Woody Plant Medium

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Malaysia is a country that is experiencing the equatorial climate which is hot and humid all over the year. It has several of forest types which are the tropical rain forests, mangrove forests, mountain forests, and coastal forests. These forests give a lot of benefit to human and animals. In addition, for agro-tourism activities, plants could also be used as sources of nutrition and oxygen supply to the surrounding

ecosystem wildlife. Besides, plant can be categorized into a few types such as climbers, ferns and aliens, shrubs, cycads, palms and trees. It is categorized according to their growth habits or types.

1.2 Plant

There are many different types of plants in the world including in Malaysia. Malaysia has thousands of types of plants. In order to align the name of the plants, an international system was introduced known as taxonomy. Taxonomy is the science of describing, classifying and naming organisms. Classification means arranging organisms into groups based on their similarities. The organisms are classified by biologist into taxa. Taxa / taxon is the name for one category or group of organism at one level or rank in the taxonomic hierarchy scheme. Taxonomy has two branches that are nomenclature and systematics. Nomenclature refers to the naming of organisms and the taxa to which they belong. It is also refers to the correct usage of scientific names used in taxonomy. It is based on the binomial system. While, systematics (grouping) is the discovery and scientific study of biological diversity. Systematics classify organisms into groups using classification methods by their evolutionary relationships. Taxonomy is very important because the former is the standard worldwide system for communicating the identity of plants (Min *et.al*, 2006). There are some advantages of taxonomy in which it will help to identify the

universal name of organisms, to precisely identify plants and other organisms by avoiding confusion of one organism having many common names, to manage the information and data that can be kept and catalogued in a good system, allow to study the diversity of biology and the natural connection among the organism in the web of life, allow to study the other related field such as morphology and anatomy, able to distinguish between beneficial and harmful varieties, to support evolution theory and lastly to develop strategies for protection and conservation of endangered species.

1.2.1 Plants and Energy Consume

In the world, the total number of plant species is estimated between 300,000 and 500,000. Of these, approximately 250,000 have been identified and classified (Frusciante, 2000). While, according to Willis (2017), almost 80% of the food derived from plants comes from seventeen plant families and in total, 452 vascular plant families have been identified by botanists across the world.

Plants are either herbaceous plants or woody plants. They also vary in structure, yet all plants have the same basic body plan. There are three basic organ that belongs to the plants. They are root, stem, and leaf. It is also organized into a root

and shoot system. Normally, in tissue culture, herbaceous plants will regenerate easier than woody plants.

Besides that, plant is very special because it can do photosynthesis process. Photosynthesis is the process by which photoautotrophic organisms use sunlight as energy to make glucose (as product) and oxygen gas (as by product) from carbon dioxide and water (as raw materials). There is equation of photosynthesis process:

$$6CO + 6H_2O \text{ ---------- } > C_6H_{12}O_6 + 6O_2$$

The green plants use the light energy, thus they converted it to the chemical energy and stored in the bonds of organic molecules such as carbohydrates. Example of the organic molecules is carbohydrate. It takes place inside the chloroplast on leave. Carbohydrates is one of the energy form that can give the benefit for humans and animals. Humans and animals depend on plants as the source of food, which provided oxygen and energy. Because of that, tissue cultures play an important role to fulfil the needs for human and animals. (ISAAA, 2004)

1.2.2 Plants Reproduction

Furthermore, plants reproduction important in order to make sure that the continuity of the generation of some species. Reproduction is a production process of new individual (offspring) by sexual or asexual reproduction. Without reproduction, plants heritage will be extinct. It is a fundamental characteristic of life; to increase number of some species. In reproduction, a parent generation effectively passes on a copy of itself, in the form of genetic material to another generation (offspring). The period of time from the beginning of an organism‟s life until it reproduces is called the life cycle. In this life cycle, young organism must first grow and develop before they can reproduce after maturity. Reproduction can be classified into two types. There are sexual and asexual reproductions. Sexual reproduction is involving two gametes fuse to form a zygote, which then grows into a new individual. Sexual also means with sex, fertilization and with the fusion of gametes. While asexual reproduction means „non-sexual‟ in which no gametes are formed in asexual reproduction and a single organism produces new individuals. Normally, the cells of the new offspring are produced by mitosis. Asexual reproduction can be divided into two. There are vegetative growth and spore formation. In vegetative growth, there are three types of reproduction. There are asexual reproduction by modified stem, plant propagation and micropropagation. Micropropagation or *in vitro* propagation is the clonal propagation of plants by tissue, cell and organ culture methods. It involves the aseptic culture of

explants of tissues and organs in closed vessels using defined culture media in a controlled environment. (Samir & Usha, 2020)

1.2.3 Plant Nutrients

Several components in media that composed in plant tissue culture are macronutrients, vitamins, micronutrients, complex nutritive mixtures, growth regulators, amino acids, carbon source (sugars), agar or gelrite and water. All these compounds fulfil one or more functions of the *in vitro* growth of plants. The salts in media can be divided into macronutrients and micronutrients. Macronutrients include Calcium (Ca), Magnesium (Mg), Sulphur (S), Phosphorus (P), Nitrogen (N) and Potassium (K) while micronutrients include Iron (Fe), Cuprum (Cu), Manganese (Mn), Boron (B), Cobalt (Co), Molybdenum (Mo), Iodine (I), Chlorine (Cl), Nickel (Ni), and Aluminium (Al). The subdivision in macronutrients and micronutrients is mainly based on the need of the plant for these elements. Most micronutrients are present in micromolar quantities. The requirement for macronutrients is superior and therefore in media presents in milimolar concentrations. Most components found in the culture media are required for whole plant growth. (Cseke *et.al*, 2004)

1.2.4 Climate Change

Lately, climate change causes severe impact on human health and plants growth. Unpredictable weather conditions causes the rate of crop production becomes unstable and uncertain. The farmers suffered significant losses due to the effects of climate change. For example, flooding and crop diseases give affects to the farmers. The mechanisms should be approaches to resolve these issues because it involves the cost and impact on the human food supply.

1.3 Problem Statement

The improvement of crop using conventional methods has several limitations (Gana, 2010). Crop cultural techniques by using conventional methods in soil or sand medium have often faced technical, environmental and time constraints problem. For example, plant propagation through seeds usually require a long time period and the results are different from its parent. Another obstacle that is faced is natural disturbance, either caused by living bodies, such as pests and diseases, and also environmental stresses that can interfere with the success of plant propagation in the field. The need of plant seeds in large quantities, quality, free of pests and diseases, availability in a short time, it often cannot be met with conventional methods either

generative or vegetative (Triwibowo, 2006). In addition, land preparation started 45 days before planting to grow this cabbage with harvesting activities between 70 and 120 days after planting (Amir et al*.,* 2007). In horticultural crops, vegetative propagation in the maintenance of genetic uniformity and preservation of cloning identity is essential, so that an efficient vegetative propagation technique is required (Mumo, Rimberia, Mamati, & Kihurani, 2013).

Cabbage found difficult to germinate in low land area. Warland, McKeown and McDonald (2006), stated that the physiological process of cabbage were affected by high temperature and will reduce the quality and marketable yield. Safaryani*,* et al.,(2007) also stated cabbage does not withstand high temperature and it is suitable to be planted in high land area.

Besides that, cabbage was being infected by insect. These insect pests can cause damage directly by sucking or chewing sap from leaves and roots. Indirectly they will transmit diseases to the cabbage. Although, it can also lower the value of the cabbage heads causing unsightliness such as the presence of insect cast skins and detritus (Znidarcic et al., 2008). According to Shamsudin et al., (2010), the use of pesticides in vegetables cultivation can be harmful for humans. It is often argued that pesticide is often applied in appropriate amounts to cabbage in order to ensure the unblemished and fresh looking produce.

1.4 Research Objective

The objectives of these study are :

i. To identify and establish the most efficient regeneration system for *Brassica oleraceae* L. var *capitata* through tissue culture.

ii. To establish callus induction of *Brassica oleraceae* L. var *capitata.*

iii. To produce synthetic seeds from the regeneration of *Brassica oleraceae* L. var *capitata.*

iv. To achieve acclimatization process for *Brassica oleraceae* L. var *capitata.*

1.5 Significance of Study

Plant breeding in biotechnology and molecular biology has ability to overcome some of the problems that occur in conventional breeding (Muhammad, Sriani, & Rahmi, 2015). According to Yusnita (2004), tissue culture has advantages compared with conventional plant propagation, they are: able to produce the number of seeds of plants in a relatively short period, does not require a large area, can be executed throughout the year without depending on the season, produced more healthy seeds and enabling genetic manipulation.

In the last 20 years, tissue culture techniques have assisted researchers, plant growers, and nursery industry in increasing a large quantity plants (Yusnita, 2004). The studies on plant propagation in tissue culture especially for *Brassica oleracea* L. var *Capitata* have not been widely implemented. Finally, this study was expected to contribute to other researchers, as a knowledge for further research. Moreover, this research can be applied in maximum in producing cabbage that are resistant to disease, so it can help the farmers to get the better plant quality and quantity.

1.6 Scope and Limitation of Study

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