COMPARISON OF DNA EXTRACTION METHODS FROM ORCHID LEAVES

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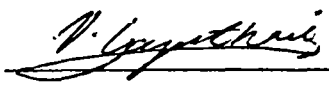

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ABSTRACT

DNA extraction is an important step in obtaining molecular data. In this study, three DNA extraction methods were compared based on the quantity and quality of the obtained DNA. The Dellaporta, SDS and CTAB methods were used to extract DNA from leaf samples of four different orchid species namely *G. speciosum*, *P. amabilis*, *R. bella* and *Oncidium* species with each having four replicates. Results indicated that there was interaction between the extraction methods and the species used in terms of the obtained DNA quantity ($p<0.05$).However, there was no significant interaction between the methods and species in terms of quality ($p>0.05$). Based on the results the highest mean of DNA quantity for the CTAB method was obtained for *G. speciosum* (84.40 µg). Furthermore, for the Dellaporta method the highest mean quantity was obtained by Oncidium species (48.10 µg). Apart from that, the highest mean quantity for the SDS method was obtained by *G. speciosum* (30.95 µg). On the other hand, based on the quality of the obtained DNA, the Dellaporta and CTAB method gave the highest mean ratio of A_{260}/A_{280} for all the species with mean values of 1.678 and 1.582 respectively. Conversely, the SDS method gave the lowest mean ratio of 1.323.

PERBANDINGAN KAEDAH PENGEKSTRAKAN DNA DARIPADA DAUN ORKID

ABSTRAK

Kaedah pengekstrakan DNA merupakan suatu langkah yang penting dalam mendapatkan data molekular. Dalam kajian ini, perbandingan antara tiga kaedah pengekstrakan DNA dari segi kuantiti serta kualiti DNA dilakukan. Kaedah Dellaporta, SDS serta CTAB digunakan untuk mengekstrak DNA daripada empat spesies sampel orkid liar iaitu *G. speciosum*, *P. amabilis*, *R. bella* serta spesies *Oncidium*. Sebanyak empat replikasi digunakan untuk setiap spesies. Keputusan menunjukkan bahawa wujud interaksi yang signifikan antara kaedah dan spesies dari segi kuantiti DNA ($p<0.05$). Walau bagaimanapun, dari segi kualiti pula tidak wujud interaksi yang signifikan antara kaedah dan spesies ($p> 0.05$). Berdasarkan keputusan yang diperoleh, min kuantiti DNA yang tertinggi untuk kaedah CTAB diperoleh oleh spesies *G. speciosum* (84.40 µg). Selain itu, untuk kaedah Dellaporta pula spesies Oncidium yang memperolehi min kuantiti yang tertinggi (48.10 µg). Di samping itu, spesies yang memberikan min kuantiti yang tertinggi untuk kaedah SDS adalah *G. speciosum* (30.95 µg). Dari segi kualiti pula, min nisbah A_{260} / A_{280} yang paling tinggi untuk kesemua spesies diberikan oleh kaedah Dellaporta serta kaedah CTAB iaitu masing-masing sebanyak 1.67 dan 1.582. Kaedah SDS pula telah memberikan min nisbah yang terendah iaitu sebanyak 1.323.

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LIST OF SYMBOLS AND ABBREVIATIONS

%	percentage
°C	degree Celsius
µl	microliter
H_o	Null hypothesis
H_a	Alternative hypothesis
g	gravitational force
g	gram
M	mole
bp	base pair
v/v	volume over volume
w/v	weight over volume
A_{260}	Absorbance at 260 nanometer
A_{280}	Absorbance at 280 nanometer
min	minutes
µg	microgram
$\mu g ml^{-1}$	microgram per milliliter
ml	milliliter

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Orchids belong to the largest flowering plant family which is known as Orchidaceae and it is the largest in the plant kingdom comprising of 750 genera and a minimum of 25, 000 native species and 30 000 hybrids (Teo, 1979; Hew & Yong, 1997). Malaysia houses approximately 3 000 species of beautiful and exotic orchids which makes up of almost 10 % of the world's population of these plants. Sabah and Sarawak alone have more than 2 000 species of wild orchids (Nor Ain, 1999). The diversity of orchids originates from years of evolution that developed for the purpose of adaptation to the environment (Teo, 1985).

There is a need in today's world to study the morphology, anatomy and chemistry of the orchids to get a better understanding of the factors that have made these flowers the largest flowering plant family in the plant kingdom. There is a difficulty in doing this as

such efforts will only prove to be time consuming since the family is so large and diverse. Therefore deoxyribonucleic acid (DNA) analysis proves to be an effective solution because molecular data is quicker to collect and easier to asses. The advancement in gene cloning and transfer has also come hand in hand with the development of technology for the removal and analysis of DNA. It is possible now to isolate DNA from an organism and use it for molecular research. This conserved DNA has various uses such as in the study of the molecular phylogenetics of extant and extinct taxa (Adams, 1997).

The classification of orchids has been attempted since a very long time ago. One of the common methods of classifying orchids is based on the comparison of different anatomical structure such as the flowers. Despite this, there is still skepticism lying behind these methods as the classifications of morphological structures prove to be insufficient. There are cases where closely related species are placed in different genera and vice versa. Even though the orchids may belong to a similar group or class, there may be variation of the orchids at a molecular level. Such differences can only be identified by obtaining molecular data through DNA analysis such as DNA sequencing. This method has been proven a success on many other types of angiosperms as well as several other groups of orchids (Chase, 1999).

Apart from the study of phylogenetics, DNA can also be used for the production of genomic probes for research laboratories. Here DNA can be extracted and immobilized onto nitrocellulose sheets where it can be probed with numerous cloned genes. Furthermore, the DNA can also be applied in various biomolecular study processes such

as polymerase chain reaction where specific genes from a whole mixture of genomic DNA can be routinely amplified (Adams, 1997). In addition to that DNA could be analyzed and used in some instances to detect the presence of foreign genes by 'dot blotting methods'. This method is a test that verifies the nature of the tissue to identify if it is transformed (Nicholl, 2002).

In this study several DNA extraction methods are used to isolate genetic materials from orchid leaf tissue. The DNA Mini Prep Method using sodium dodecyl sulphate (SDS) which is recommended by Dellaporta *et al.* (1985) does not require the use of an ultracentrifugation procedure with CsCl. This technique has been reported to produce a high yield of DNA and is suitable for genetic blot analysis. The usage of the detergent SDS is emphasized because it plays a role in stripping of proteins from nucleo-protein complexes so that free DNA can be obtained (Dellaporta *et al.*, 1983; Draper & Scott, 1988). It also solublizes the membranes in the cell. SDS will form a potttasium dodecyl sulphate complex with proteins and polysaccharides in the presence of high potassium ion concentrations. This is why potassium acetate is added in this method. In the original method the slurry is then filtered through cheesecloth because the complexes formed with SDS are flocculent and hard to remove yet in the current modified version that is to be applied no filtration is required do to the minimal scale of the extraction procedure.

The second method described by Pich & Schubert (1993) is suitable for tissues that are high in secondary products such as polyphenolics and tannins that may bind to DNA after cell lysis. Polyvinylpyrrolidone (PVP) is used to form complexes with these

secondary products and hence will be precipitated and centrifuged so that the complexes could be removed and DNA can be obtained. This method incorporates a phenol,chloroform and isoamylalcohol extraction to remove contaminants and the DNA is precipitated with isopropanol. It is a quick method of isolation and will yield clean DNA that is suitable for restriction, Southern and PCR analysis (Pich & Schubert, 1993).

The third method is obtained from the works of Bhushan *et al.* (2003). It is a modified cetyl-trimethylammonium bromide (CTAB) method. CTAB is a cationic detergent that is also widely used in various methods of DNA isolation. The DNA method using CTAB uses this chemical to precipitate DNA. CTAB has the ability to precipitate contaminants or DNA depending on the concentration of sodium chloride (NaCl). At a low salt concentration (NaCl <0.5 M), the DNA is precipitated into the aqueous layer leaving the proteins and polysaccharides in the solution. The DNA can then be obtained from the aqueous layer. On the other hand, if salt concentrations are high (NaCl > 0.8 M) then the opposite occurs with the DNA being left behind in the solution. The latter is usually applied to obtain RNA (Draper & Scott, 1988; Kidwell & Osborn, 1992). The method recommended by Bhushan *et al.* (2003) is efficient to successfully extract DNA from plant extracts that are highly acidic with secondary compounds. This protocol is the quickest among the three and does not use phenol but just a chloroform-isoamylalcohol extraction step. The DNA obtained is high in molecular weight and is suitable for usage in polymerase chain reaction (Bhushan *et al.*, 2003).

1.2 Objective

The objective of the study is to compare the Modified Mini Prep DNA extraction recommended by Dellaporta *et al.* (1985), modified Mini Prep DNA extraction recommended by Pich & Schubert (1993) and modified CTAB DNA extraction method recommended by Bhushan *et al.* (2003) among orchid leaves of different morphology in terms of quality and quantity.

1.3 Scope

The scope of this research is to determine the DNA concentration by optical density readings from the plant extract using spectrophotometer followed by the visualization of bands formed from gel electrophoresis. The materials are *Grammatophyllum speciosum* Bl, *Phaleonopsis amabilis*, *Oncidium* spp. and *Renanthera bella*. These orchids show distinct morphologies and the study is done to compare and determine which method is suitable for particular leaf morphology.

CHAPTER 2

LITERATURE REVIEW

2.1 Orchidaceae

Orchids belong to the family Orchidaceae and are unique members of the flowering plants since they have various features that makes them special from the rest of the angiosperms. Orchids are monocotyledonous and the first major feature that highlights their specialty is the flower. An orchid flower like any other flower has three outer segments called sepals and three inner segments called petals (Teo, 1985). Despite that the specialty of the orchid lies in the fact that the sepals are incorporated in the flower itself unlike other flowers where the sepal serves the purpose of holding the three petals together. Another unique feature is that the sepals are not solely green but instead are highly coloured like the petals (Teo, 1979). The petals on the other hand have a distinct feature where one of it is called lip or labellum (Teo, 1985). The lip has two types of lobes comprising of two side lobes and a single mid lobe (Teo, 1979). Besides the outer structure of the flower, another unique feature of the orchids that makes it different from other flowers is the structure of the reproductive organ. Unlike most flowers that have separate male and female organs, orchids have a single reproductive organ called column which consists of a stamen and

pollen that are fused as one single structure (Teo, 1985). The pollen grains occur as a single sticky mass which is known as pollinium which plays a significant role in securing the production of a huge mass of seeds to ensure the perpetuation of the species (Teo, 1985).

Orchids can be classified according to habitat that they grow in thus can be categorized into three categories which are terrestrials, epiphytes and saprophytes (Cribb, 1999). Terrestrial orchids are those that grow on the ground where the roots are ground dwelling and cannot withstand exposure to air (Teo, 1985). The roots of these orchids are thick, fleshy and sometimes contain storage fat. Some of the roots of the terrestrials can appear to be tuber like. The roots contain mycorrhizal fungi that infect the plant at the seed stage. These fungi serve the purpose of providing carbohydrate and mineral nutrients to the plant (Hew & Yong, 1997). The terrestrials in Malaysia can be divided into two groups where the first has a limited root system and the second with a large root system. The former has thick stems and some may have tubes. These terrestrial orchids have thin leaves. The latter have pseudobulbs and leathery leaves. Some of the common genus in this group is *Paphiopedillum*, *Phaius* and *Liparis*.

Epiphytes such as *Phaleonopsis* orchids are another category that can be further divided into sympodials and monopodials (Teo, 1979; Teo, 1985). Sympodials have a limited growth hence they are short in nature and contain pseudobulbs. The auxiliary buds of the sympodials usually grow laterally. *Dendrobium* is one of the common orchid types that fall under this group (Hew & Yong, 1997). Monopodials on the other hand are

usually climbers and their growth occurs on the stem tip without termination (Teo, 1985). This group of epiphytes displays a continuous growth pattern and usually consists of orchids such as *Arandas*, *Vandas* and *Mokaras and Renanthera* (Beaman *et al.*, 1993; Hew & Yong, 1997). Apart from the two main classifications of epiphytic orchids, both these groups display some common features. Most epiphytes have roots that are covered by a dead sliver tissue called the valamen layer. The valamen serves as a protection from excess light and heat since the root is not buried in the ground (Teo, 1985). The aerial roots of epiphytes have a green or reddish tip and the remainder of the root is covered by the valamen (Hew & Yong, 1997).

Saprophytes are orchids that do not perform photosynthesis. This is due to the lack of chlorophyll in the plant. Several of the common saprophytic genuses are *Vanilla* and *Corybas* (Teo, 1985). There are a few saprophytic orchids which are leafless and also those that have leaves which are reduced to colourless scales due to the lack of chlorophyll (Cribb, 1999).

Furthermore, apart from the diversity of habitat, orchids can vary in size and shape (Teo, 1979). The smallest orchids could be in a miniature size that is only 3 mm to 4 mm tall. In contrast the giant orchid, *Grammatophyllum speciosum* can weigh up to many hundred kilograms (Northen, 1990). This variation can also extend to the many different morphological structures of the plant (Cribb, 1999). Orchid leaves can come in a diverse range of shapes and sizes (Teo, 1979). The shape of the orchid leaves can be simple, linear, lanceolte, oblanceolate, ovate or elliptic. Apart from these shapes some orchids can

possess other forms of leaf shapes such as fan-shaped, palmate, sagittate and spirally twisted. Leaf size is another variable feature. Some orchids such as *Bulbophyllum minutissimum* has a length of 1mm long yet *Bulbophyllum fletcherianum* in contrast has leaves as long as 1 m. Leaf texture can vary as well such as hard, leathery, fleshy or soft depending on the type of orchid (Cribb, 1999). The form of leaf that an orchid possesses usually displays the habitat that it belongs to because the leaf structure itself plays a specific function in the plant. Orchids such as *Spathoglottis* have thin, pleated leaves that functions as a food manufacturer by carrying out photosynthesis. Certain orchids have large pleated leaves to absorb as much sunlight as possible so photosynthesis can be carried out. The presence of such features implies that such plants possibly originated from a shady but moist habitat of the forest floor. Furthermore, orchids such as *Phaleonopsis* have thick and fleshy leaves that have an additional purpose of storing water apart from performing photosynthesis. Certain orchids do not have leaves and thus the functions of photosynthesis and water storage are both played by the roots and stems (Teo, 1979).

2.1.1 *Grammatophyllum speciosum* BL

The genus *Grammatophyllum* can be found scattered throughout Burma, Thailand, Malaysia, Phillipines, Indonesia, Papua New Guinea and Polynesia. There are around eight species that belong to this genus and generally the orchids in this genus have leaves that are lanceolate (Figure 2.1) and can grow up to 60 cm in length.

Figure 2.1 Leaves of *G. speciosum*

The species in this genus can be further divided into two distinct groups. The first type is known as Pattonia and it has short ovoid or ellipsoidal pseudobulbs with two to eight apical leaves. *G. scriptum* is one of the species that belong to this group. The second type is known as Gabertia which in contrast have very elongated pseudobulbs that appear as fleshy stems that have many leaves (Andeson *et al.*, 1991).

G. speciosum is the largest species in the genus *Grammatophyllum* and it belongs to the Gabertia group (Teo, 1985; Anderson *et al.*, 1991). This species is native to Malaysia and can weigh up up to many hundreds of kilograms (Teo, 1985; Cribb, 1999). The plant has been known previously with several names such as *G. giganteum* Bl, *G.*

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