UNIVERSITI PUTRA MALAYSIA

AGRO-MORPHOLOGICAL, GLUCOMANNAN CONTENT AND MOLECULAR CHARACTERIZATION OF MALAYSIAN Amorphophallus spp. BLUME GERMPLASM COLLECTION

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By

SURISA PHORNVILLAY

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

September 2015

I dedicate this dissertation works in memory of beloved my mother and grandmother, who I deeply miss.

A special dedication to my father, sisters and other family members for always be here, by my side through many ups and downs till the completion of my master study

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Master of Science

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Chairman : Siti Hajar Ahmad, PhD
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Amorphophallus has attracted much attention as it contains glucomannan and also possess other medicinal properties. Prior to the collection of propagating materials and cultivation, identification and diversity information of the *Amorphophallus* species are essential as different species perform differently under cultivation. However, limited research has been conducted on morphological and genetic variations of *Amorphophallus* spp. in Peninsular Malaysia. Therefore, the present study was conducted with objectives, namely collection and establishment of the *Amorphophallus* spp. in Peninsular Malaysia, morphological and genetic variations, and glucomannan (GM) content of the established *Amorphophallus* spp. accessions. The current observation from the collection of *Amorphophallus* spp. from six locations found that *Amorphophallus* spp. were abundantly spotted in disturbed areas of secondary forest. They were found thriving well in damp, moist and shady areas, mostly near a riverbank. The accessions were established, and their life cycle was observed. *Amorphophallus* had three distinct growth phases, namely, vegetative, generative and dormancy. The plant had a vegetative growing phase with the leaf development for about 5 months and then underwent a dormancy period for about 2 to 3 months. The plant resumed its growth in the next season or followed by generative life cycle, whereby the inflorescence was produced. Sixty accessions of *Amorphophallus* spp., with 10 accessions representing six locations, were used to assess morphological vegetative characters and molecular variations. The morphological variation data were also supplemented with the assessment of 20 *Amorphophallus* inflorescence accessions. There were variations in morphological characteristics among accessions of *Amorphophallus* spp. based on corm size, corm shape, cormel number per corm, petiole nature and distinct inflorescence characters, spathe colour, appendix colour and appendix shape and pattern. Genetic variations of the plants were assessed using nine screened microsatellite primers. Principle coordinates and cluster analysis results for morphological and molecular characters clearly separated Kota Bahru, Kelantan (KKB) accessions from the other 50 accessions, thus grouping the accessions into two major groups. The KKB population accounted for 22.22% genetic variations while the remaining five populations varied 77.78 to 100% genetically. Nonetheless, high inbreeding coefficients (0.21 to 0.88) were detected within each of the population. For the determination of glucomannan (GM) content, the 3,5-dinitrosalycyclic acid colorimetric assay was employed. The Hulu Langat, Selangor accession SHL24 was found to contain the highest GM content at 8.29%. The lowest GM content was from Perak, Taiping; PT37 at only 2.55%. The results also showed that there was a significant interaction effect between the populations and the corm size of GM content. Based on the results, PUK population of 500 g had the highest GM content. In conclusions, both the morphology and molecular analysis results suggest that KKB accessions are identified as *A. paeoniifolius* while other accession are *A. prainii* particularly based on distinct inflorescence characteristics. This study provides valuable insight and a better understanding of the habitat, growth, phenotypic and genotypic characteristics and GM content of the available *Amorphophallus* spp. accessions in Peninsular Malaysia. These findings could be used as the basic information for breeding, improvement and conservation program of this plant in Malaysia.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

AGRO-MORFOLOGI, KANDUNGAN GLUKOMANAN DAN PENCIRIAN MOLEKUL BAGI KOLEKSI GERMPLASMA *Amorphophallus* spp. BLUME MALAYSIA

Oleh

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Amorphophallus telah menarik banyak perhatian kerana kandungan glukomanan serta memiliki ciri perubatan. Pengenalpastian dan pengumpulan maklumat tentang kepelbagaian spesies *Amorphophallus* adalah penting kerana spesies yang berlainan menunjukkan prestasi yang berbeza semasa penanaman dan diikuti dengan pengumpulan bahan-bahan tanaman dan penanaman. Namun begitu, penyelidikan yang dijalankan terhadap variasi morfologi dan genetik tumbuhan *Amorphophallus* spp. di Semenanjung Malaysia masih terhad. Oleh itu, kajian ini dijalankan dengan menggunakan dua objektif iaitu pengumpulan dan pengukuhan bagi spesies *Amorphophallus* di Semenanjung Malaysia, variasi morfologi dan genetic, serta kandungan glukomanan (GM) daripada aksesi *Amorphophallus* spp. yang dikumpulkan. Hasil pemerhatian daripada pengumpulan *Amorphophallus* spp. daripada enam lokasi mendapati bahawa *Amorphophallus* spp. boleh ditemui dengan banyak di kawasan hutan sekunder yang telah diterokai. Ia didapati tumbuh dengan subur di kawasan lembap dan teduh yang kebanyakannya adalah berhampiran dengan tebing sungai. Aksesi *Amorphophallus* spp. telah diperoleh dan kitaran hidup tumbuhan ini diperhatikan. *Amorphophallus* mempunyai tiga fasa pertumbuhan yang berbeza iaitu, vegetatif, generatif dan dorman. Tumbuhan tersebut mempunyai fasa vegetatif dengan pertumbuhan daun selama kira-kira lima bulan dan kemudian menjalani satu tempoh dorman selama lebih kurang 2 hingga 3 bulan. Pertumbuhan diteruskan semula pada musim berikutnya atau diikuti dengan kitaran hidup generatif yang menghasilkan bunga akan dihasilkan. Sebanyak 60 aksesi *Amorphophallus* spp. dengan 10 aksesi mewakili enam lokasi telah digunakan untuk penilaian morfologi aksara vegetatif dan variasi molekul. Penambahan terhadap data bagi variasi morfologi dilakukan dengan penilaian terhadap 20 aksesi bunga *Amorphophallus.* Terdapat kepelbagaian pada ciri-ciri morfologi di antara aksesi *Amorphophallus* spp. berdasarkan saiz dan bentuk umbisi, bilangan anak umbisi per umbisi, sifat petiol dan ciri-ciri jambak bunga yang jelas dengan warna *spathe*, dan umbaian (appendix) serta bentuk dan corak umbaian (appendix).Variasi genetik tumbuh-tumbuhan dinilai menggunakan sembilan primer mikrosatelit. Prinsip koordinasi dan analisis berkelompok bagi pencirian morfologi dan molekul telah memisahkan aksesi Kota Bahru, Kelantan (KKB) daripada 50 aksesi yang lain dengan jelas dan membentuk dua kumpulan yang besar. Populasi KKB

menyumbang kepada 22.22% variasi genetik dan lima populasi dengan 77.78%-100% adalah variasi selebihnya. Namun begitu, koefisyen pembiakbakaan yang tinggi (0.21 hingga 0.88) dikesan bagi setiap populasi. Penentuan kandungan glukomanan (GM) daripada spesies *Amorphophallus* menggunakan asai kolorimetrik asid 3,5-dinitrosalisilik. Aksesi Hulu Langat, Selangor, SHL24 didapati mengandungi kandungan GM yang paling tinggi pada 8.29%. Kandungan GM yang paling rendah adalah daripada PT37 dengan hanya 2.55%. Keputusan juga menunjukkan bahawa terdapat kesan interaksi di antara aksesi dan saiz umbisi dengan kandungan GM. Kesimpulannya, berdasarkan kedua-dua analisis morfologi dan molekul mencadangkan bahawa aksesi KKB dikenal pasti sebagai *A. paeoniifolius* manakala aksesi lain adalah *A. prainii*,terutamanya berdasarkan ciri bunga yang berbeza. Kajian ini memberikan pandangan yang berharga dan pemahaman yang mendalam tentang habitat, pertumbuhan, ciri-ciri fenotip dan genotip serta kandungan GM di dalam aksesi *Amorphophallus* spp. di Semenanjung Malaysia. Penemuan ini boleh digunakan sebagai asas untuk pembiak-bakaan dan program pemuliharaan tumbuhan ini di Malaysia.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

GM	Glucomannan
NS	North-south
EW	East-west
DAP	Days after planting
M	Molar
WHO	World Health Organization
NAA	α-naphthaleneacetic acid
MS	Murashige and Skoog medium
DNA	Deoxyribonucleic acids
PCR	Polymerase chain reaction
RAPD	Random amplified polymorphic DNA
AFLP	Amplified fragment length polymorphisms
ISSR	Inter simple sequence repeat
SSR	Simple sequence repeat
EST	Expressed sequence tag
PCA	Principal component analysis
PCoA	Principal coordinate analysis
PC	Principal component
UPGMA	Unweighted pair group method based on arithmetic averages
UPM	Universiti Putra Malaysia
CRD	Completely Randomized Design
ANOVA	Analysis of variance
LSD	Least significant different
PUK	Ulu Kenas, Perak

PT	Taiping, Perak
KKB	Kota Bahru, Kelantan
KKP	Kubur Panjang, Kedah
PBJ	Bukit Jambul, Penang
SHL	Hulu Langat, Selangor
$MgCl_2$	Magnesium chloride
dNTP	2'-deoxynucleoside 5'-triphosphate
TBE	Tris-borate-EDTA
I	Shanon's information index
H_o	Observed heterozygosity
H_e	Expected heterozygosity
PIC	Polymorphic information content
CF	Crude flour
NTSYS-pc	Numerical taxonomy multivariate analysis system
PAST	Paleontological statistics
EDTA	Ethylene diamine tetraacetic acid
g	Gram
µl	Microliter
µM	Micromolar
min	Minute
s	Second
°C	Degree celsius
w/v	Weight per volume
ng	Nanogram
kg	Kilogram

mM	Milimolar
ml	Milliliter
h	Hour
nm	Nanometer
syn	Synonym
bp	Base pair
cm	Centimeters
mg	Milligram
T_a	Annealing temperatures
F_{is}	Fixation index
F_{ST}	Coefficient of gene differentiation
N_m	Estimate of gene flow
n_a	Average number of alleles
n_e	Number of effective alleles
6-BAP	6-benzylaminopurine
2,4-D	2, 4-dicholophenoxy acetic acid
3,5-DNS	3,5-dinitrosalicyclic

CHAPTER 1

GENERAL INTRODUCTION

Amorphophallus spp. is a perennial plant belonging to the Araceae family, consisting of approximately 200 species. They have been found distributed across the tropical and subtropical regions ranging from West Africa to Polynesia (Hetterscheid and Ittenbach, 1996; Sedayu et al., 2010). The *Amorphophallus* spp. has an underground storage organ known as the corm. The corm produced enormous solitary leaves consisting of trunk-like petiole, often with mottled and dissected umbrella shaped leaf lamina (Hetterscheid and Ittenbach, 1996; Hejnowicz and Barthlott, 2005). In Peninsular Malaysia, *A. paeoniifolius* and A. *prainii* are reported to be in abundance, followed by *A. muelleri* and *A. elegans* (Mansor et al., 2012; Shahbudin, 2012).

Plants of the *Amorphophallus* have been long used as folk medicine, food source, animal fodder, and ornamental and religious ceremony (Long, 1998; Bown, 2000; Follet and Douglas, 2002). *A. praiini*, *A.aphyllus*, *A. commutatus*, *A. paeoniifolius* and *A. sylvaticus*, for instance, are used to treat snake bite, as well as for arrow poison and an analgesic (Quattrocchi, 2012). *A. paeoniifolius* is grown commercially in India. The corm, young shoot and flower are eaten, either as boiled or baked vegetables, and used in Ayurvedic medicine. The ash of the corm is prescribed to treat piles, haemorrhoids, gout, asthma, bronchitis and stomach indigestion while the petiole juice is used to cure diarrhoea (Khare, 2004). The corm extract possesses antitumour, antioxidant and cytotoxic properties and has synergistic depressant effect when used with diazepam (Madhurima et al., 2012). In China, *A. konjac* is used traditionally for detoxification, as well as an antitumor and to treat asthma; cough and skin disorder (Bown, 2000; Niwa et al., 2000).

However, recent interest is towards the flour content extracted from the corm tissues of some of *Amorphophallus* spp., such as *A. konjac*, *A. albus* and *A. muelleri*. The corm contains high glucomannan (GM) content which is a water-soluble polysaccharide, fermentable dietary fibre or hemicelluloses (Keithley and Swanson, 2005). Traditionally, the flour has been used to prepare noodles, tofu and snacks (Long, 1998; Chua et al., 2010). As the flour has no calorific content, therefore, the purified form is potential to be used in health food products such as supplement, diet cookies, noodles and rice. Many studies have reported that GM is an effective treatment of obesity, diabetes, hypertension, cardiovascular disease and high cholesterol problems (Liu et al., 1998; Kraemer et al., 2007). Consumption of GM based food products increased high-density lipoprotein cholesterol and total cholesterol ratio (Keithley and Swanson, 2005; Kraemer et al., 2007).

Development of GM based-food products is significant, especially for developed and developing countries, including Malaysia, whereby the above diseases are listed as the primary cause of major health problem and death. According to the World Health Organization (WHO), approximately 3.4 million adults die each year due to diabetes, ischaemic heart disease, cancer and obesity (WHO, 2014). The diseases are caused by the consumption of high calorific content of foods. Thus, consuming GM food-based products, with little calories, could help to overcome the problem.

Purified konjac flour (>~90.0% GM) has the potential to be developed as a pharmaceutical excipient to control drug delivery system due to its unique gelling and rheological properties (Zhang et al., 2014). As a natural biopolymer, GM is also used to make the capsule for medicine, whereby it can replace gelatin as edible coating for postharvest produce and to restructure foods (Parry, 2010). As a gelatin replacer, GM has gained much attention in the Muslim world because gelatin is derived from the animal source, considered as non-halal. GM seems to be the most promising source of a plant-based medicinal capsule for the halal industry. Therefore, due to its benefits and market demand, Japan, China and Indonesia regard these plants as a high-value crop and are commercially planting *A. konjac*, *A. albus* and *A. muelleri*. China is the world largest producer and exporter of GM flour with 60% of total global production followed by Japan at 28% (Liu, 2004).

Despite the economic importance and benefits of *Amorphophallus* plants, there is still lack of detail information available for the Malaysian *Amorphophallus* spp. in Peninsular Malaysia. To date, only a few studies have been conducted on *Amorphophallus* spp. cultivation and glucomannan content, much less on genetic diversity of this plant in Malaysia. Therefore, in order to promote breeding program, such fundamental knowledge of morphological and genetic variation is crucial as it underlies the crop improvement and selection. Moreover, *Amorphophallus* plant is easy to grow under plant shade, and the climate in Malaysia provides suitable conditions for the plant to grow. It is apparent that *Amorphophallus* spp. has the potential to be cultivated as a new crop.

Therefore, the objectives of this study were to (i) collect and establish the accessions of *Amorphophallus* spp. from selected locations in Peninsular Malaysia, (ii) determine phenotypic and genotypic characteristics, as well as the GM content of the accessions.

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