
**UNIVERSITI PUTRA MALAYSIA**

# *PROPAGATION, GENETIC VARIATION AND EVALUATION OF KEMUNTING (Rhodomyrtus tomentosa (AITON) HASSK) POPULATIONS IN MALAYSIA FOR LANDSCAPE USE*

***TAN SIAO HUE***

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# PROPAGATION, GENETIC VARIATION AND EVALUATION OF KEMUNTING (*Rhodomyrtus tomentosa* (AITON) HASSK) POPULATIONS IN MALAYSIA FOR LANDSCAPE USE

By

**TAN SIAO HUE**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy**
**July 2015**

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

**PROPAGATION, GENETIC VARIATION AND EVALUATION OF KEMUNTING (*Rhodomyrtus tomentosa* (AITON) HASSK) POPULATIONS IN MALAYSIA FOR LANDSCAPE USE**

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**Chairman: Associate Professor Thohirah Lee Abdullah, PhD**
**Faculty: Agriculture**

Kemunting (*Rhodomyrtus tomentosa*) is a multipurpose edible ornamental shrub. Its natural populations are fast diminishing and conservation of the species is urgently needed. No previous study has been conducted in terms of its propagation, genetic variation and evaluation of population for landscape use. A series of experiment were conducted to (i) determine the rooting performance of *R. tomentosa* populations as affected by different IBA concentrations and types of cutting (ii) document the seed traits and the effects of temperature on germination performance of *R. tomentosa* from different sources (iii) access the genetic variability among *R. tomentosa* populations in Malaysia and (iv) evaluate *R. tomentosa* populations suitable for ornamental landscape.

Softwood, semi hardwood and hardwood cuttings from nine natural populations (C01, C02, D01, D02, K01, M01, N01, T01 and T02) were arranged in a three factorial (locations, types of cutting, IBA concentrations) RCBD design with four replications. Subsequently, only softwood cuttings from four natural populations (J01, J02, J03 and Q01), arranged in RCBD with 4 replications were used for rooting assessment. The data obtained were analyzed together with softwood data from the previous nine populations. The cuttings were treated with 0, 800, 1600, 2400 and 3200 mg/L IBA. Histological examination was done to follow the root initiation process. Softwood cuttings gave the best rooting performance regardless of IBA treatments and it should be used in the propagation of *R. tomentosa*. Cuttings from different populations showed variation in rooting performance, with cuttings from J03 rooted the best (87.3%). Root primordium started to develop from phloem region three weeks after the cuttings were planted.

Only seeds from five populations (C02, K03, M01, SA01 and T01) were obtained during the collection and subjected to light, scanning electron microscopy and water imbibition test. Seeds from other populations (C01, D01, D02, J01, J02, J03, K01, K02, N01, Q01, SA02, S01, T02) were obtained from the plants established at Field 2, UPM. Seed morphology from each population was measured. The seeds were germinated at constant (10°C, 15°C, 20°C, 25°C, 30°C, 35°C, 40°C) and fluctuating temperatures (33°C/ 25°C), arranged in CRD with 4 replications. Seeds from all the

populations varied in their seed traits and germination performance. Seed structure affect water uptake but not germination. Seeds from all locations except C01, K03 and T01 germinated to a higher percentage in fluctuating temperatures. *R. tomentosa* seeds germinated slowly and erratically both under constant and fluctuating temperatures.

ISSR was utilized to ascertain the genetic variation of 15 *R. tomentosa* populations. All 18 collections were included in morphological study. Twenty one morphological traits were measured. The 11 primers generated 95.29% polymorphism. Population N01 was the most variable (PPB = 42.35%). The significant population pairwise PhiPT suggested that the populations were isolated. The groupings of populations were according to their geographical origins based on molecular data but not based on morphological characters. ITS analysis showed that it is possible for M01 and N01 to be classified as new variety.

The perception of nursery owners, final year students of Bachelor of Horticultural Science (BSHort) and Bachelor of Landscape Architecture (BLA) towards *R. tomentosa* were assessed using questionnaire with pictorial simulations. *R. tomentosa* is better known among students (BSHort, 34.8%; BLA, 15.7%) through university (BSHort, 52.9%; BLA, 44.4%). Of the 7.9% of nursery owners, 50% of them know *R. tomentosa* through friends. Suitablity of *R. tomentosa* as landscape plant was perceived by 96.6% of the respondents. *R. tomentosa* is most suitable to be utilized as ornamental hedges (71.9%). The most attractive landscaping features were rounded plant form (56.2%); elliptic leaf shape (60.7%); flower with rounded petals and dense petal arrangement (73%) and rounded fruit with reflexed calyx lobes (42.7%), which was possessed by sample K03 from Langkawi, Kedah except the elliptic leaf shape.

In conclusion, *R. tomentosa* should be propagated using softwood cuttings without IBA or with 1600 mg/L IBA. All the *R. tomentosa* populations should be conserved *in-situ* and *ex-situ* since there is a huge potential for it to be introduced into the Malaysian landscape industry.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PEMBIAKAN, VARIASI GENETIK DAN PENILAIAN POPULASI KEMUNTING (*Rhodomyrtus tomentosa* (AITON) HASSK) DI MALAYSIA UNTUK KEGUNAAN LANSKAP**

Oleh

**TAN SIAO HUE**

**Julai 2015**

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Kemunting (*Rhodomyrtus tomentosa*) adalah tumbuhan hiasan renek pelbagai guna. Taburannya di Malaysia semakin berkurangan dan pemuliharaan species ini adalah sangat diperlukan. Tiada lagi kajian yang dijalankan terhadap pembiakan, variasi genetik dan penilaian kegunaan lanskap ke atas tumbuhan ini. Beberapa eksperimen telah dijalankan untuk menilai (i) potensi pengakaran populasi *R. tomentosa* berdasarkan kepekatan IBA dan jenis keratan batang yang berbeza (ii) ciri-ciri biji benih dan percambahan biji benih dalam suhu yang berbeza untuk sumber biji benih yang berlainan (iii) variasi genetik di kalangan populasi di Malaysia dan (iv) populasi *R. tomentosa* yang sesuai untuk lanskap.

Keratan batang lembut, separa-lembut dan keras dari sembilan lokasi semulajadi (C01, C02, D01, D02, K01, M01, N01, T01 and T02) telah disusun dalam rekabentuk rawak berblok lengkap (RCBD) tiga faktor (lokasi, jenis keratan dan kepekatan IBA) dalam empat replikasi. Seterusnya, hanya keratan batang lembut dari empat lokasi semulajadi (J01, J02, J03 and Q01), disusun dalam rekabentuk rawak berblok lengkap (RCBD) dalam empat replikasi digunakan untuk ujian pengakaran. Data yang diperolehi telah dianalisa bersama dengan data untuk keratan batang lembut dari sembilan lokasi sebelumnya. Dalam kedua-dua eksperimen, keratan telah dirawat dengan 0, 800, 1600, 2400 dan 3200 mg/L IBA. Kajian histologi telah dijalankan untuk menkaji proses pengakaran. Keratan batang lembut mencatatkan prestasi pengakaran yang terbaik tanpa mengambil kira rawatan IBA dan ia adalah sesuai untuk digunakan dalam pambiakan *R. tomentosa*. Keratan dari tempat berlainan adalah berbeza dalam prestasi pengakaran. Keratan dari J03 mencatatkan prestasi pengakaran yang terbaik (87.3%). Primordia akar mula terbentuk dari floem tiga minggu selepas keratan ditanam.

Hanya biji benih daripada lima lokasi boleh diperoleh (C02, K03, M01, SA01 and T01) semasa pengumpulan sample dan biji tersebut diperhatikan dengan microskop

cahaya dan mikroskop imbasan electron. Ujian penyerapan air juga dijalankan terhadap biji tersebut. Biji benih daripada lokasi lain (C01, D01, D02, J01, J02, J03, K01, K02, N01, Q01, SA02, S01, T02) telah diperoleh dari Ladang 2, UPM. Morfologi biji benih dari setiap lokasi telah diukur. Biji-biji tersebut telah dicambahkan dalam julat suhu tetap (10°C, 15°C, 20°C, 25°C, 30°C, 35°C, 40°C) dan tidak tetap (33°C/ 25°C), disusun dalam rekabentuk lengkap rawak (CRD) dalam empat replikasi. Biji benih dari semua lokasi menunjukkan perbezaan dalam ciri-ciri dan juga prestasi percambahan. Struktur biji mempengaruhi penyerapan air tetapi tidak mempengaruhi percanbahan biji. Biji benih dari semua lokasi bercambah dengan lebih baik dalam suhu tidak tetap berbanding dengan suhu tetap kecuali biji dari lokasi C01, K03 dan T01. Biji benih *R. tomentosa* menunjukkan trend percambahan yang lambat dan tidak tetap pada suhu tetap dan tidak tetap.

ISSR telah digunakan untuk mengkaji variasi genetik untuk 15 populasi R. tomentosa. Kesemua 18 koleksi telah digunakan dalan kajian morfologi. Dua puluh satu ciri morfologi telah diukur. Sebelas primer yang digunakan telah menghasilkan 95.29% jalur polimorfik. Populasi N01 mempunyai variasi yang paling tinggi (PPB = 42.35%). Nilai PhiPT yang nyata antara populasi menunjukkan bahawa populasi adalah terpisah.

Populasi adalah dikategorikan berdasarkan asal-usul geografi dalam kajian molekul tetapi tidak dalam kajian morfologi. Variasi ISSR menunjukkan pembezaan genetik yang sebenarnya tetapi variasi morfologi tidak. Analisa ITS telah menunjukkan bahawa populasi M01 dan N01 mungkin boleh diklasifikasikan sebagai varieti baru.

Persepsi pemilik nurseri, pelajar tahun akhir Bacelor Sains Hortikultur (BSHort) dan Bacelor Seni Bina Lanskap (BLA) terhadap *R. tomentosa* telah dikaji melalui soal selidik dengan simulasi bergambar. *R. tomentosa* adalah lebih popular di kalangan pelajar (BSHort, 34.8%; BLA 15.7%) melalui university (BSHort, 52.9%; BLA, 44.4%). Daripada 7.9% pemilik nurseri, 50% mengenali sepsis ini melalui rakan mereka. Kebanyakan responden (96.6%) menganggap *R. tomentosa* adalah sesuai digunakan dalam lanskap. *R. tomentosa* adalah paling sesuai digunakan sebagai tumbuhan pepagar hiasan (71.9%). Ciri-ciri lanskap yang paling menarik ialah bentuk tumbuhan bulat (56.2%); bentuk daun eliptik (60.7%); bunga dengan kelopak yang bulat dan susunan kelopak yang padat (73%) dan bentuk buah bulat dengan lobus kaliks yang terbuka (42.7%). Sampel K03 daripada Langkawi, Kedah mempunyai kesemua ciri-ciri yang tersebut kecuali bentuk daun eliptik.

Kesimpulannya, *R. tomentosa* paling sesuai dibiakkan dengan keratan batang lembut tanpa kegunaan IBA atau dengan 1600 mg/L IBA. Kesemua populasi *R. tomentosa* patut dipulihara secara in-situ dan ex-situ memandangkan ia mempunyai potensi yang besar untuk diperkenalkan kepada industri lanskap Malaysia.

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I certify that a Thesis Examination Committee has met on 14 July 2015 to conduct the final examination of Tan Siao Hue on her thesis entitled "Propagation, Genetic Variation and Evaluation of Kemunting (*Rhodomyrtus tomentosa* (Aiton) Hassk) Populations in Malaysia for Landscape Use" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philopsophy.

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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 Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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| | |
|---|---|
| ANOVA | Analysis of Variance |
| AFLP | Amplified fragment length polymorphism |
| bp | Basepair |
| Ca | Calcium |
| cpDNA | Chloroplast DNA |
| CRD | Completely randomized design |
| DNA | Deoxyribonucleic acid |
| dNTPs | Deoxyribonucleotide triphosphates |
| Fe | Ferum |
| FAA | Formalin-acetic acid-alcohol |
| GeneAlEx | Genetic Analysis in Excel |
| IAA | Indole-3-acetic acid |
| IBA | Indole-3-butyric acid |
| ISSR | Inter simple sequence repeat |
| ITS | Internal transcribed spacer |
| K | Potassium |
| K-IBA | Potassium salt of indole-3-butyric acid |
| LM | Light microscope |
| LSD | Least significant differences |
| MEGA | Molecular Evolutionary Genetics Analysis |
| Mg | Magnesium |
| mtDNA | Mitochondrion DNA |
| MVSP | Multivariate Statistical Package |

| | |
|---|---|
| Na | Sodium |
| NAA | α-naphthalene acetic acid |
| nrDNA | Nuclear ribosomal DNA |
| NTSYS | Numerical Taxonomy and multivariate analysis system |
| P | Phosphorous |
| POPGENE | Population genetic analysis software |
| PCR | Polymerase Chain Reaction |
| RAPD | Random Amplified Polymorphic DNA |
| RCBD | Randomized complete block design |
| RFLP | Restriction fragment length polymorphism |
| SAS | Statistical Analysis System |
| SEM | Scanning electron microscope |
| SNP | Single nucleotide polymorphism |
| SSR | Simple sequence repeat |
| TBA | Tertiary butyl alcohol |
| UPM | Universiti Putra Malaysia |
| Zn | Zinc |

# CHAPTER 1

## INTRODUCTION

Malaysia, being one of the world's twelve mega diversity hotspots, harbors a vast variety of flora. These flora possess vast potentials that benefit the country's economy, either in the pharmaceutical sector, food and beverages, tourism, floriculture and landscape industries, just to name a few. However, many of them are still unknown to us. In view of the above, intense explorations and researches need to be conducted to place them into full utilization. The clearing of their natural habitats by developments has threatened their survival potential (Lai, 2013). Thus, information on how to propagate the species for economic and conservation purposes is needed. These native floras harbour immense genetic variations that can be used to improve the present day commercial cultivars through plant breeding.

One of the native plant species found growing abundantly in Malaysia is *Rhodomyrtus tomentosa*. It is commonly known by its vernacular name Kemunting or Kalimunting by the locals, especially the older Malay generations (Latiff, 1992). Due to the outer appearance of its ripened fruits which resembled that of blueberry, it is sometimes known as the 'Malaysian Blueberry'. The older Malay generation considered it as their childhood plant and consumed their berries which are dark purple in colour when ripened and sweet to taste (Latiff, 1992). Besides fresh consumption, the ripened berries are processed into jams by some in small scale (Latiff, 1992). Most of the young generations do not know about this plant while those who know from the elderly. This would mean that *R. tomentosa* has become less known among us and consequently less exploited and utilized and neglected. Although *R. tomentosa* is wide spread in Malaysia (Ridley, 1967), its population has started to decrease in recent time due to destruction of its habitat to pave way for housing projects and agricultural activities. Thus, proper documentation and conservation program should be initiated before it become extinct in Malaysia.

*R. tomentosa* is found growing in South East Asia, Sri Lanka, Southern China and Taiwan (Chen, 1984; Latiff, 1992; U.S. Dept. Agr., Agr. Res. Serv., 2008), and it has been introduced into the United States (PIER, 2003) and Japan (Kato, 2007). In the United States, it has been widely used as a landscaping plant (Langeland and Craddock, 1998) attributed by its showy pink flowers (Zhao *et al*., 2006), together with its dark purple ripened berries that are bird attracting. In countries such as China, Thailand and Vietnam, the benefits and economical values of this plant is well documented and numerous studies have been conducted (Zhao *et al*., 2006; Limsuwan *et al*., 2009; Wei *et al*., 2009) mainly on the phytochemical compounds, antibacterial activity, medicinal values, and ecology of *R. tomentosa*. The chemical properties of its ripened fruit have also been studied in depth (Lai *et al*., 2015), and has been processed into pies, jams and nutritious drink (Liu and Deng, 1997; Zhang *et al*., 2008). In addition, researchers from China and Japan have also processed the leaves extracts of *R. tomentosa* into skin cosmestics, health food and drink, besides functions as a Chinese traditional medicine in the form of oral liquid and powder. These products

have been patented (Miyake and Nojima, 2006; Wei, 2006a; 2006b). However, to date, very little or even none of these studies has been initiated in Malaysia and it is only being used as traditional folk medicine (Ong and Nordiana, 1999).

*R. tomentosa* is still underutilized and underexploited locally despite its multiple uses. Since it is growing natively, *R. tomentosa* can adapt to the local growing conditions and this would be an added advantage. In view of this, more study on *R. tomentosa* should be initiated to document and subsequently to promote this valuable landscape plant to the local communities. Introduction of this native edible ornamental into the Malaysian landscape industry may be one of the initial steps. Most of the local landscape industry has been and is still utilizing exotic plants in landscaping to a large extent despite the enormous varieties of local floras that have potential to be used in landscaping. Exotic plants may become invasive and displaces native plant communities. Nevertheless, the trend now sees that native floras have started to gain popularity in the ornamental nursery industries.

Sufficient planting materials should be obtained in order for it to be reintroduced and promoted for use in the landscape industry. *R. tomentosa* can be propagated both by seeds and by stem cuttings (Campbell, 1977; Latiff, 1992). Zhang and Deng (2008) have shown that the seed germination and emergence rate was 25.5% and 24% at laboratory and nursery condition after 60 days at 4°C respectively. Study done by Yang (2005) has shown that only 21.4% of the softwood cuttings rooted. These were probably the only report concerning the propagation aspect of *R. tomentosa*. From the results obtained, it has shown that planting materials for *R. tomentosa* is difficult to procure, except from the wild. Propagation of *R. tomentosa* through both seeds and stem cuttings should be studied to select the best method to produce planting materials. Propagation by seeds and by cuttings possessed its advantages and disadvantages. Plants propagated by cuttings will have a shorter juvenile period as compared to plants propagated through seeds (Kesari *et al*., 2009). On the other hand, plants propagated through seeds will not fully resemble the mother plant and the superior characteristics of mother plant may fail to get transmitted to the next generation (Henrique *et al*., 2006).

It is well known that the environmental factors where the mother plants thrive have great influences not only on the rooting ability of stem cuttings (Radosta *et al*., 1994), but also on the seed germination performance (Baskin and Baskin, 2014). It is such that mother plants grown in different locations will experience different environmental variations and this will subsequently lead to the differences in the rooting performance and seed germination performance of stem cuttings and seeds obtained from the respective locations, referred to as provenance or geographical effects (Lacey, 1998; Puri and Swamy, 1999). The environmental stimuli that lead to these differences will be transmitted to the next generations through cuttings and seeds (Roach and Wulff, 1987; Haissig and Riemenschneider, 1988).

Since *R. tomentosa* propagates naturally by seeds in the wild (Crurhes and Hankamer, 2011), genetic variation might occur within as well as among its populations. Little is known regarding these variations in the wild populations of *R. tomentosa* in Malaysia and it has not been studied before. Plants with higher ornamental value and good landscape characters might occur in the wild. Evaluation of *R. tomentosa* with attractive plant form and showy blooms that is suitable for landscaping need to be carried out and explored in order to access its adaptability for landscape planting.

Several studies has been undertaken where *R. tomentosa* was collected from their wild populations in Malaysia, and subsequently its propagation aspects, genetic variations and assessment of the landscape characteristics of each of the populations were evaluated. This study might be the first of such in Malaysia that will contribute to the knowledge regarding this plant, and also the first in the region that will contribute to the general knowledge regarding the propagation of this plant using seeds and cuttings. Although numerous studies on phytochemical compound, antibacterial activity and medicinal values was and is still extensively carried out, however over harvesting and over exploitation of *R. tomentosa* from the wild might lead to the suffering of its population size. Thus besides testing on its numerous economical values, the most efficient method to propagate this plant should not be overlooked.

The objectives of this study were to:

(i) Assess the rooting performance of *R. tomentosa* populations as affected by different IBA concentrations and types of cutting.

(ii) Study the seed morphometric traits and the effect of temperatures on germination of different *R. tomentosa* seed sources.

(iii) Study the genetic variability of *R. tomentosa* populations.

(iv) Evaluate the populations of *R. tomentosa* suitable for ornamental landscape.

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