VARIABILITY OF WORMWOOD CAPILLARY (*ARTEMISIA CAPILLARIS* THUNB.) IN PENINSULAR MALAYSIA

MOHAMMAD SHAFIE BIN SHAFIE

MASTER OF SCIENCE
UNIVERSITI MALAYSIA TERENGGANU
MALAYSIA

2011

VARIABILITY OF WORMWOOD CAPILLARY (*ARTEMISIA CAPILLARIS* THUNB.) IN PENINSULAR MALAYSIA

MOHAMMAD SHAFIE BIN SHAFIE

Thesis Submitted in Fulfillment of the Requirements for the Degree of Master of Science in the Faculty of Agrotechnolgy and Food Science Universiti Malaysia Terengganu

March 2011

Dedication To My Beloved Family... :p

Friends & Those Who Had Been

Involved In Making This

Thesis The Succes

Mohammad Shafie Bin Shafie...

2011

Abstract of thesis presented to the Senate of Universiti Malaysia Terengganu in fulfillment of the requirement for the degree of Master of Science.

VARIABILITY OF WORMWOOD CAPILLARY (*ARTEMISIA CAPILLARIS* THUNB.) IN PENINSULAR MALAYSIA

MOHAMMAD SHAFIE BIN SHAFIE

March 2011

Chairperson : Professor Sayed Mohd Zain S. Hasan, Ph.D.

Member : Associate Professor Hj. Abdullah Md. Zain, M.Sc.

Faculty : Agrotechnology and Food Science

Artemisia capillaris Thunb. is a herb from the family Asteraceae which had a wide range of morphological and genetic variability. The different among individuals within and between populations of the species is complex and difficult to identify. In Malaysia, the data and information on the biology of *A. capillaris* particularly concerning with the variability is still lacking. Hence, an assessment on the morphological and genetic variations of *A. capillaris* from Malaysia has been conducted in order to identify the most variable characters and to assess the genetic relationship among the populations of the species.

Forty populations of *A. capillaris* collected from differences locations and habitats in eight states of Peninsular Malaysia were used in this study. The live plants of the populations were maintained under the green house condition at Universiti Malaysia Terengganu (UMT). The polymorphic data of 35 morphological characters, 218 RAPDs

and 191 ISSRs were recorded and subjected to the computer for similarity index (SI) analysis, cluster analysis (CA) and principal component analysis (PCA).

Analysis of similarity index based on the morphological traits revealed that the similarity among forty populations of *A. capillaris* in Malaysia ranging from 0.06 to 0.63. The Euclidean distance coefficient value of dendrogram generated from the morphological marker data ranged from 4.62 to 9.86. Both CA and PCA are capable to identify and group *A. capillaris* in Malaysia into seven and five groups respectively. The results of PCA indicated that the most variable characters (with eigenvalue $\geq$ 1.0 and having correlation coefficient value > 0.6) in *A. capillaris* were mostly associated with nine leave characters (LL, LW, LWLB, LWML, LWPL, LLI, LBI, LNLAI and LLWI) and were subsequently used in distinguishing groups of *A. capillaris* variants. The two dimension plot of the first two PCs showed a clear separation between the populations.

Out of five DNA extraction methods tested, the Sarkosy method was found to be the best method to extract DNA from *A. capillaris* producing a high quantity and quality of DNA, and thus was adopted for DNA extraction in this study. Out of fifty-seven oligonucleotide primers screened, ten primers (OPA 04, OPA 09, OPA 16, OPA 17, OPA 18, OPG 03, OPG 05, OPG 09, OPG 15 and 391) were selected for generating RAPD fragments marker. Meanwhile, out of twenty-five ISSR primers screened, also ten primers (807, 809, 825, 834, 841, 862, 866, 876, nIssr 1 and nIssr 3) were selected for amplifying ISSR fragments marker.

RAPD and ISSR primers successfully generated 218 and 191 amplification products respectively, which all (100%) were polymorphic. The size of the fragments for RAPD marker ranges from 150 bp to 3000 bp. While, the fragments size for ISSR marker ranges from 150 bp to 3000 bp. The similarity index (SI) values among forty populations of *A. capillaris* in Malaysia based on the RAPDs and ISSRs data ranged from 0.00 to 1.00 (mean ± 0.41) and 0.00 to 0.96 (mean ± 0.49) respectively. Meanwhile the similarity index values derived from the combined RAPDs and ISSRs data ranged from 0.00 to 0.94 (mean ± 0.45).

The dendrogram of CA based on molecular markers revealed that the populations of *A. capillaris* in Malaysia can generally be divided into three major groups. Member of each group however is exchangeable depending on the markers of either RAPDs, ISSRs or combined markers (RAPDs + ISSRs) are used. The dendrogram dissimilarity coefficient values generated from the RAPD and ISSR markers data ranged from 0.00 to 1.65 and from 0.00 to 1.36 respectively. Meanwhile the dendrogram dissimilarity coefficient generated from the combined data of the both markers (RAPD + ISSR) ranges from 0.00 to 1.71.

The two dimensions scatter plot of the first two PCs based on RAPD and ISSR markers are clearly showed the separation among the populations of *A. capillaris* in Malaysia. This dispersion was highly associated with the marker identified as OPG13.2500, OPG13.2000, OPG13.1500, OPG13.1750, OPG03.1750, OPA17.2500, OPA09.1116, OPA09.1500 and OPG13.1200 for RAPD marker and 866.400, 807.1350, 807.1750,

834.1750, 825.600, 876.700, 834.1116, nIssr1.2000, 866.350 and 876.850 for ISSR marker in first two PCs.

Both the morphological and molecular markers systems were found to be appropriated for use in genetic diversity study of *A. capillaris* in Malaysia. The grouping of *A. capillaris* populations as revealed by morphological traits were not significantly correlated ($p < 0.5$) with those grouping based on the molecular (RAPD and ISSR) markers. The result of this study proved that *A. capillaris* in Malaysia consists of several group of genotypes that need to be evaluated for the genetic improvement. The present of vast genetic resource of *A. capillaris* in Malaysia would provide a better prospect in promoting the use of *A. capillaris* genetic resource in Malaysia.

Abstrak tesis yang dikemukakan kepada Senat Universiti Malaysia Terengganu sebagai memenuhi keperluan untuk Ijazah Sarjana Sains.

KEPELBAGAIAN RU NYAMUK (*ARTEMISIA CAPILLARIS* THUNB.) DI SEMENANJUNG MALAYSIA

MOHAMMAD SHAFIE BIN SHAFIE

Mac 2011

Pengerusi : Profesor Sayed Mohd Zain S. Hasan, Ph.D.

Ahli : Profesor Madya Hj. Abdullah Md. Zain, M.Sc.

Fakulti : Agroteknologi dan Sains Makanan

Artemisia capillaris Thunb. merupakan sejenis herba yang berasal dari keluarga Asteraceae yang mana mempunyai ciri kepelbagaian morfologikal dan genetik yang sangat luas. Perbezaan antara individu-individu di dalam dan di antara populasi dalam spesies ini sangat rumit dan sukar untuk dikenalpasti. Data dan maklumat biologi bagi *A. capillaris* terutamanya berkenaan kepelbagaian masih lagi berkurangan di Malaysia. Maka, kajian terhadap penilaian tahap variasi morfologi dan genetik bagi spesies ini yang berasal dari Malaysia telah dijalankan dalam usaha mengenalpasti ciri yang paling bervariasi dan juga untuk mencari hubungan genetik di antara populasi.

Empat puluh populasi *A. capillaris* yang dipungut daripada lokasi dan habitat yang berbeza di lapan negeri di Semenanjung Malaysia telah digunakan dalam kajian ini. Pokok bagi setiap populasi ini telah ditanam dan diselengara di rumah hijau, Universiti Malaysia Terengganu (UMT). Data polimorfik daripada 35 sifat morfologi, 218 RAPDs

dan 191 ISSRs telah direkodkan dan digunakan untuk komputer analisis bagi indeks kessamaan (SI), analisis kelompok (CA) dan analisis komponen prinsipal (PCA).

Analisis indeks kesamaan berdasarkan sifat-sifat mofologi menunjukkan bahawa persamaan antara empat puluh populasi *A. capillaris* di Malaysia berjulat antara 0.06 hingga 0.63. Nilai bagi koefisien dendrogram ketaksamaan Euclidean berdasarkan data penanda morfologi berjulat antara 4.62 hingga 9.86. Kedua-dua CA dan PCA mampu mengenalpasti dan mengkelaskan *A. capillaris* di Malaysia kedalam tujuh dan lima kumpulan. Keputusan PCA menunjukkan sifat kepelbagaian paling tinggi (dengan nilai eigen $\geq$ 1.0 dan mempunyai nilai koefisien korelasi > 0.6) di dalam *A. capillaris* kebanyakannya dikaitkan dengan sembilan sifat daun (LL, LW, LWLB, LWML, LWPL, LLI, LBI, LNLAI dan LLWI) dan kemudiannya digunakan dalam membezakan variasi kumpulan-kumpulan *A. capillaris*. Plot dua dimensi bagi dua PCs yang pertama menunjukkan pemisahan yang jelas antara populasi.

Daripada lima kaedah pengekstrakan DNA yang dikaji, kaedah Sarkosyl telah didapati menjadi kaedah terbaik untuk ekstrak DNA dari *A. capillaris* dengan menghasilkan kuantiti dan kualiti DNA yang tinggi, dan ianya telah diterima untuk kaedah ekstrak DNA di dalam kajian ini. Daripada lima puluh tujuh pencetus oligonukleotida yang telah disaring, sepuluh pencetus (OPA 04, OPA 09, OPA 16, OPA 17, OPA 18, OPG 03, OPG 05, OPG 09, OPG 15 dan 391) telah dipilih untuk penjanaan jujukan-jujukan penanda RAPD. Sementara itu, daripada dua puluh lima pencetus ISSR yang telah disaring, juga

sebanyak sepuluh pencetus (807, 809, 825, 834, 841, 862, 866, 876, nIssr 1 dan nIssr 3) dipilih untuk pengandaan jujukan-jujukan bagi penanda ISSR.

Pencetus RAPD dan ISSR telah berjaya menghasilkan 218 dan 191 produk-produk amplifikasi (pengandaan) masing-masing, di mana semua (100%) serpihan adalah polimorfik. Saiz jalur segmen untuk penanda RAPD berjulat antara 150 bp hingga 3000 bp. Manakala, saiz jalur segmen untuk penanda ISSR berjulat antara 150 bp hingga 3000 bp. Nilai indeks kesamaan (SI) antara empat puluh populasi *A. capillaris* dengan berdasarkan data RAPDs dan ISSRs adalah masing-masingnya diantara 0.00 hingga 1.00 (purata ± 0.41) dan 0.00 hingga 0.96 (purata ± 0.49). Sementara itu nilai-nilai indeks kesamaan berasarkan daripada pergabungan data RAPDs dan ISSRs adalah berjulat antara 0.00 hingga 0.94 (purata ± 0.45).

Dendrogram bagi CA berdasarkan penanda molekular menunjukkan bahawa populasi *A. capillaris* di Malaysia boleh dibahagikan kepada tiga kumpulan utama secara umumnya. Ahli setiap kumpulan bagaimanapun boleh ditukar bergantung pada penanda-penanda yang digunakan sama ada RAPDs, ISSRs atau penanda yang digabungkan (RAPDs + ISSRs). Nilai-nilai jarak ketaksamaan dendrogram yang dihasilkan daripada data penanda RAPD dan ISSR adalah masing-masingnya berjarak antara 0.00 hingga 1.65 dan antara 0.00 hingga 1.36. Sementara itu, jarak ketaksamaan dendrogram yang dihasilkan daripada data yang digabungkan kedua-dua penanda (RAPD + ISSR) adalah berjulat antara 0.00 hingga 1.71.

Plot taburan dua dimensi bagi dua komponen prinsipal (PC) pertama berdasarkan penanda RAPD dan ISSR jelas menunjukan pemisahan antara populasi-populasi *A. capillaris* di Malaysia. Taburan ini sangat berkait rapat dengan penanda yang dikenalpasti sebagai OPG13.2500, OPG13.2000, OPG13.1500, OPG13.1750, OPG03.1750, OPA17.2500, OPA09.1116, OPA09.1500 dan OPG13.1200 bagi penanda RAPD, dan 866.400, 807.1350, 807.1750, 834.1750, 825.600, 876.700, 834.1116, nIssr1.2000, 866.350 dan 876.850 bagi penanda ISSR melalui PC pertama dan kedua.

Kedua-dua sistem penanda molekular dan mofologi didapati sesuai untuk digunakan di dalam kajian kepelbagaian genetik *A. capillaris* di Malaysia. Pengkelasan populasi *A. capillaris* yang ditunjukan melalui sifat-sifat mofologi tidak berhubung kait ($p < 0.5$) dengan pengkelasan berdasarkan kepada penanda molekul (RAPD dan ISSR). Hasil kajian ini membuktikan *A. capillaris* di Malaysia mengandungi beberapa kumpulan genotip yang perlu untuk dinilai bagi tujuan pembaikan genetik. Kehadiran sumber genetik yang luas bagi *A. capillaris* di Malaysia memberikan prospek yang baik dalam manggalakkan penggunaan sumber genetik *A. capillaris* di Malaysia.