UNIVERSITI PUTRA MALAYSIA

GENETIC DIVERSITY OF *CURCULIGO LATIFOLIA* BASED ON MORPHOLOGICAL CHARACTERISTICS AND ISSR MARKERS

ALI RANJBARFARD I

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GENETIC DIVERSITY OF *CURCULIGO LATIFOLIA* BASED ON MORPHOLOGICAL CHARACTERISTICS AND ISSR MARKERS

By

ALI RANJBARFARD

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

May 2011

DEDICATIONS

Dedicated to my mother Robab Sattarirafieh, my father Elias Ranjbarfard and my beloved wife Norlida Binti Ali Ahmad for their endless and boundless love, support, encouragement, and most of all for their ever continuous do'a for my life.

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 : Professor Ghizan Saleh, PhD

Faculty : Agriculture

Studies were conducted to establish a germplasm collection of *Curculigo latifolia* from Peninsular Malaysia, to describe the morphological characteristics of the collection, to analyze diversity and relationships within and among the populations based on morphological characteristics and to analyze molecular diversity and relationships within and among the populations using ISSR markers. *Curculigo latifolia* is a perennial shrub from the family Hypoxidaceae with great potential for the pharmaceutical industry. A germplasm collection consisted of 225 samples representing 45 populations from 11 states of Peninsular Malaysia was established. *Curculigo latifolia* was found growing under various environmental conditions including primary forests, undisturbed secondary forests, plantations (especially rubber plantations) and along roadsides, thus proving its high adaptability to a wide variety of ecological niches in Peninsular Malaysia.

The genetic diversity of the germplasm collection was estimated using 28 qualitative and quantitative morphological characteristics and seven ISSR molecular markers. Results indicate that high morphological and molecular variations existed within and among the 45 populations of *C. latifolia* collected. Red leafstalk, hard leaf texture and hairy abaxial leaf surface were found to be identical in Populations 1, 2, 4, 13, 14, 15 and 40, while there were common qualitative characteristics among populations such as green leaf colour and glabrous adaxial leaf surface. Results of analysis of variance revealed that there were significant differences among the populations studied for all the quantitative characteristics measured.

The 45 populations collected were grouped into several diverse clusters based on their morphological characteristics using UPGMA clustering method. The three dendrograms constructed based on qualitative morphological characteristics, quantitative morphological characteristics and a combination of both sets of data, revealed that Populations 1, 2, 4, 13, 14, 15 and 40 were distinctly detached from the other populations studied.

ISSRs were found to be informative molecular markers for investigating genetic diversity among the *C. latifolia* populations as indicated by the high Nei's gene diversity coefficient and Shannon's information index (0.37 and 0.55, respectively). Results showed that AG and CA microsatellite repeats exhibited high polymorphism. The populations collected from Pahang were found to have the highest number of polymorphic bands among the populations studied. The relatively high coefficient of

genetic differentiation (G_{ST}) obtained (0.48) revealed that *C. latifolia* is a cross-pollinating species.

Populations 1, 2, 13, 14, 15 and 40 were found to be distinctly separated from all other populations studied. The results were similar to those revealed by the cluster and PCA analyses based on morphological characteristics. Although similarity coefficients among the populations studied obtained from morphological characteristics and molecular markers were found not to be correlated with each other, both morphological and molecular characterizations revealed that Populations 1, 2, 13, 14, 15 and 40 were distinctly different from the other populations. This indicates major differences in morphology and genome composition between these populations and the other populations studied.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagi memenuhi keperluan untuk ijazah Master Sains

KEPELBAGAIAN GENETIK *CURCULIGO LATIFOLIA* BERDASARKAN CIRI-CIRI MORFOLOGI DAN PENANDA ISSR

Oleh

ALI RANJBARFARD

Mei 2011

Pengerusi : Profesor Ghizan Saleh, PhD

Fakulti : Pertanian

Kajian telah dijalankan untuk membangunkan koleksi germplasma *Cucurligo latifolia* dari Semenanjung Malaysia, bagi menganalisis kepelbagaian dan hubungkait diantara dan dikalangan populasi berdasarkan ciri morfologi dan penanda ISSR. *Curculigo latifolia* ialah tumbuhan saka berasal dari famili Hypoxidaceae dengan potensi yang besar dalam industri farmaseutikal. Satu koleksi germplasma yang mengandungi 225 sampel mewakili 45 populasi dari 11 negeri di Semenanjung Malaysia telah dibangunkan. *Curculigo latifolia* boleh tumbuh di dalam pelbagai keadaan persekitaran termasuk hutan prima, hutan sekunder yang tidak terusik, ladang-ladang (terutama ladang getah) dan di sepanjang jalan. Ini membuktikan tumbuhan ini mempunyai kebolehan penyesuaian yang tinggi dalam pelbagai ekologi di Semenanjung Malaysia.

Kepelbagaian genetik di dalam koleksi germplasma telah dianggarkan menggunakan 28 ciri-ciri morfologi kualitatif dan kuantitatif serta tujuh penanda molekul ISSR.

Keputusan menunjukkan bahawa terdapat variasi morfologi dan molekul yang wujud di dalam dan di antara 45 populasi *C. latifolia* yang telah dikumpul. Ciri tangkai daun berwarna merah, tekstur daun yang kasar dan permukaan daun abaksial berbulu telah didapati sama dalam populasi 1, 2, 4, 13, 14, 15 dan 40, sementara terdapat ciri-ciri kualitatif yang biasa di antara populasi seperti warna daun yang hijau dan permukaan daun adaksial berkilat. Keputusan analisis variasi menunjukkan bahawa terdapat perbezaan yang signifikan di antara populasi yang dikaji untuk semua ciri kuantitatif yang diukur.

Empat puluh lima populasi yang dikumpul telah dibahagikan kepada beberapa kluster yang berlainan berdasarkan kepada ciri morfologi menggunakan kaedah pengklusteran UPGMA. Tiga dendrogram telah dibina berdasarkan ciri kualitatif morfologi, ciri kuantitatif morfologi dan kombinasi antara kedua-dua set data, menunjukkan bahawa Populasi 1, 2, 4, 13, 14, 15 dan 40 telah jauh dipisahkan daripada populasi lain yang dikaji.

ISSR telah dikenalpasti sebagai penanda molekul yang informatif untuk mengkaji kepelbagaian genetik di antara populasi *C. latifolia* sepertimana yang ditentukan oleh nilai pekali kepelbagaian gen Nei's dan index informasi Shannon's yang tinggi (masing-masing dengan nilai 0.37 and 0.55). Keputusan menunjukkan bahawa jujukan berulang mikrosatelit AG dan CA mempamerkan kadar polimorfisma yang tingi. Populasi-populasi yang dikumpul dari Pahang didapati mempunyai bilangan jalur polimorfik yang tinggi berbanding populasi lain yang dikaji. Nilai relatif yang

tinggi untuk pekali perbezaan genetik (G_{ST}) yang diperoleh iaitu 0.48 menunjukkan bahawa *C. latifolia* ialah spesis yang menjalankan pendebungaan kacuk.

Populasi 1, 2, 13, 14, 15 dan 40 telah didapati terpisah jauh daripada populasi lain yang dikaji. Keputusannya adalah selari dengan apa yang ditunjukan melalui analisis kluster dan PCA berdasarkan ciri-ciri morfologi. Walaupun nilai pekali kesamaan di antara populasi yang didapati daripada ciri morfologi dan penanda molekul menunjukkan bahawa populasi-populasi tersebut tidak mempunyai korelasi antara satu sama lain, kedua-dua analisis morfologi dan molekul menunjukkan bahawa Populasi 1, 2, 13, 14, 15 dan 40 adalah berbeza daripada populasi-populasi lain. Ini menunjukkan bahawa terdapat perbezaan yang besar dari segi morfologi dan komposisi genom di antara populasi tersebut dengan populasi-populasi lain yang dikaji.

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I certify that a Thesis Examination Committee has met on 13 May 2011 to conduct the final examination of Ali Ranjbarfard on his thesis entitled "Genetic Diversity of *Curculigo latifolia* based on Morphological Characteristics and ISSR Markers" in accordance with the Universities and Univesity Colleges Act 1971 and the Constitution of the Pertanian Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

ALI RANJBARFARD

Date: 13 May 2011

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