ESTABLISHMENT OF CORE COLLECTION OF SWEETPOTATO (*IPOMOEA BATATAS* L.) GERMPLASM USING RAPD MARKERS

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DOCTOR OF PHILOSOPHY
UNIVERSITI PUTRA MALAYSIA

2006

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By

SOW HAROUNA

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

May 2006

Dedicated to

My mother,
Who has inspired me to do this
My father,
Who has taught me how to struggle and persevere
My family Ramatoulaye BA, Mumthaz Seyd Mohideen and my lovely Mohamed Mahathir SOW
My brothers and sisters
Ibrahima, Ndeye Binta, Fatoumata, Mamadou Demba and Ousmane Boukar
My uncle,
Sadio Cisse
My aunty,
Aissa Cisse
Your constant encouragement, sacrifice and support
are highly appreciated.

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

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Chairman : Associate Professor Mohd Said Saad, PhD.

Faculty : Agriculture

Sweetpotato (*Ipomoea batatas* LAM.) is among the world's most important, versatile and underexploited food crop after rice, wheat, maize and cassava based on fresh-weight basis in developing countries. The presence of high level of genetic diversity in germplasm of sweetpotato is reflected in the big number of accessions being maintained at various genebanks around the world. Maintenance of these germplasm collections can be a problematic and costly activity. Germplasm rationalization can facilitate maintenance and utilization of the available germplasm. Identification of core collection is one of the essential steps in rationalization. Core collection normally established based on qualitative and quantitative characteristics of morphological data. Morphological markers, however, have some disadvantages because they are often subjected or converged according to development and environmental variation / changes. Establishment of a core collection based on genetic variation using molecular markers can be advantageous due to the absence of environmental and developmental stage variation. The main objectives of this study were to use RAPD markers to investigate the

genetic diversity of sweetpotato germplasm, and consequently to establish a core collection based on the RAPD polymorphism.

A total of 134 accessions of sweetpotato germplasm originated from six countries namely Malaysia (52), Indonesia (35, of which 17 were from Irian Jaya), Thailand (24), Philippines (6), AVRDC-Taiwan (7) and from CIP-Peru (10) were used in the study. They were subjected to RAPD using initially 54 primers and finally 5 primers selected mainly on their ability to produce polymorphic bands. Cluster analysis was done using Jaccard's similarity matrix based on the RAPD data. Consequently, core collection was established based on the groups revealed by the cluster analysis. The core collection was validated through three field trials with single, two and three replications.

The five primers yielded 30 – 46 amplification products and the detectable DNA bands ranged between 101 – 2939 bp in size. A total of 202 reproducible bands were amplified by the five primers and they were all polymorphics. The Jaccard's similarity index among the 134 sweetpotato accessions ranged from 0.02 to 1.00. Cluster analysis based on matrix of pairwise genetic similarity using UPGMA grouped the 134 accessions into thirteen distinct groups at 0.124 genetic similarities indicating the presence of wide genetic diversity within the germplasm. In general, many of the accessions were grouped on the basis of their geographical origin.

A core collection was established by random sampling (25 %, with a minimum of one) from each of the 13 groups. A total of 34 accessions from the entire collection were

incorporated into the core collection. Accessions from all the countries were represented in the core collection except those from the Philippines.

Newman-Keuls test (for difference in mean) and Levene test (for homogeneity of variance) showed no significant differences between means, except for petiole length in trial with single replicate, while Levene's test showed homogeneous variances between the entire and core collections for all characters.

Mean difference percentage (MD %) and variance difference percentage (VD %) were analyzed to compare the entire collection and core collections in the three field evaluations. The MD% average value for all three trials (8.26), (4.85) and (3.19) was less than 20% indicating the core had captured all the variability in the entire collection. The VD% average value for the single replication trial (21.3%) was higher than 20%, where it revealed that the core did not represent the entire collection, while for trial with more replications (2 and 3), the core collection was shown to be representative of the entire collection (VD% 11.36 and 7.16 respectively).

Coincidence rate (CR %) and variable rate (VR %) average values increased from single replication to two and three replications trials. The high average value of CR% and VR% retained in the core collection indicated that it was representative of the entire collection.

The validation generated from the twelve qualitative characters showed similar trend of frequency distribution except for immature leaf colour and petiole colour between the

entire and core collections. It was noted that these two classes in the two characters occurred at low frequency (2 accessions over 134) within the entire collection. The Shannon-weaver index calculated for the twelve qualitative characters showed similar average values between the entire and core collections, 0.361 and 0.364 respectively.

Results of characterization and evaluation on the sweetpotato core collection showed that the coefficient of variation values of quantitative characters generally indicated high variation. Cluster analysis used on the core collection revealed the presence of five groups. However, this grouping was found to be based on morphological and no parallelism between the geographical and genetic diversity.

Phenotypic correlation on characters measured showed positive phenotypic correlations between vine length and internode length and petiole length; between vine thickness and dry matter and starch, between yield and yield components (root number and mean root weight). But negative phenotypic correlation was observed between yields (its components) and dry matter and starch content.

The highest broad-sense heritability estimates were revealed by starch (89.9 %), dry matter content (87.0%), vine length (83.8%), vine thickness (75.9%) and root number (75.0%). Broad-sense heritability for other characters ranged from 72.0% for internode length character to 58.5% for mean root weight which was the lowest broad-sense heritability value.

Finally this study identified five accessions namely 11080, 10209, 11079, 10057, 10082, and 10236 from the core collection with potential for breeding due to their high yield, nutritional contents and favorable root flesh colour.

This study has proven that random amplified polymorphic DNA (RAPD) was suitable for assessing the genetic diversity in sweetpotato germplasm, consequently useful for core collection development by being an alternative to morphological markers.

Few primers (5) used was found to be enough to reveal the genetic diversity with RAPD-PCR which can reduce cost and time. The core collection established was found to be representative and captured all the genetic diversity present in the sweetpotato entire collection. This core collection can serve as a logical and efficient starting point for projects involving screening of the germplasm collection for sources of desirable alleles. It also provides a logical subset of germplasm to examine when it is not feasible to examine the entire collection. For example this sweepotato core collection can be evaluated for high yield, important nutritional element like dry matter, starch, sugar, carbohydrates and beta-carotenes.

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

PEMBENTUKAN HIMPUDAN TERAS GERMPLASMA KELEDEK (*IPOMOEA BATATAS* L.) MENGGUNAKAN PENANDA RAPD

Oleh

SOW HAROUNA

Mei 2006

Pengerusi: Profesor Madya Mohd Said Saad, PhD.

Fakulti : Pertanian

Keledek (*Ipomoea batatas* LAM.) merupakan tanaman makanan utama dunia selepas padi, gandum jagung dan ubikayu berasasban berat kering di Negara membangun. Tanaman ini mempunyai kepelbagaian genetik yang luas berdasarkan banyaknya bilangan aksesi germplasma yang disimpan di beberapa bankgen diseluruh dunia. Pemuliharaan himpunan germplasma yang besar merupakan aktiviti yang sukar dan mahal. Bagaimanapun rationalisasi germplasma boleh memudahkan pengurusan dan penggunaan germplasma. Pembentukan Himpunan Teras merupakan langkah utama dalam proses rasionalisasi germplasma. Himpunan teras biasanya dibentuk berdasarkan data pencirian sifat morfologi, kuantitatif dan kualitatif. Bagaimana pun sifat morfologi boleh dipengaruhi oleh persekitaran dan peringkat perkembangan pokok. Oleh itu, membentuk himpunan teras berdasarkan data dari penanda molekul adalah lebih baik kerana ia tidak dipengaruhi oleh faktor persekitaran dan peringkat perkembangan pokok. Matlamat utama kajian ini adalah untuk menggunakan penanda RAPD untuk

mengnalisis kepelbagaian genetik varsasi pada germplasma keledek dan seterusnya membentuk himpunan teras berdasarkan pada polimorfisme RAPD yang terhasil.

Sejumlah 134 aksesi germplasma keledek diperolehi dari 6 negara iaitu Malaysia (52), Indonesia (35, dimana 17 dari Irian Jaya), Thailand (24), Philippines (6), AVRDC-Taiwan (7) dan CIP-Peru (10) telah digunakan dalam kajian ini. Analisis RAPD telah dijalankan terhadap semua aksesi ini dengan menggunakan pada mulanya 54 primer dan akhirnya 5 primer yang dipilih berdasarkan pembentukan jalur yang polimorfik. Analisis kelompok telah dijalankan dengan menggunakan Indeks kesamaan Jaccard yang dikira menggunakan data RAPD tersebut. Seterusnya himpunan teras dibangun berdasarkan kelompok hasil dari analisis kelompok tersebat. Himpunan teras ini akhirnya di sahkan melalui tiga ujian diladang dengan menggunakan 1, 2 dan 3 replikasi.

Kelima primer yang digunakan menghasilkan 30 – 46 jaluran yang bersaiz antara 101 – 2939 bp. Jumlah keseluruhan jaluran yang dihasilkan oleh lima primer tersut ialah 202 dan kesemuanya adalah polimorfik. Indek kesamaan Jaccard antara 134 aksesi tersebut adalah antara 0.02-1.00. Analisis kelompok menggunakan kaedah UPGMA membahagikan aksasi keledek tersebut kepada 13 kumpulan yang berbeza pada nilai indek kesamaam 0.124. Ini menunjukan kewujudan kepelbagaian genetik yang luas dalam himpunan germplasma keledek yang dikaji. Amnya, kebanyakan aksasi dikelompokan mengikut negara asalnya.

Himpunan teras telah di bentuk melalui pensampelan rawak (25%, minima 1 aksasi) dari tiap 13 kelompok dari analisis kelompok. Hasilnya sejumlah 34 aksasi telah dimasukan kedalam himpunan teras dan ia mewakili aksasi dari semua negara kecuali Filipina.

Ujian Newman-Keuls (perbandingan min) dan ujian Levene (kehomogenen varians) telah tidak menunjukan sebarang perbezaan bererti antara min dan varians semua sifat antara himpunan teras dan himpunan asal kecuali sifat panjang tangkai daun dari ujian satu replikasi.

Peratus perbezaan purata (MD %) dan peratus perbezaan varians (VD %) telah dikira untuk membandingkan himpunan teras dan himpunan asal germplasma tersebut. Nilai purata MD% untuk ketiga ujian (8.26), (4.85) and (3.19) adalah kurang dari 20% menunjukan bahawa himpunan teras itu telah memperolehi kepelbagaian yang hampir sama yang terdapat pada himpunan asal. Nilai purata VD% dari ujian satu replikasi (21.3%) adalah sedikit lebih tinggi dari 20%, bermakna himpunan teras itu mungkin tidak mewakili kepelbagaian himpunan asal tetapi nilai VD% dari ujian dengan 2 dan 3 replikasi adalah lebih rendah (11.36 dan 7.16) menunjukan himpunan teras itu sebenarnya mewakili himpunan asal dari segi kepelbagaian.

Nilai purata kadar "*coincidence"* (CR %) dan kadar "*variable"* (VR %) meningkat mengikut bilangan replikasi ujian. Walau bagaimanapun, nilai purata CR% dan VR% masih menunjukan bahawa himpunan teras adalah mewakili himpunan asal.

Pengesahan berdasarkan taburan kekerapan 12 ciri kualitatif, kacuali ciri warna daun muda dan warna tangkai daun, menunjukan taburan yang hampir serupa pada himpunan teras dan himpunan asal. Bagaimanapun, hanya 2 kelas dari dua ciri berlaku dalam frekuensi yang rendah iaitu 2 dari 134 berbanding dengan keseluruhan limpunan. Nilai indeks Shannon-weaver untuk 12 ciri kualitatif tersebut adalah hampir serupa di antara himpunan asal (0.361) dengan himpunan teras (0.364). Ini menunjukan himpunan teras dapat mewakili himpunan asal.

Pencirian dan penilaian himpunan teras tersebut menunjukkan wujudnya kepelbagaian yang tinggi pada semua sifat. Analisis kelompok terhadap himpunan teras ini menunjukkan kehadiran 5 kumpulan utama tetapi taburan aksasi dalam kelompok tersebut tidak mengikut negara asalnya. Ini kerana pengelompokan ini dilakukan dengan menggunakan data morfologi.

Korelasi fenotip antara sifat menunjukkan nilai positif antara panjang jalar, panjang ruas dan panjang tangkai daun; dan antara tebal batang, kandunag bahan kering dan kanji; dan antara hasil dan komponen hasil. Korelasi negatif diperolehi antara hasil dan komponen hasil dengan kandungan kanji dan bahan kering

Nilai heritabiliti luas yang tinggi diperolehi untuk kandungan kanji (89.9 %), bahan kering (87.0%), panjang jalar (83.8%), tebal batang (75.9%) dan bilangan ubi (75.0%). Nilai heritabiliti umum untuk ciri lain adalah antara 72.0% untuk panjang ruas hingga 58. 5% untuk purata berat ubi.

Sebanyak lima aksesi telah dikenalpasti berpotensi kerana memberihan hasil yang tinggi, kandungan nutrien dan warna ubi yang menarik. Aksesi tersebut adalah 11080, 10209, 11079, 10057, 10082, dan 10236.

Kajian ini membuktikan bahawa RAPD merupakan kaedah molekul yang amat sesuai digunakan untuk analisis kepelbagaian dalam germplasma keledek dan seterusnya untuk membentuk himpunan teras. Ia merupakan alternatif kepada penggunaan data morfologi. Menggunakan bilangan *primer* yang agah sedikit, lima (5), adalah mencukupi dan ini dapat menjimatkan kos dan masa. Himpunan teras yang terbentuk juga terbukti dapat mewakili kepelbagaian himpunan asal. Himpunan teras ini merupakan sumber permulaan dalam saringan himpunan germplasma untuk alel yang dikehendaki. Ia juga merupakan bahagian germplasma yang boleh digunakan sebagai wakil kepada himpunann asal. Contohnya, himpunan teras keledek ini boleh dinilai untuk memperolehi sumber gen untuk hasil, bahan kering , kanji, gula,dan karotina.

ACKNOWLEDGEMENTS

Praise to Almighty Allah (SWT) the most Benevolent, Merciful and Compassionate, for giving me the utmost strength, patience and guidance to have this work completed.

I would like to express my most sincere gratitude and deepest appreciation to the chairman of my supervisory committee, Assoc. Prof. Dr. Mohd Said Saad, Department of Crop Science, Faculty of Agriculture, UPM for his invaluable contribution, inputs and careful supervision. Without his constant encouragement this thesis would have never been written.

I am indebted to the members of my supervisory committee, Assoc. Prof. Dr. Abdul Ghani Yunus, Department Crop Science and Dr. Mihdzar Abdul Khadir, Department of Agrotechnology, Faculty of Agriculture, UPM for their encouragement, constructive suggestion and guidance.

Furthermore, I would like to acknowledge Malaysian Technical Cooperation Programme (MTCP) for supporting the research and granting me a scholarship under the Malaysian government in this cooperation between both countries. Also, to IPGRI and IRPA for providing the financial support for the research.

I also wish to convey my thanks to Ramisah and Khairoul Hasni for their kind assistance during my laboratory work. Thanks are also due to the staff, especially Mr. Tajuddin of Institute of Bio-Science, UPM for their help and management of the germplasm.

I would also like to extent my heartfelt appreciation to all my friends who were there to help during field experiments, Jebril Ali, Abdukhadr, Ahmed Sharif, Mendefro and Roslina from the Tissue Culture laboratory for their hospitality and help.

Words are not enough to express my heartfelt feelings to my brothers Mamadou Demba, Cherif Thiam and Siba Cisse and to my sisters Ndeye Binta and Fatoumata for their constant support and concern and motivation throughout the study period.

I specially acknowledged my boundless gratitude to my uncle Sadio Cisse and my aunty Aissa Cisse for their constant encouragement, support and concern during my stay in Malaysia.

Lastly, keeping the best for them, my beloved deceased parents, Oumar Binta and Aby, I would like to express my deepest appreciation and respect for their untiring guidance, support and sacrifices since my childhood. Their spiritual support, *do'a* and motivation inspired me to do this research. I owe a depth gratitude to them, which can never be repaid.

I certify that an Examination Committee has met on 29th May 2006 to conduct the final examination of Sow Harouna on his Doctor of Philosophy thesis entitled "Establishment of Core Collection of Sweetpotato (*Ipomoea batatas* L.) Germplasm using RAPD Markers" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

Name: **SOW HAROUNA**

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