UNIVERSITI PUTRA MALAYSIA

WEAK NECK PROBLEM IN *MUSA* SP. CV. RASTALI POPULATIONS IN RELATION TO MAGNESIUM, BORON AND SILICON AVAILABILITY

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BY
EKA TARWACA SUSILA PUTRA

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

April 2011

DEDICATION

I would like to dedicate my thesis to:

My beloved parents,

Sapardi

Marsih

For giving me do'a, support, sacrifices and encouragement

and

My dearest wife and sons,

Atin

Khadafi

Ilham

for their do'a, motivation, inspiration and love

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

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Chairman : Assoc. Prof. Zakaria bin Wahab, PhD

Faculty : Agriculture

Weak neck is the most significant physiological damage in *Musa* sp. cv. Rastali accessions. It has the ability to reduce fruit quality and selling value. A holistic approach is needed to solve the weak neck problem. The objectives of the research were: (1) to determine the genetic diversity of the Rastali population based on morphological and molecular markers, (2) to identify the specific allele that is associated with weak neck in the Rastali populations in comparison to other cultivars, and (3) to elucidate the relationship between fertilizer recommendation containing Mg, B and Si and the weak neck problem of the Rastali population.

A total of 78 accessions of Rastali (74 accessions of Rastali from Peninsular Malaysia and 4 accessions from Central Java, Indonesia), and six different cultivars with no occurrence of weak neck were selected for comparison. Out of these, only 29 accessions were able to be morphologically characterized because many of them were without reproductive organ. A total of eight qualitative

reproductive descriptors were used in this experiment. These 78 accessions were used for the molecular study. Genomic DNA were extracted from leaf samples using GeneAll® Plant DNA extraction kit and quantified using a spectrophotometer. Polymerase chain reaction (PCR) was performed in a total volume of 15µL. A total of 10 SSR primer pairs were used in this study. Meanwhile, forward primers were labelled with FAM fluorescent label. The detection of the DNA fragments was made on ABI Prism® 310 automated sequencer and the allele sizes were also obtained.

The field experiment was done at Universiti Putra Malaysia under field conditions arranged in split-split plot design with five blocks. The main factor was NPK fertilizer which was recommended by Department of Agriculture Malaysia (DOA) and United Plantation Berhad (UPB). The sub-factors were applications and non-application (as control) of kieserite (Magnesium), boric acid (Boron) and sodium silicate (Silicon). The sub-sub factors were the *Musa* sp. cv. Rastali accessions, consisting of four groups, which were chosen for their different morphological characters. In particular, the *Musa* sp. cv. Berangan accession was selected as the control treatment.

The findings showed that there is genetic diversity among Rastali accessions. Two male reproductive morphological descriptors (namely, male neuter flower along the rachis and male bud shape) were found to be the most important morphological characters which have caused the variation among the Rastali accessions. Existence of variations among Rastali was further supported by

genetic analysis using 10 SSR markers. The SSRs markers were able to separate the accessions of Rastali. A significant 39% of genetic diversity was found among 78 accessions of Rastali. Out of the 78 Rastali accessions, eight were excluded from the main cluster, and showed higher heterogeneity compared to those in the main group (the coefficients of similarity ranging from 0.13 to 0.24). In the selection of the overall allele at each Rastali accession, one allele with the size of 238 bp, was found to be always present in all the accessions of Rastali, but was absent in the other six cultivars. This allele was located at locus A-25 and was considered the most common allele. Meanwhile, the allele which was located on locus A-13 and with the size of 277bp was only found in the other six cultivars but absent in all the Rastali accessions.

The findings also showed that combined application of magnesium, boron and silicon could improve the Mg, B and Si contents in the leaves and fruit of the Rastali accessions and Berangan, in combination with the NPK fertilizer doses from DOA or UPB. These conditions increased the stomatal length and width and physiological activities of the Rastali accessions and Berangan at low level of NPK fertilizer (UPB), in combination with Mg, B and Si applications. In particular, the fingers of the R08 (in combination with the NPK fertilizer doses from UPB) and R62 (in combination with the NPK fertilizer doses from DOA) which had received Mg, B and Si were found to be lighter and smaller than those without any Mg, B and Si applications. Meanwhile, the bunches of the R08, R62 and Berangan which had received Mg, B and Si were found to be lighter and smaller than those without any Mg, B and Si applications, in combination with the

NPK fertilizer doses from DOA. These characters have been shown to have the potential in reducing the intensity of the weak neck problem in the Rastali accessions, especially for the R08 and R62.

Although the applications of magnesium, boron and silicon enhanced stomatal length and width and physiological activities of the Rastali accessions and Berangan at low level of NPK fertilizer (UPB), they decreased polygalacturonase (PG) and pectinmethylesterase (PME) activities in the Rastali accessions, except the PG on Day 4 after harvesting for the R08 and R62. Nevertheless, the PG and PME activities in Rastali were still higher compared to those of Berangan. The applications of Mg, B and Si also increased the lignin and cellulose content in the fruit neck zone of the R34 (in combination with the NPK fertilizer doses from DOA) and R08 (in combination with the NPK fertilizer doses from UPB). The decline in the PG and PME activities, as well as the increase in the lignin and cellulose content in the R08 (in combination with the NPK fertilizer doses from UPB) and R34 (in combination with the NPK fertilizer doses from DOA) which had received Mg, B and Si were able to increase the relative thickness of the cell walls during fruit growth. Application of Mg, B and Si resulted in small-sized cell but relatively thicker cell wall. Meanwhile, the applications of Mg, B and Si decreased the ethylene activity in the R08 (in combination with the NPK fertilizer doses from DOA or UPB), R34 and R12 (in combination with the NPK fertilizer doses from UPB).

The decrease in ethylene, PG and PME activities delayed the accumulation of soluble soilids content (SSC) in the R08 (in combination with the NPK fertilizer doses from DOA) and chlorophyll degradation in the R12, which had obtained Mg, B and Si. The slowing down of chlorophyll degradation and the lowering of SSC resulted in maintained finger firmness of the fruit at a high level. Thus, the fruit has been found to remain strong and hard, especially in the abscission layer, although it has been ripened. The R08 and R12 which had obtained Mg, B and Si were able to produce fruit with stronger abscission layer (higher firmness), in combination with the NPK fertilizer doses from DOA. The stronger abscission layer is not easy to crack and it is also not prone to weak neck. Therefore, the applications of Mg, B and Si in combination with the NPK fertilizer doses from DOA have the potential to reduce the weak neck intensity in the R08, although totally eliminating the weak neck problem as in Berangan has yet to be achieved.

Based on the data, it can therefore be concluded that (1) Rastali cultivar has weak neck problem, (2) there are early indications that the 238bp and 277bp alleles have the possibilities to be associated with weak neck, and (3) the combined applications of Mg, B and Si was able to reduce the weak neck intensity from 80.00% to 24.33% in the Rastali accessions and Berangan, in combination with the NPK fertilizer doses from DOA.

Abstrak Tesis yang dikemukakan kepada senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KEPERLUAN MAGNESIUM, BORON DAN SILIKON DAN HUBUNGANNYA DALAM MASALAH LERAI BUAH PADA POPULASI *MUSA* SP. CV. RASTALI

Oleh

EKA TARWACA SUSILA PUTRA

April 2011

Pengerusi : Prof. Madya Zakaria bin Wahab, PhD

Fakulti : Pertanian

Lerai buah merupakan kerosakan fisiologi yang paling banyak didapati pada *Musa* sp. cv. Rastali. Ia mampu mengurangkan kualiti dan harga pasaran buah. Pendekatan holistik diperlukan dalam menyelesaikan masalah lerai buah. Objektif kajian ini adalah untuk: (1) menentukan kepelbagaian genetik populasi Rastali berdasarkan morfologi dan penanda molekul (2) mengenalpasti alel khusus yang terdapat pada populasi Rastali berbanding kultivar yang lain dan (3) menentukan hubungan di antara baja yang disyorkan yang mengandungi magnesium (Mg), boron (B), dan silikon (Si) terhadap lerai buah pada populasi Rastali.

Sejumlah 78 aksesi Rastali (74 aksesi Rastali dari Semenanjung Malaysia dan 4 aksesi dari Jawa Tengah, Indonesia) dan enam kultivar berbeza yang tidak mempunyai lerai buah dipilih untuk perbandingan. Daripada jumlah ini, hanya 29 aksesi sahaja yang dapat dicirikan morfologinya kerana ketiadaan organ

reproduktif pada kebanyakan kultivar yang mengalami lerai buah. Sebanyak lapan nilai kualitatif untuk ciri-ciri reproduktif digunakan dalam eksperimen ini. Untuk kajian molekular, 78 aksesi telah digunakan. DNA diekstrak daripada daun menggunakan *GeneAll® Plant DNA extraction kit* dan kuantitinya diukur dengan menggunakan spektrofotometer. Reaksi Rantaian Polimerase dihasilkan dalam jumlah 15µL. Sepuluh primer SSR digunakan untuk kajian ini. Primer kehadapan dilabel menggunakan FAM fluorescent. Serpihan bahagian DNA dikenalpasti menggunakan *ABI Prism® 310 automated sequencer* dan saiz alel diperolehi.

Kajian lapangan telah dijalankan di Universiti Putra Malaysia, yang mana ia disusun dalam plot yang dipisah-pisahkan dengan lima blok. Faktor utama adalah baja NPK, iaitu yang disyorkan oleh Jabatan Pertanian Malaysia (DOA) dan United Plantation Berhad (UPB). Faktor-faktor sampingan adalah diaplikasikan dan tidak diaplikasikan (sebagai pengawal) kieserite (Mg), asid borik (B) dan natrium silikat (Si). Faktor sampingan-sampingan bagi aksesi Rastali, mengandungi empat kumpulan yang dipilih untuk ciri-ciri morfologi yang berbeza. *Musa* sp. cv. Berangan dipilih sebagai rawatan kawalan.

Hasil kajian menunjukkan terdapat kepelbagaian genetik pada aksesi Rastali. Dua morfologi reproduktif jantan (iaitu bunga jantan mandul sepanjang rakis dan bentuk kudup jantan) merupakan ciri morfologi yang paling penting yang menyebabkan kepelbagaian di antara aksesi Rastali. Perbezaan yang wujud di antara Rastali kemudian disokong oleh analisis genetik menggunakan 10 penanda SSR mampu mengasingkan aksesi Rastali. Kepelbagaian genetik yang signifikan

dijumpai di antara 78 aksesi Rastali. Daripada 78 aksesi Rastali, 8 aksesi terkeluar daripada kumpulan utama dan menunjukkan heteroginiti yang tinggi berbanding yang terdapat di kumpulan utama (skala persamaan koefisien bermula 0.13 hingga 0.24). Keputusan ini juga menunjukkan bahawa dalam pemilihan daripada keseluruhan aksesi Rastali, satu alel yang bersaiz 238bp ditemui sentiasa ada dalam keseluruhan aksesi Rastali, tetapi tiada dalam enam kultivar yang lain. Alel ini berada pada lokus A-25 dan boleh dipertimbangkan sebagai alel yang paling biasa. Sementara itu, alel yang bersaiz 277 bp yang berada pada lokus A-13, hanya ditemui pada enam kultivar yang lain tetapi tiada pada semua aksesi Rastali.

Hasil penyelidikan ini juga menunjukkan gabungan aplikasi Mg, B dan Si dapat meningkatkan kandungan Mg, B dan Si di dalam daun dan buah pada aksesi Rastali dan Berangan, dengan kombinasi baja NPK dos daripada DOA dan UPB. Keadaan ini meningkatkan panjang dan lebar stomata dan aktiviti fisiologi aksesi Rastali dan Berangan pada tahap rendah baja NPK (UPB), dengan kombinasi aplikasi Mg, B dan Si. Secara khusus, buah R08 (dalam kombinasi dengan baja NPK dos dari UPB) dan R62 (dalam kombinasi dengan baja NPK dos dari DOA) yang telah menerima Mg, B dan Si didapati lebih ringan dan lebih kecil berbanding dengan yang tidak diaplikasikan dengan Mg, B, and Si. Sementara itu, tandan bagi R08, R62 dan Berangan yang telah mendapat Mg, B dan Si didapati ringan dan kecil berbanding dengan yang tidak diaplikasikan dengan Mg, B and Si, dengan kombinasi baja NPK dos daripada DOA. Ciri ini mempunyai

potensi untuk mengurangkan intensiti masalah lerai buah pada aksesi Rastali, terutamanya untuk R08 dan R62.

Walaupun aplikasi Mg, B dan Si dapat meningkatkan panjang dan lebar stomata serta aktiviti fisiologi pada aksesi Rastali dan Berangan pada kadar rendah baja NPK (UPB), ia akan mengurangkan aktiviti poligalakturonase (PG) dan pectinmethylesterase (PME) dalam aksesi Rastali, kecuali PG pada hari ke 4 selepas dituai untuk R08 dan R62. Bagaimanapun, aktiviti PG dan PME dalam Rastali masih lebih tinggi berbanding dengan Berangan. Aplikasi Mg, B, dan Si juga dapat meningkatkan kandungan lignin dan selulosa di zon leher buah R34 (dalam kombinasi dengan baja NPK dos daripada DOA) dan R08 (dalam kombinasi dengan baja NPK dos daripada UPB). Penurunan aktiviti PG dan PME, serta peningkatan lignin dan selulosa dalam R08 (dalam kombinasi dengan baja NPK dos daripada UPB) dan R34 (dalam kombinasi dengan baja NPK dos daripada DOA) yang menerima Mg, B dan Si mampu meningkatkan ketebalan relatif dinding sel semasa pertumbuhan buah. Aplikasi Mg, B dan Si menghasilkan saiz sel yang kecil tetapi dinding sel yang lebih menebal. Sementara itu, aplikasi Mg, B dan Si menurunkan aktiviti etilena pada R08 (dalam kombinasi dengan baja NPK dos daripada DOA atau UPB), R34 dan R12 (dalam kombinasi dengan baja NPK dos daripada UPB).

Penurunan aktiviti etilena, PG dan PME akan menangguhkan pengumpulan kandungan pepejal boleh larut (SSC) pada R08 (dalam kombinasi dengan baja NPK dos daripada DOA) dan degradasi klorofil pada R12, yang telah menerima

Mg, B dan Si. Perlambatan degradasi klorofil dan penurunan SSC akan mengekalkan ketegasan buah pada peringkat tinggi. Oleh itu, buah akan didapati tetap kuat dan keras, terutamanya pada lapisan luruhan, walaupun buah telah masak. R08 dan R12 yang mendapat Mg, B dan Si mampu menghasilkan buah dengan lapisan luruhan yang lebih kuat (ketegasan lebih tinggi), dalam kombinasi dengan baja NPK dos daripada DOA. Lapisan luruhan yang kuat tidak mudah retak dan tidak mudah terdedah kepada lerai buah. Oleh sebab itu, aplikasi Mg, B dan Si dengan kombinasi baja NPK dos daripada DOA mempunyai potensi untuk menurunkan intensiti lerai buah pada R08, walaupun secara keseluruhannya penyelesaian masalah lerai buah seperti pada Berangan masih belum dapat dicapai.

Berdasarkan data tersebut, dapatlah disimpulkan bahawa (1) kultivar Rastali mempunyai masalah lerai buah, (2) terdapat penunjuk awal bahawa alel 238bp dan 277bp berkemungkinan berkaitan kepada masalah lerai buah, (3) kombinasi aplikasi Mg, B dan Si mampu mengurangan intensiti masalah lerai buah dari 80.00% kepada 24.33% pada aksesi Rastali dan Berangan, dalam kombinasi dengan baja NPK dos daripada DOA.

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I certify that a Thesis Examination Committee has met on 27 April 2011 to conduct the final examination of Eka Tarwaca Susila Putra on his thesis entitled "Weak Neck Problem in *Musa* Sp. Cv. Rastali Populations in Relation to Magnesium, Boron and Silicon Availability" in accordance with the Universities and University College 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 Philosophy.

Members of the Thesis Examination Committee were as follows:

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Date: 26 July 2011

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

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Date:

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 institution.

EKA TARWACA SUSILA PUTRA

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