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PHYSIOLOGICAL AND BIOCHEMICAL CAHNGES OF VITRO PROPAGATED BAHANA PLANTLETS INOCULATED WITH RHIZOBACTERIA AND AGROBACTERIA

ZURAIDA AB. RAHMAN.

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By

ZURAIDA AB. RAHMAN

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

July 2005

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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July 2005

Chairman : Professor Marziah Mahmood, PhD

Faculty : Biotechnology and Biomolecular Sciences

A series of experiments were carried out to observe the effects of rhizobacterial and agrobacterial inoculation, singly or combined on the total content, concentration and distribution of the biochemical components (total soluble protein, soluble nitrogen, proline, peroxidase activity, total soluble phenolic, nitrate reductase activity, nitrate, chlorophyll), physiological characteristics (percentages of growth, number of roots, fresh and dry weight of roots, maximum and total length of roots) and mineral contents (N, P, K, Ca and Mg) of *in vitro* banana plantlets using MS (Murashige and Skoog, 1962) basal medium. The effects of rhizobacterial inoculation in modified MS medium containing sodium chloride (0.2%) and boron (1 μM and 10 μM) on the biochemical components, physiological characteristics and mineral content of the *in vitro* banana plantlets were also studied. Growth of banana plantlets cultured in modified MS liquid medium supplemented with

different forms and concentrations of nitrogen or carbon sources and inoculated with *Bacillus sphaericus* UPMB10 was estimated.

Results from the inoculation study using MS basal medium indicated that inoculation with rhizobacteria (*Azospirillum brasilense* Sp7, *Bacillus sphaericus* UPMB10 and *Microbacterium oxydens* UPMB11) or agrobacteria (*Agrobacterium rhizogenes* strains AR9402 and A4) showed positive response on growth of *in vitro* banana plantlets compared to uninoculation after one month of culture. The inoculation treatment also increased the number of root, fresh and dry weight of roots and total length of root. At the same time, with inoculation the total content or concentration of the respective biochemical activity as total soluble protein, peroxidase, nitrate reductase, proline, nitrate, soluble nitrogen, phenolic and chlorophyll of the host plants increased and varied according to the type of bacteria used. Inoculation with these bacteria also enhanced the accumulation of N and P in the plantlets. Co-inoculation with rhizobacteria (*Azospirillum brasilense* Sp7, *Bacillus sphaericus* UPMB10 and *Microbacterium oxydens* UPMB11) and agrobacteria (*Agrobacterium rhizogenes* strains AR9402 and A4) also showed similar response as in single inoculation; UPMB10+AR9402 treatment was the most effective treatment. The presence of rhizobacteria in the medium supplemented with 0.2% sodium chloride resulted in an improvement in growth and root biomass compared to the control (uninoculated). This rhizobacterial inoculation also produced an increase in protein, nitrate, soluble nitrogen and chlorophyll contents of the plantlets cultured in MS modified medium containing 0.2% sodium chloride. The

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descending order of effectiveness of the rhizobacteria in medium containing 0.2% sodium chloride was:UPMB11>UPMB10>Sp7. Similar response was shown when *Bacillus sphaericus* UPMB10 was inoculated into medium containing boron at two concentrations: 1 µM and 10 µM. An increase in percentage of growth (> 295%) was shown when boron was applied into medium inoculated with *Bacillus sphaericus* UPMB10. Results from the experiment of modified MS medium supplemented with different concentrations and forms of nitrogen also strongly indicated that inoculation with *Bacillus sphaericus* UPMB10 has the potential to improve the *in vitro* plant growth especially in the absence of nitrogen. Inoculation with *Bacillus sphaericus* UPMB10 showed significant increased plant growth in treatment without nitrogen (- nitrogen) at 166% compared to un-inoculated only at 115%. Inoculation with *Bacillus sphaericus* UPMB10 to enhance growth of *in vitro* plantlets could partly replace the expensive chemical nitrogen requirement for the plants. *Bacillus sphaericus* UPMB10 seem to have the ability to increase growth of plantlets in medium supplemented with asparagine, potassium nitrate and urea. The descending order of effects of rhizobacterial inoculation on growth of plantlets varied according to the following N-sources in the MS modified media: asparagine (392%) > potassium nitrate (376%)> urea 291%. There was a negative response of *Bacillus sphaericus* UPMB10 inoculation in promoting growth of plantlets in media containing KNO_3 (a range of 0 mM -300 mM) or $(NH_4)_2SO_4$ (a range of 0 mM-80mM) at all concentrations used. At 1.5% to 6.0% concentrations of sucrose, inoculated plantlets with *Bacillus sphaericus* UPMB10 showed increased growth within a range of 250% to 304% compared to un-inoculated plantlets. It also indicate

that inoculation with *Bacillus sphaericus* UPMB10 into the media containing carbon successfully enhanced growth of *in vitro* plantlets. The descending order of effect of UPMB10 inoculation on plant growth varied according to the following carbon sources in the MS modified media: fructose (421%)> sucrose (356%)> glucose (354%)> maltose (221%)> sorbitol (78%)> mannitol (51%). Therefore, inoculation with *Bacillus sphaericus* UPMB10 into the medium containing carbon sources produced positive response on the host plant, an effect which is dependent on the forms and concentrations of the carbon sources. The above finding provided evidence that *Azospirillum brasilense* Sp7, *Bacillus sphaericus* UPMB10, *Microbacterium oxydens* UPMB11, *Agrobacterium rhizogenes* strains AR9402 and A4, singly or combined are potentially effective in promoting growth of *in vitro* banana plantlets. Inoculation of rhizobacteria were showed beneficial to the plantlet in saline conditions through increment of growth and improvement in rooting system The effectiveness of inoculation is increased when associated with boron, nitrogen or carbon into the medium. Thus these bacterial strains could be used as a bioenhancer for growth of *in vitro* banana plantlets.

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

PERUBAHAN FISIOLOGI DAN BIOKIMIA KE ATAS ANAK PISANG *IN VITRO* YANG DIINOKULASI DENGAN RHIZOBACTERIA DAN AGROBACTERIA

Oleh

ZURAIDA AB. RAHMAN

Julai 2005

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Beberapa siri eksperimen telah dijalankan untuk memerhatikan kesan inokulasi rhizobakteria dan agrobakteria secara tunggal atau digabungkan ke atas jumlah kandungan, kepekatan dan pengagihan komponen-komponen biokimia (jumlah protein terlarut, nitrogen terlarut, prolin, aktiviti peroxidase, jumlah fenolik terlarut, aktiviti reduktase nitrat, nitrat, klorofil), ciri-ciri fisiologi (Peratus pertumbuhan, jumlah akar, berat basah dan kering akar, jumlah dan panjang akar maksimum) dan kandungan mineral (N,P,K, Ca dan Mg) anak-anak pokok pisang *in vitro* dengan menggunakan media basal MS. Kesan inoculasi rhizobakteria dalam media MS (Murashige and Skoog, 1962) ubahsuai yang mengandungi natrium klorida (0.2%) dan boron (1 μM dan 10 μM) ke atas kandungan biokimia, ciri-ciri fisiologi dan kandungan mineral anak-anak pokok pisang *in vitro* juga dikaji. Pertumbuhan anak-anak pokok pisang dalam media cecair ubahsuai yang dibekalkan dengan bentuk dan kepekatan nitrogen atau sumber karbon yang berbeza serta diinokulasi dengan *Bacillus sphaericus* UPMB10 juga telah dianggarkan.

Hasil daripada kajian inokulasi yang menggunakan media basal MS menunjukkan bahawa inokulasi dengan rhizobakteria (*Azospirillum brasilense* Sp7, *Bacillus sphaericus* UPMB10 dan *Microbacterium oxydens* UPMB11) atau agrobakteria (*Agrobacterium rhizogenes* strain AR9402 dan A4) menunjukkan respon positif terhadap pertumbuhan anak-anak pokok pisang *in vitro* jika dibandingkan dengan yang tidak diinokulasi selepas dikultur satu bulan. Rawatan inokulasi juga meningkatkan bilangan, berat basah dan kering akar dan jumlah panjang akar. Pada masa yang sama, melalui inokulasi jumlah kandungan protein terlarut, peroxidase, reduktase nitrat, nitrat, prolin, nitrogen terlarut, fenolik dan klorofil dari pokok perumah meningkat dan berbeza mengikut jenis bakteria yang digunakan. Inokulasi dengan bakteria ini juga merangsang pengumpulan kandungan N dan P dalam anak-anak pokok. Gabungan inokulasi antara rhizobakteria (*Azospirillum brasilense* Sp7, *Bacillus sphaericus* UPMB10 dan *Microbacterium oxydens* UPMB11) dan agrobakteria (*Agrobacterium rhizogenes* strain AR9402 dan A4) menunjukkan respon yang sama seperti inokulasi tunggal; di mana rawatan UPMB10+AR9402 ialah rawatan yang paling efektif. Kehadiran rhizobakteria di dalam media yang dibekalkan dengan 0.2% natrium klorida menunjukkan peningkatan dalam pertumbuhan dan biojisim akar berbanding dengan kawalan (tidak diinokulasi). Inokulasi dengan rhizobakteria ini juga meningkatkan kandungan protein terlarut, nitrat, nitrogen terlarut dan klorofil dalam anak-anak pokok yang dikultur dalam media ubahsuai MS yang mengandungi 0.2% natrium klorida. Keberkesanan dalam urutan menurun adalah: UPMB11>UPMB10>Sp7. Respon yang sama juga dilihat apabila *Bacillus sphaericus* UPMB10 diinokulasi dalam

media yang mengandungi boron dalam dua kepekatan: 1 μM dan 10 μM. Peningkatan peratus pertumbuhan (>295%) dilihat apabila boron digunakan dalam media yang diinokulasi dengan *Bacillus sphaericus* UPMB10. Hasil daripada ujikaji media MS ubahsuai yang dibekalkan dengan kepekatan dan bentuk nitrogen yang berbeza juga menunjukkan bahawa inokulasi dengan *Bacillus sphaericus* UPMB10 mempunyai potensi untuk memperbaiki pertumbuhan pokok *in vitro* terutamanya dalam ketidakhadiran nitrogen. Inokulasi dengan UPMB10 menunjukkan peningkatan yang bererti terhadap pertumbuhan pokok dalam rawatan tanpa nitrogen (-nitrogen) pada 166% berbanding dengan yang tidak diinokulasi pada hanya 115%. Inokulasi dengan *Bacillus sphaericus* UPMB10 boleh merangsang pertumbuhan anak-anak pokok *in vitro* di mana ia boleh menggantikan sebahagian daripada keperluan bahan-bahan kimia nitrogen yang mahal untuk pokok. *Bacillus sphaericus* UPMB10 didapati mempunyai keupayaan untuk meningkatkan pertumbuhan anak-anak pokok dalam media yang dibekalkan dengan asparagin, kalium nitrat dan urea. Kesan inokulasi rhizobakteria terhadap pertumbuhan anak-anak pokok secara urutan menurun mengikut sumber N- dalam media ubahsuai MS: asparagin> kalium nitrat> urea. Terdapat respon negatif inokulasi *Bacillus sphaericus* UPMB10 dalam menggalakkan pertumbuhan anak-anak pokok dalam media yang mengandungi KNO_3 (julat antara 0mM-300mM) atau $(NH_4)_2SO_4$ (julat antara 0mM-80 mM) dalam semua kepekatan yang digunakan. Pembekalan sukrosa pada kepekatan 1.5% hingga 6.0% dan diinokulasi dengan *Bacillus sphaericus* UPMB10 menunjukkan peningkatan pertumbuhan anak-anak pokok di antara julat 250-304% berbanding dengan yang tidak diinokulasi. Ia juga menunjukkan bahawa inokulasi dengan

Bacillus sphaericus UPMB10 dalam media yang mengandungi sumber karbon dengan jayanya merangsang pertumbuhan anak-anak pokok *in vitro*. Kesan inokulasi UPMB10 secara urutan menurun terhadap pertumbuhan pokok berbeza mengikut sumber karbon di dalam media ubahsuai: fruktosa (421%)> sukrosa (356%)> glukosa (354%)> maltosa (221%)> sorbitol (78%)> mannitol (51%). Maka, inokulasi dengan *Bacillus sphaericus* UPMB10 ke dalam media yang mengandungi sumber karbon menghasilkan respon yang positif kepada pertumbuhan pokok perumah, di mana kesannya begantung kepada bentuk dan kepekatan sumber karbon. Penemuan ini membuktikan bahawa *Azospirillum brasilense* Sp7, *Bacillus sphaericus* UPMB10, *Microbacterium oxydens* UPMB11, *Agrobacterium rhizogenes* strain AR9402 dan A4 secara tunggal atau digabungkan berpotensi dalam menggalakkan pertumbuhan anak-anak pokok *in vitro* secara efektif. Inokulasi dengan rhizobakteria juga memberi kebaikan kepada anak-anak pokok dalam persekitaran garam melalui peningkatan pertumbuhan dan pengembangan sistem pengakaran. Keberkesanan inokulasi meningkat apabila disatukan dengan boron, nitrogen atau karbon dalam medium. Maka strain bakteria ini boleh digunakan sebagai perangsang biologi ('bioenhancer') bagi pertumbuhan anak-anak pokok pisang *in vitro*.

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I certify that an Examination Committee met on 13th July 2005 to conduct the final examination of Zuraida Ab. Rahman on her Doctor of Philosophy thesis entitled "Physiological and Biochemical Changes of *in vitro* Propagated Banana Plantlets Inoculated with Rhizobacteria and Agrobacteria" 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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This thesis 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 are as follows:

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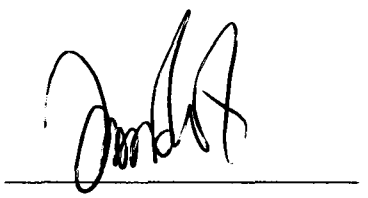
DECLARATION

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.



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Date:. 11- 10- 2005

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PGPR	Plant Growth Promoting Rhizobacteria
PGRs	Plant growth regulators
UPMB10	*Bacillus sphaericus* UPMB10
UPMB11	*Microbacterium oxydens* UPMB11
Sp7	*Azospirillum brasilense* Sp7
NRA	Nitrate reductase activity
C/N ratio	Carbon/nitrogen ratio
NAA	a-Napthalene acetic acid
BAP	6- Benzylaminopurine
MS	Murashige and Skoog
IAA	3-Indoleacetic acid
ABA	Abscisic acid
NEU	Nitrogen use efficiency
WUE	Water use efficiency
ACC	1-aminocyclopropane-1-carboxylate