UNIVERSITI PUTRA MALAYSIA

INDUCING HOST RESISTANCE AGAINST FUSARIUM WILT OF BANANA THROUGH APPLICATION OF CHITOSAN

MOHAMMED YAHYA ABDULLAH AL HETAR

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INDUCING HOST RESISTANCE AGAINST FUSARIUM WILT OF BANANA THROUGH APPLICATION OF CHITOSAN

By

MOHAMMED YAHYA ABDULLAH AL HETAR

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

May 2013

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بِسْمِ اللَّـهِ الرَّحْمَـٰنِ الرَّحِيمِ

﴿ رَبِّ أَوْزِعْنِي أَنْ أَشْكُرَ نِعْمَتَكَ الَّتِي أَنْعَمْتَ عَلَيَّ وَعَلَىٰ وَالِدَيَّ وَأَنْ أَعْمَلَ صَالِحًا تَرْضَاهُ وَأَدْخِلْنِي بِرَحْمَتِكَ فِي عِبَادِكَ الصَّالِحِينَ ﴾

"My Lord, enable me to be grateful for Your favor which You have bestowed upon me and upon my parents and to do righteousness of which You approve. And admit me by Your mercy into [the ranks of] Your righteous servants."

صدق الله العظيم
سورة النمل ـ الآية (19)

DEDICATION

Dedicated to my beloved parents

Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirements of Doctor of Philosophy

INDUCING HOST RESISTANCE AGAINST FUSARIUM WILT OF BANANA THROUGH APPLICATION OF CHITOSAN

By

MOHAMMED YAHYA ABDULLAH AL HETAR

May 2013

Chairman: Associate Professor Zainal Abidin Mior Ahmad, PhD

Faculty: Agriculture

This study was undertaken with the aim to evaluate the effects of chitosan on the activation of defense related compounds to increase tolerance to *Fusarium oxysporum* f. sp. *cubense* Race 4 (FocR4) in susceptible banana seedlings var Berangan. In the *in vitro* studies, chitosan reduced the hyphal growth of FocR4 on Potato Dextrose Agar (PDA) medium and gave maximum inhibition of 76.36 % at 8 mg/ml. Inhibition of fungal growth was more efficient in Potato Dextrose Broth (PDB) where complete inhibition of mycelial growth of FocR4 was accomplished at all concentrations tested. Chitosan inhibited the sporulation of FocR4 by a maximum of 96.53 % at 8 mg/ml chitosan and 100% inhibition of spore germination was recorded at all concentrations tested. Chitosan was also found to induce morphological and cytological changes in FocR4 characterized by agglomeration of hyphae, abnormal shapes, and formation of vesicles or empty cells devoid of cytoplasm in the mycelia. Chitosan sprayed at concentrations of more than 0.3 mg/ml was found to cause damage to banana leaves. There was no significant difference in photosynthesis parameters including photosynthetic rates, stomatal

conductance and chlorophyll contents within four days after spraying with chitosan at 0.3 mg/ml. However, chitosan was found to increase significantly the activities of peroxidase (PO), phenylalanine ammonia lyases (PAL), lignothioglycolic acid (LTGA), chitinases and glucanase enzymes in banana roots. The occurrence of induced resistance enhanced tolerance of the seedlings to Fusarium wilt based on parameters such as delay in onset of foliar disease symptoms, lower in percentage of disease severity (DS), lower in percentage of disease incidence (DI), area under disease progress curve (AUDPC), and epidemic rate. Vegetative growth were measured based on increased in plant height, number of leaves, diameter of pseudostem, root and shoot dry mass showing significant improvement only between treatments challenged by inoculation with FocR4 (T1 and T2). The growth of banana plants was not influenced by chitosan itself as a growth stimulator but it enhanced the growth in those infected with FocR4. The increase in PO, PAL, LTGA, chitinase and β-1,3-glucanase activity in seedlings treated with chitosan had been shown to be associated with plant defense through the decrease percentage of disease severity, disease incidence and area under disease progress curve. This indicated effective role by chitosan in controlling banana seedlings against wilt disease caused by FocR4. It showed promise as an inducer in the activation of host defense systems and increasing tolerance to FocR4 infection in susceptible banana var Berangan. Further studies regarding application frequency and combination techniques are essential to more effective of control this disease.

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

KETAHANAN RANGSANGAN TERHADAP LAYU FUSARIUM PISANG MELALUI PERMOHANAN KITOSAN

Oleh

MOHAMMED YAHYA ABDULLAH AL HETAR

Mei 2013

Pengerusi: Profesor Madya Zainal Abidin Mior Ahmad, PhD

Fakulti: Pertanian

Kajian ini telah dijalankan dengan tujuan untuk menilai kesan kitosan dalam pengaktifan sebatian berkaitan pertahanan untuk meningkatkan toleransi kepada *Fusarium oxysporum* f. sp. *cubense* Race 4 (FocR4) dalam benih pisang rentan var Berangan. Kitosan mengurangkan pertumbuhan hifa FocR4 atas medium PDA dan memberi perencatan maksimum 76.36% pada 8 mg/ml. Kitosan adalah lebih berkesan dalam PDB di mana ia menghalang sepenuhnya pertumbuhan miselium FocR4 pada semua kepekatan yang diuji. Kitosan menghalang pensporulan FocR4 di tahap maksimum 96.53% pada 8 mg/ml kitosan dan perencatan 100% untuk percambahan spora telah direkodkan pada semua kepekatan yang diuji. Kitosan juga telah didapati merangsang perubahan morfologi dan sitology pada FocR4 melalui ciri pengumpulan hifa, bentuk yang tidak normal, pembentukan vesikel atau sel-sel kosong tanpa sitoplasma dalam miselia. Kitosan disembur pada kepekatan melebihi lebih daripada 0.3 mg/ml menyebabkan kerosakan pada pisang. Tiada perbezaan yang bererti dalam parameter fotosintesis termasuk kadar fotosintesis, penyaluran stomata dan kandungan klorofil dalam masa empat hari selepas disembur dengan

kitosan pada 0.3 mg/ml. Walau bagaimanapun, kitosan telah didapati meningkatkan dengan bererti aktiviti peroksidase, fenilalanine ammonia liase, asid lignithioglicolic, kitinases dan glucanases dalam akar pisang. Pengeluaran kompaun teraruh meningkatkan ketahanan benih pisang terhadap layu Fusarium berdasarkan kepada parameter seperti kelewatan dalam kemunculan symptom, lebih rendah di peratusan keterukan penyakit (DS), lebih rendah dalam peratusan kejadian penyakit (DI), kawasan di bawah kemajuan penyakit keluk (AUDPC), dan kadar wabak. Tumbesaran tampang diukur berdasarkan ketinggian pokok daun, garispusat batang pseudo, jisim kering akar dan pucuk yang menunjukkan pembaikan hanya antara rawatan yang disuntik cabar dengan FocR4 (T1 dan T2). Tumbesaran tumbuhan pisang tidak dipengaruhi oleh kitosan sendiri sebagai perangsang pertumbuhan tetapi turut meningkatkan tumbesaran tumbuhan yang dijangkiti oleh FocR4. Peningkatan PO, PAL, LTGA, kitinase dan aktiviti β-1 ,3-glucanase dalam benih dirawat dengan kitosan telah ditunjukkan berkait dengan pertahanan tumbuhan melalui pengurangan peratusan keterukan penyakit, kejadian penyakit dan keluasan di bawah keluk kemajuan penyakit. Ini menunjukan kesan efektif oleh kitosan dalam pengawalan anak benih pisang terhadap penyakit layu oleh FocR4. Ia menunjukkan kebaikannya sebagai perangsang dalam pengaktifan sistem pertahanan perumah dan peningkatan toleransi kepada jangkitan FocR4 pada pisang var Berangan yang rentan. Kajian lanjut mengenai kekerapan dan teknik gabungan adalah penting untuk lebih berkesan untuk mengawal penyakit ini.

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I certify that a Thesis Examination Committee has met on 17 May 2013 to conduct the final examination of Mohammed Yahya Abdullah Al Hetar on his thesis entitled "Inducing Host Resistance Against Fusarium Wilt of Banana Through Application of Chitosan" in accordance with the Universities and University Colleges 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.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. 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.

_____

MOHAMMED YAHYA A. AL HETAR

Date: 17 May 2013

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