UNIVERSITI PUTRA MALAYSIA

MECHANISMS INVOLVED IN THE BIOLOGICAL CONTROL OF TOMATO BACTERIAL WILT CAUSED BY *RALSTONIA SOLANACEARUM USING* ARBUSCULAR MYCORRHIZAL FUNGI

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By

MONTHER MOHUMAD TAHAT

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

2009

DEDICATION

Special dedication to:

My dearest Father; Mohumad Tahat (Abu Faruq), my Mother; (Um Faruq), Sisters, Brothers and to my wife, endless and boundless love, understanding, supporting waiting and encouragement throughout my study.

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

MECHANISMS INVOLVED IN THE BIOLOGICAL CONTROL OF TOMATO BACTERIAL WILT CAUSED BY *RALSTONIA SOLANACEARUM* USING ARBUSCULAR MYCORRHIZAL FUNGI

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March 2009

Chairman: Associate Professor Kamaruzaman Sijam, PhD

Faculty: Agriculture

Glasshouse experiment was done to study the ability of two local mycorrhizal fungi species (*Glomus mosseae, Scutellospora* sp.,) and introduced species *(Gigaspora margarita*) to colonize and enhance some tomato growth parameters. *G. mosseae* showed the best preformance among species used. *G. mosseae* was able to increase significantly plant height (60%), shoot dry weight (135%) and flowers number (58%) compared to the control plant at the 7th weeks of plant growth. *G. mosseae* alter root structures such as root dry weight (42%), root tips (120%), root length(83%), root surface area (106%), and root volume (59%), which can increase nutrient absorption and enhance plant growth. *G. mosseae* was adapted to the local environmental conditions which resulted in more root colonization (300%) and more spores number (300%), different from the introduced species *G. margarita*. The overall data presented in

this study showed that local species can be used for enhancing yield growth more than the introduced species. Three mechanisms were described to explain by how arbuscular mycorrhizal fungi (AMF) inhibit or control the bacterial wilt disease. Nutrient uptake, biochemical changes and root morphological changes were the mechanisms studied. The concentrations of N (41%), P (133%), K (49%), Fe (44%), and Zn (33%) in tomato shoots were increased after the colonization of *G. mosseae*, indicating that AMF was able to increase the shoot nutrient uptake due to the hyphal net were produced by AMF which allow the roots to absorb more nutrient. The root morphological characteristics (root dry weight, root tips, root volumes, root length and root surface area) were changed significantly in *G. mosseae* treatment compared to all other treatments. The SEM and TEM images provided evidence that AMF can modify the root cortex cells and root structure which finally helps the plant to prevent the disease infection totally. The *G. mosseae* hyphal structures were seen inside the cortex cell. Disease symptoms were not seen in the *G. mosseae +R. solanacearum* treated plants. The extensive colonization by AMF was the reason behind the high concentration of chlorophyll (a) and chlorophyll (b) which could contribute to the increase of photosynthetic rate in tomato leaves and enhance plant growth. Ch.(a) and ch.(b) in *G. mosseae* treated plants was significantly higher compared to the rest of the treatments. *G. mosseae* can be used as a bio-protection agent because it can provide root with hyphal net which can minimize the bacterial wilt infection. The production of healthy, huge number and clean *G. mosseae* spores were the targets of another glasshouse experiment. The results obtained from this experiment showed that the harvest date and the type of the

crops were played a critical role in AMF spore production. Corn was the most suitable host for *G. mosseae* sporulation (167 spore/10gm soil). Lentil, green bean, and barley showed low AMF sporulation and colonization related to the inability of these crops to grow under glasshouse conditions. Several important factors must be considered in AMF mass production, included plant host species, environmental conditions, soil types, nutrient regime, pot size, inoculum amount and the source of primary inoculum. *In vitro* experiments were done to study the effects of different root exudates with and without pre-inoculation with *G. mosseae* on the control of *R. solanacearum* and to study the indirect interaction between *G. mosseae* and *R. solanacearum*. In general, the influence of root exudates produced from tomato and corn plants on *G. mosseae* spore germination showed different response. The spores germination number was decreased using different volumes of mycorrhizal tomato root exudates (MTRE) and mycorrhizal corn root exudates (MCRE). It was increased when non-mycorrhizal tomato root exudates (NMTRE) and non-mycorrhizal corn root exudates (NMCRE) were applied in different volumes. *G. mosseae* spores germinated in all types of media used. The spore germination number was increased by increasing the original number of spores cultured and this indicated that the volatiles compounds produced from bacterial pathogen did not inhibit the spore's germination. The overall results concluded from these studies confirm that the local species of AMF were more able to support and enhance plant growth compared to the introduced species. *G. mosseae* was able to control totally the bacterial wilt causal agent *R. solanacearum* under glasshouse conditions. Nutrient uptake, biochemical changes and root morphological

changes were the three mechanism tested. The production of huge number of AMF spores is a critical area for mycorrhizal research using suitable host plant as a trap.

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

MEKANISMA YANG TERLIBAT DALAM KAWALAN BIOLOGI LAYU BAKTERIA TOMATO DISEBABKAN OLEH *RALSTONIA SOLANACEARUM* MENGGUNAKAN KULAT MIKORIZA ABUSKULAR

Oleh

MONTHER MOHAMMAD TAHAT

March 2009

Pengerusi: Profesor Madya Kamaruzaman Sijam, PhD

Fakulti: Pertanian

Kajian rumah kaca telah dijalankan untuk mengkaji keupayaan kolonisasi dan meningkatkan beberapa parameter pertumbuhan pokok tomato oleh dua spesies kulat mikoriza tempatan iaitu *Glomus mosseae, Scutellospora* sp., dan spesies luar, *Gigaspora margarita*. *G. mosseae* menunjukkan prestasi yang terbaik di antara spesies yang digunakan. *G. mosseae* berkemampuan meningkatkan tinggi pokok (60%), berat kering pokok (135%) dan bilangan bunga (58%) berbanding pokok kawalan pada minggu ke-7 pertumbuhan. *G. mosseae* mengubah struktur akar seperti berat kering akar (42%), hujung akar (120%), panjang akar (83%), luas permukaan akar (106%) dan jumlah akar (59%), yang mana boleh meningkatkan penyerapan nutrien dan meningkatkan pertumbuhan pokok. *G. mosseae* dapat beradaptasi dengan persekitaran yang mana menyebabkan peningkatan kolonisasi akar (300%) dan jumlah spora (300%) berbeza dengan spesies luar, *G. margarita*. Secara keseluruhannya

data yang dipersembahkan dalam kajian ini menunjukkan bahawa spesies tempatan dapat digunaka untuk meningkatkan hasil lebih berbanding dengan spesis luar. Tiga mekanisma telah diperjelaskan untuk menerangkan bagaimana kulat mikoriza abuskular (AMF) merencat atau mengawal penyakit layu bakteria. Pengambilan nutrient, perubahan biokimia dan perubahan morfologi akar adalah mekaisma yang dikaji. Kepekatan N (41%), P (133%), K (49%), Fe (44%) and Zn (33%) dalam pucuk daun tomato meningkat selepas dikolonisasi oleh *G. mosseae*, ini menunjukkan bahawa AMF berupaya meningkat pengambilan nutrien disebabkan jaringan hifa yang dihasilkan oleh AMF membenarkan akar menyerap lebih nutrient. Ciri-ciri morfologi akar (berat kering akar, hujung akar, jumlah akar, panjang akar, luas permukaan akar) telah berubah secara berkesan pada rawatan *G. mosseae* berbanding dengan rawatan yang lain. Gambar SEM dan TEM memberi bukti bahawa AMF dapat mengubah sel kortek akar dan struktur akar yang akhirnya membantu pokok daripada dijangkiti penyakit secara total. Struktur hifa *G. mosseae* dapat dilihat di dalam sel kortek. Simptom jangkitan tidak dilihat pada pokok yang dirawat dengan *G. mosseae* + *R. Solanacearum.* Kolonisasi secara ektensif oleh AMF adalah sebab peningkatan kepekatan ch(a) dan ch(b) yang mana menyumbang peningkatan kadar fotosistesis pada daun tomato dan meningkatkan pertumbuhan pokok. Ch(a+b) pada pokok yang dirawat dengan *G. mosseae* adalah lebih tinggi berbanding dengan rawatan yang lain. *G. mosseae* boleh digunakan sebagai egen kawalan biologi dan dengan penghasilkan jaringan hifa dapat meminimumkan jangkitan layu bakteria. Penghasilan spora *G. mosseae* yang sihat, banyak dan bersih merupakan sasaran eksperiment rumah kaca yang

lain. Keputusan daripada eksperiment ini menunjukkan bahawa tarikh tuaian dan jenis tanaman memainkan peranan yang penting dalam pengeluaran spora AMF. Jagung adalah perumah yang sangat sesuai untuk pembentukan spora *G. mosseae* (167 spora/10g tanah). Kacang kuda, kacang hijau dan barli menghasilkan spora AMF yang rendah dan kolonisasi adalah berkait dengan keupayaan tanaman ini dibiak di dalam rumah kaca. Beberapa faktor penting perlu dipertimbangkan dalam pengeluran spora AMF yang besar iaitu jenis perumah, cuaca persekitaran, jenis tanah, regim nutrien, saiz pasu, jumlah inokulum dan punca inokulum. Perhubungan negatif di antara *G. mosseae dan R. solanacearum* telah dilihat semasa ujian tanpa pokok tomato dijalankan. Eksperiment *in vitro* telah dijalankan untuk mengkaji kesan perbezaan rembesan akar tanpa dan pre-inokulasi oleh *G. mosseae* untuk mengawal *R. solanacearum* dan mengkaji perhubungan tidak langsung di antara *G. mosseae* and *R. solanacearum*. Umumnya, pengaruh rembesan akar tomato dan jagung pada percambahan spora *G. mosseae* memberi reaksi yang berbeza. Percambahan spora menurun menggunakan rembesan akar tomato mikoriza (MTRE) dan rembesan akar jagung mikoriza (MCAR) pada jumlah yang berbeza. Apabila rembesan akar tomato (NMTRE) dan rembesan akar jagung (NMCRE) digunakan dalam jumlah yang berbeza ia dapat meningkatkan kadar percembahan spora. Spora G.mosseae bercambah didalam semua jenis media yang digunakan. Jumlah percambahan spora meningkat dengan meninjugkatuya jumlah asla spora yang fikultur dan ini menunjukhan bahawa kompaun meruap daripada bakteria pathogen tidak merencat percembahan spora. Keputusan keseluruhan kajian merumukan bahawa dan meringkatakan

pertumbuhan pokok berbanding dengan spesies luar. G. Mosseae berupaya menguwal penyakit layu bakteria yang disebabkan oleh. Pengambilan nutrien, perubahan biokimia dan perubahan morfologi akare adalah tiga mekanisma yang telahdikaji. Pengeluran spora AMF secara besr-besaran adalah bidang kritical dalam penyelidikan mikoriza dengan menggunakan perumah sebagai perangkap.

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I certify that an Examination Committee has met on **date of viva voce** to conduct the final examination of **Monther Mohumad Yuesef Tahat** on his **Doctor of Philosophy** thesis entitled "**Biological Control of Tomato Bacterial Wilt Caused by *Ralstonia solanacearum* Using Endomycorrhizal Fungi**" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Examination Committee were as follows:

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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 Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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Professor and Dean
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Date: 8 June 2009

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.

_____

Monther Mohumad Yuesef Tahat

Date:

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