



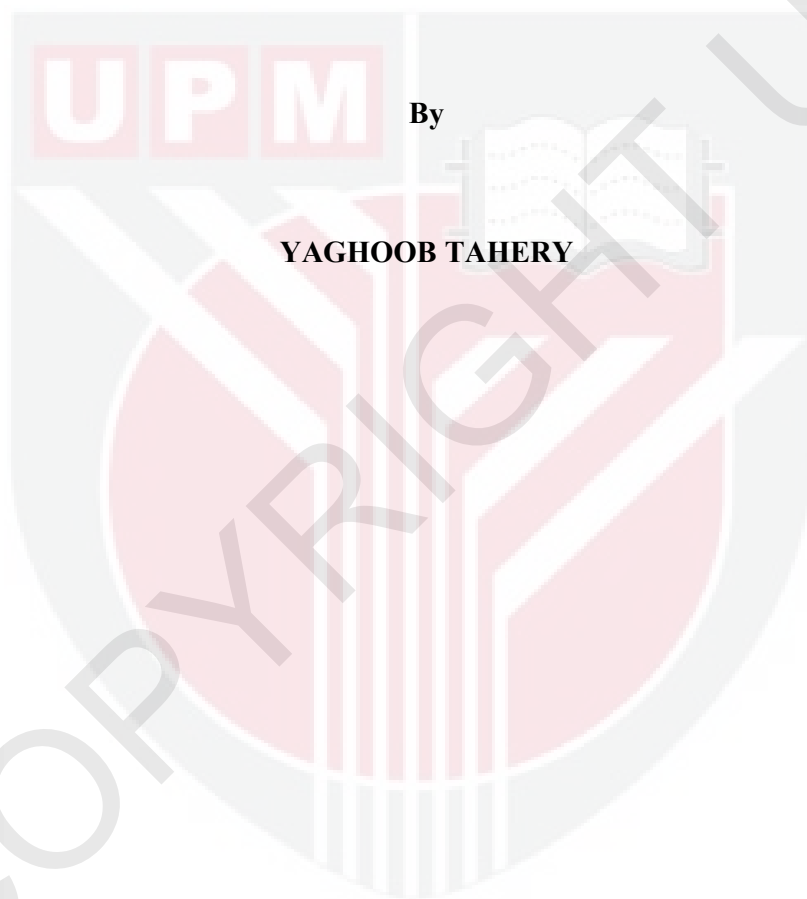
UNIVERSITI PUTRA MALAYSIA

***RESPONSES OF SOME KENAF (*Hibiscus cannabinus* L.) VARIETIES TO
ROOT KNOT NEMATODE INFECTION***

YAGHOOB TAHERY

IPTPH 2012 1

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By

YAGHOOB TAHERY

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

June 2012

DEDICATION

Dedicated to:

All members of my family, especially my parents, brothers and sister who have supported and encouraged me.

My dear wife and sons Mohammad and Ali for their love, moral support, patience and understanding during the course of my study.

ABSTRACT of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

**RESPONSES OF SOME KENAF (*Hibiscus cannabinus* L.) VARIETIES TO
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June 2012

Chairman: Professor Nor Aini Ab Shukor, PhD

Institute: Institute of Tropical Forestry and Forest Products

Kenaf is a fast growing crop cultivated for its natural fibre. It is one of the potential multipurpose crop species in Malaysia which thrives relatively well in less fertile BRIS (Beach Ridges Interspersed with Swales) soils. Despite growing relatively well in this area, there is a threat of infection with Root Knot Nematode (RKN) which can potentially reduce the growth and yield production of this crop. However, the knowledge on nematode infection that may affect gas exchange characteristics and enzymatic defence mechanisms of kenaf still remains limited. Moreover, as traditional method of nematode management is time consuming, laborious and associated with health and environmental concerns, thus the host resistance and screening based on molecular markers would be a preferable choice to apply.

The study evaluated disease incidence of RKN and its severity on kenaf plants cultivated in sandy BRIS soils in Telaga Papan, Terengganu, Malaysia following which a subsequent screening was made on the *Hibiscus cannabinus* varieties for

their growth, productivity, resistance, physiological and enzymatic responses to RKN infections. In addition, the study determined the genetic mode of the RKN resistance inheritance in the F₂ populations those were derived from crosses of resistant and susceptible plants which was further verified by the ISSR markers which were associated with this resistance.

Investigation on kenaf cultivated in BRIS soils exhibited a severe nematode infection (*Meloidogyne incognita*) of Race 1, which was significantly correlated with the reduction in height ($R^2 = -0.73$), diameter ($R^2 = -0.60$) and number of nodes ($R^2 = -0.70$). Screening of sixteen kenaf varieties from different origin was conducted using three levels of artificial inoculation viz; 1000, 5000 and 10000 eggs per pot under controlled condition with untreated plant as control showed that the average height and stalk diameter of the infected plants were reduced by 10.62% and 9.95% respectively during harvesting time of 120 days. The effects were significantly different between varieties and time of measurement. Variety KK60 (M) outperformed the others with height (214.44 cm), stem diameter (14.19 mm) and number of nodes (52) while variety 3740 performed the poorest in terms of height (115.89 cm) and stem diameter (9.94 mm) and variety 113 produced the lowest number of nodes (32) upon RKN infection.

Inoculation with *M. incognita* decreased kenaf height and diameter at a range of 0.37% to 24.79% and 1.4% to 16.5% respectively. Variety 113, KK60 (Aust) and Everglades 41 recorded the highest reduction in height (24.79%), diameter (16.5%) and internodes number (18.5%) respectively. On the contrary, variety 4638 recorded the least decrement in height (0.37%) and diameter (1.4%) respectively. Resistant

variety (Gregg) recorded reduction in height and stalk diameter of 9.84% and 3.04% respectively while the susceptible variety (Everglades 41) produced bigger reduction of 14.55% and 8.46% for similar parameters. The highest reduction of height and diameter occurred in the third month and the first month respectively. Different varieties reacted differently with levels of nematode inoculation for all characteristics studied here. In addition, infected plants also recorded bigger losses in yield than the control.

Measurement of gas exchange characteristics in infected plant showed that nematode has decreased the values of Assimilation rate (A), transpiration rate (E), intercellular CO₂ concentration (ci) and Vapour Pressure Deficit based on Leaf temperature (VPDL) by 22.50%, 32.30%, 21.62% and 34.80% respectively whilst increased both water use efficiency and intrinsic water use efficiency. The different varieties used in the experiment also showed different responses of gas exchange to nematode infection.

Susceptible variety showed higher assimilation rate and water use efficiency than the resistant variety. In turn, the value of stomatal closure, transpiration rate, VPDL and intrinsic water use efficiency ($A: gs$) for susceptible was lower than the resistant variety.

Examining of individual plant based on three (3) parameters i.e. gall index ratings, egg mass index and egg per gramme root of plant showed that variety Everglades 41 is more susceptible to *M. incognita* Race 1 having value of 4.33, 4.89 and 16985 as compared with variety Gregg that exhibited resistance response with value of 1.67,

2.33 and 2691 for the above mentioned parameters respectively.

Study on biochemical basis of resistance to *M. incognita* in kenaf showed a significant increase of peroxidase activity in the roots of both susceptible and resistant plants when compared to the non-inoculated control plants. The resistant host exhibited five times higher peroxidase activity than the susceptible ones upon infection with nematode.

Examining of resistance to RKN using gall index in F₁ and F₂ populations showed segregation pattern that fits the Mendelian ratio of 1: 3 (resistant: susceptible). Further investigation using sixty (60) Inter Simple Sequence Repeat (ISSR) primers revealed polymorphism between resistant and susceptible parents. Two markers ISSR 807 and ISSR 837 were found to be associated with RKN resistance trait. The phenotype variation explained by these markers accounted by 33.88% and 33.71% respectively.

In conclusion, variety KK60 is the most recommended variety to be planted on sites which are prone to RKN infestation such as BRIS soils eventhough it was not found to be resistant against RKN. This is based on its overall growth performance and yield productivity upon infection with RKN. The study also revealed that screening for resistance against RKN using ISSR markers is an important reliable tool to be incorporated in kenaf improvement programme.

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

**TINDAKBALAS BEBERAPA VARIETI KENAF (*Hibiscus cannabinus*)
TERHADAP JANGKITAN ROOT KNOT NEMATOD**

Oleh

YAGHOOB TAHERY

Jun 2012

Pengerusi: Profesor Nor Aini Bt Ab Shukor, PhD

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Kenaf ialah tanaman yang berkembang pesat sebagai sumber serat semulajadi berkos rendah. Ia adalah tanaman baru di Malaysia di mana ia boleh ditanam terutamanya di tanah BRIS yang kurang subur. Kajian terhadap penanaman kenaf di tanah BRIS bagaimanapun mendapati bahawa kebanyakannya mengalami jangkitan nematod yang teruk (*Meloidogyne incognita*) yang mampu menjejaskan pertumbuhan dan kualiti pengeluaran hasilnya. Pengetahuan tentang kesan jangkitan nematod terhadap ciri-ciri pertukaran gas dan mekanisma pertahanan enzim kenaf masih terhad. Oleh kerana kaedah tradisional pengurusan nematod memakan masa, sukar, dan tertakluk kepada kepentingan kesihatan dan alam sekitar, pemilihan dan saringan perumah yang mempunyai ketahanan adalah pilihan yang praktikal untuk diaplikasikan.

Kajian ini menilai kejadian jangkitan RKN dan kesan kerosakan jangkitan ke atas penanaman kenaf di tanah BRIS di Telaga Papan, Terengganu, Malaysia. Ia diikuti dengan saringan beberapa varieti kenaf ke atas pertumbuhan, produktiviti, kerintangan dan tindakbalas dari segi fisiologi dan enzim terhadap jangkitan RKN.

Kajian ini juga menentukan warisan mod genetik ketahanan RKN dalam populasi F_2 yang didapati apabila tumbuhan yang tahan dikacukkan dengan tumbuhan yang rentan bagi menentukan petanda ISSR yang dikaitkan dengan ketahanan.

Kajian ke atas kenaf yang ditanam di tanah BRIS menunjukkan jangkitan nematod yang teruk (*Meloidogyne incognita* Race 1) yang berhubungkait dengan penurunan ketara dalam ketinggian ($R^2=$ 0.73), diameter ($R^2=$ 0.06) dan bilangan ruas ($R^2=$ 0.7).

Saringan ke atas enam belas varieti kenaf / genotip yang mempunyai asal usul yang berlainan telah dijalankan dengan menggunakan kaedah inokulasi buatan menggunakan tiga tahap telur yang berbeza, 1000, 5000, 10000 di bawah persekitaran terkawal dan tumbuhan tanpa rawatan sebagai kawalan. Tumbuhan yang dijangkiti menunjukkan purata penurunan ketinggian sebanyak 10.64% dan diameter batang sebanyak 9.95% selepas 120 hari masa penuaian. Berbanding dengan semua varieti yang dikaji, varieti KK60 (M) menunjukkan nilai tertinggi dalam ketinggian (214.44 cm), diameter batang (14.19 mm) dan bilangan ruas (52). Varieti 3740 pula menunjukkan nilai terendah dalam ketinggian (115.89 cm) dan diameter batang (9.94 mm) manakala varieti 113 pula menunjukkan nilai bilangan ruas yang terendah iaitu 32 apabila dijangkitkan dengan RKN.

Inokulasi *M. incognita* dengan kenaf menyebabkan penurunan dalam ketinggian sebanyak 0.37% hingga 24.79% dan diameter sebanyak 1.4% hingga 16.5%. Varieti 113, KK60 (Aust) dan Everglades 41 masing-masing menunjukkan penurunan yang ketara dalam ketinggian (24.79%), diameter batang (16.5%) dan bilangan antara ruas

(18.5%). Sebaliknya varieti 4638 adalah yang paling kurang menerima kesan dalam ketinggian dan diameter dengan masing-masing menunjukkan penurunan sebanyak 0.37% dan 1.4%. Varieti tahan menunjukkan penurunan dalam ketinggian dan diameter batang sebanyak 9.84% dan 3.04% dan varieti rentan menunjukkan penurunan yang lebih ketara sebanyak 3.04% dan 8.46% dalam parameter yang sama. Penurunan yang ketara dalam ketinggian dapat diperhatikan pada bulan ketiga manakala penurunan dalam diameter batang pula dapat diperhatikan pada bulan pertama bagi kesemua varieti dan tahap telur yang dikaji. Varieti yang berbeza menunjukkan tindak balas yang berbeza pada setiap tahap inokulasi nematod untuk semua ciri-ciri yang dikaji. Tumbuhan yang dijangkiti mengalami kesan kehilangan kualiti hasil yang lebih besar berbanding dengan tumbuhan yang dikawal.

Pengukuran pertukaran gas terhadap jangkitan nematod menunjukkan penurunan dalam kadar asimilasi (A), kadar transpirasi (E), kepekatan intercellular CO_2 (ci) dan deficit tekanan wap berdasarkan kepada suhu daun (VPDL) dengan masing-masing mempunyai nilai 22.50%, 32.30%, 21.62% dan 34.80% sebaliknya meningkatkan nilai kecekapan penggunaan air dan kecekapan penggunaan air intrinsik. Kajian ini juga menunjukkan tindakbalas pertukaran gas yang berbeza dari varieti yang berlainan.

Varieti rentan menunjukkan nilai kadar asimilasi dan kecekapan penggunaan air yang tinggi berbanding dengan varieti tahan. Manakala kadar penutupan stomata, kadar transpirasi, VPDL dan kecekapan penggunaan air intrinsik (A:gs) bagi varieti rentan adalah rendah berbanding dengan varieti tahan.

Pemeriksaan ke atas setiap individu berdasarkan kepada tiga parameter iaitu indeks taraf bengkok, indeks jisim telur dan bilangan telur akar per gram menunjukkan varieti Everglades 41 adalah lebih rentan kepada *M. incognita* Race 1 dengan nilai masing-masing 4.33, 4.89 dan 16985 manakala variety Gregg pula menunjukkan tindakbalas ketahanan dengan nilai masing-masing 1.67, 2.33 dan 2691.

Kajian tindak balas asas biokimia tumbuhan terhadap rintangan kepada *M. incognita* dalam kenaf menunjukkan peningkatan ketara aktiviti peroksidaan dalam akar kedua-dua tumbuhan rentan dan tahan berbanding dengan tumbuhan kawalan yang tidak diinokulat. Walau bagaimanapun, perumah yang tahan mempamerkan aktiviti peroksidaan yang lebih ketara daripada yang perumah yang rentan apabila dijangkiti nematod.

Pemeriksaan terhadap heritabiliti daya tahan terhadap serangan, kacukan induk dilakukan sehingga ke peringkat F_2 menunjukkan indeks bengkok bagi rintangan terhadap serangan nematod diasingkan dalam nisbah Mendelian 1:3 (tahan: rentan). Populasi F_1 dan F_2 serta kedua-dua induk telah dikaji menggunakan 60 primer Inter Simple Sequence Repeat (ISSR), mendedahkan polymorfisma di antara ibu yang tahan dan rentan dengan serangan. Daripada jumlah ini, 2 penanda iaitu ISSR 807 dan ISSR 837 didapati berkaitan dengan sifat daya rintangan terhadap RKN dalam kenaf. Perubahan fenotip yang dijelaskan oleh ISSR 807 adalah 33.88%, diikuti oleh ISSR 837 dengan 33.71%.

Kesimpulannya varieti KK60 adalah varieti yang menjadi pilihan dan disarankan untuk ditanam dikawasan yang cenderung terhadap jangkitan RKN seperti tanah

BRIS berpasir walaupun ianya didapati tahan terhadap RKN. Ini berdasarkan kepada keseluruhan pertumbuhan dan kualiti pengeluaran hasilnya apabila dijangkitkan dengan RKN. Kajian juga menunjukkan bahawa pemeriksaan ketahanan terhadap RKN dengan menggunakan petanda ISSR adalah kaedah penting yang boleh dipercayai untuk diaplikasikan di dalam program penaiktarafan kenaf.



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I certify that a Thesis Examination Committee has met on 13 June 2012 to conduct the final examination of Yaghoob Tahery on his thesis entitled “Responses of some kenaf (*Hibiscus cannabinus* L.) varieties to root knot nematode infection” 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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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.



YAGHOOB TAHERY

Date: 13 Jun 2012

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