VARIABILITY AMONG ECOTYPES OF BARNYARDGRASS (ECHINOCHLOA CRUS-GALLI VAR. CRUS-GALLI (L.) BEAUV.) AND ITS SUSCEPTIBILITY TO EXSEROHILUM LONGIROSTRATUM

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DOCTOR OF PHILOSOPHY UNIVERSITI PUTRA MALAYSIA

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# VARIABILITY AMONG ECOTYPES OF BARNYARDGRASS (*Echinochloa crus-galli* var. *crus-galli* (L.) Beauv.) and its susceptibility to *Exserohilum longirostratum*

Ву

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

February 2005

# DEDICATION

This thesis is specially dedicated to my beloved:

Wife **Tuti Murdiati** Daughters **Mutia Febryani Arifin Anisa Nursyawaliani Arifin** Son **Rifky Rahmanda Arifin** 

for the unconditional patient, love and support

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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 currently submitted for any other degree at UPM or other institutions.

ARIFIN TASRIF

Date:

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

# VARIABILITY AMONG ECOTYPES OF BARNYARDGRASS (Echinochloa crus-galli var. crus-galli (L.) Beauv.) AND ITS SUSCEPTIBILITY TO Exserohilum longirostratum

By

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### February 2005

### Chairman : Abdul Shukor Juraimi, Ph.D.

Faculty : Agriculture

Barnyardgrass (*Echinochloa crus-galli* var. *crus-galli* (L.) Beauv) is one of the most noxious weeds found in rice fields of both Malaysia and Indonesia. Its diversity in morphological and molecular traits within and between the regions of Malaysian and Indonesian ecotypes was investigated in this study in relation to its susceptibility to potential biocontrol agent using fungal *Exserohilum longirostratum*.

A total of 85 individual ecotypes found in rice fields of the two countries were characterized. Fifty-five ecotypes from eleven different states in Malaysia and 30 ecotypes from three sub-regions (Sumatera, Java and Sulawesi islands) of Indonesia were collected.

Morphological (vegetative and reproductive characters) and molecular (RAPD-PCR) studies for diversity assessment within and between Malaysian and Indonesian ecotypes were employed. Results from morphological studies indicated that there was variability within the Malaysian and Indonesian ecotypes due to difference in characters such as plant height, panicle awn, panicle length, plant type, and number of tillers with 95.77 and 100% of total variability. To some extent, percent germination, seedling growth characters and life cycles for Malaysian ecotypes from Perlis, Kedah, Perak, Kelantan, and Johor were relatively faster than Indonesian ecotypes from Lampung, Banten, West Java, Central Java, East Java, and South Sulawesi.

Molecular (RAPD-PCR) analysis of 26 Malaysian and 14 Indonesian barnyardgrass ecotypes were amplified using four primers (A 07 A 20, OPG 06 and OPAE 12), which amplified a total of 533 and 266 repeatable fragments. The RAPD variation between Malaysian and Indonesian ecotypes ranged from 85.7 to 89% and 67 to 80%. The analysis of genetic similarity revealed four distinct clusters with an average similarity of 47% for Malaysian ecotypes, while 64% for Indonesian ecotypes. The results of the study indicated that the close relationship within and between Malaysian and Indonesian ecotypes were mainly due to the close vicinity in geographic locations.

Taking into consideration that the barnyardgrass found in both countries are quite similar in characters. The response of barnyardgrass ecotypes to the virulence of fungal *Exserohilum longirostratum*, a potential biocontrol agent was evaluated. Results of this study indicated that susceptibility varied for Malaysian and Indonesian ecotypes in glasshouse and mini-plot experiments. Plant growth of 1-2- and 2-3-leaf stages were observed to be the most susceptible growth stage to *E. longirostratum* at a concentration of  $1 \times 10^7$  and  $2.5 \times 10^7$  conidia/mL when exposed to 24 hours of dew period. However, seedlings mortality decreased slightly with increase in plant age (4-5- and 6- 7-leaf stages).

The most susceptible ecotypes were found in Kedah, Perak, Kelantan, Lampung and Central Java as indicated by the high value of the AUDPC (594 to 641) and high epidemic rate (<sup>r</sup>L) (0.358 to 0.485 unit/day), while susceptible ecotypes

were found in Melaka, Selangor, Terengganu, Banten and South Sulawesi with AUDPC value of 340 to 387 and epidemic rate (<sup>r</sup>L) value of 0.193 to 0.254 unit/day. These ecotypes also showed significant increase in seedling mortality (87 to 92%) compared to other ecotypes. These studies have demonstrated differential susceptibility within and between weed population diversity.

The results obtained from this study indicated that *E. longirostratum* has the potential to be used as a mycoherbicide against barnyardgrass ecotypes. However further studies such as increased conidia concentration with different surfactants, formulation, and characterization of phytotoxic compound to enhance the virulence for satisfactory control of barnyardgrass in rice fields conditions are required.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan<sub>v</sub>untuk ijazah Doktor Falsafah

### KEPELBAGAIAN DI ANTARA EKOTIP RUMPUT SAMBAU (*Echinochloa crus-galli* var. *crus-galli* (L.) Beauv.) DAN KERENTANAN KEATAS Exserohilum longirostratum

Oleh

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Rumput sambau (*Echinochloa crus-galli* var. *crus-galli* (L.) Beauv) merupakan salah satu rumpai noksius sawah padi di kedua-dua negara Malaysia dan Indonesia. Kajian kepelbagaian ciri morfologi dan molekular di dalam dan di antara ekotip Malaysia dan Indonesia serta hubung kait ke atas kerentanan terhadap kawalan biologi menggunakan kulat *Exserohilum longirostratum* telah dijalankan.

Sejumlah 85 ekotip dari ke dua dua negara telah dicirikan. 55 ekotip diperolehi dari sebelas negeri di Malaysia dan 30 ekotip lagi dari tiga sub-kawasan (Sumatera, Jawa dan Sulawesi) di Indonesia.

Diversiti di antara ekotip rumput sambau dijalankan berdasarkan ciri morfologi (ciri vegetatip dan reproduksi) dan teknik molekular (penanda RAPD). Kajian morfologi mendapati bahawa kepelbagaian ekotip daripada Malaysia dan Indoensia adalah disebabkan kepada beberapa ciri-ciri tinggi pokok, jejanggut, panjang panikel, panjang spikelet, jenis pokok, dan jumlah tiler dengan keseluruhan keperbagaian adalah 95.77 dan 100%. Secara am nya, ciri seperti peratus percambahan, sais cambah kenih dan kitaran hidup ekotip Malaysia (Perlis, Kedah, Perak, Kelantan dan Johor) adalah lebih cepat berbanding ekotip Indonesia (Lampung, Banten, Jawa Barat, Jawa Tengah, Jawa Timur dan Sulawesei Selatan).

Empat primer (A 01, A 20, OPG 06 dan OPAE 12) digunakan bagi pengecaman ciri molekular (RAPD-PCR) ke atas 26 ekotip Malaysia dan 14 ekotip Indonesia, dengan hasil amplifikasi 533 and 266 frakmen. Variasi RAPD di antara ekotip ke dua-dua kawasan tersebut adalah 85.7-89% dan 67-80%. Analisis persamaan genetik menampakkan empat kluster dengan purata persamaan di antara kluster ekotip Malaysia adalah 47%, manakala ekotip Indonesia adalah 64%. Hasil kajian ini mendapati bahawa hubungan kedekatan di dalam dan di antara ekotip Malaysia dan Indonesia terutamanya berdasarkan kepada kedekatan lokasi geografis.

Memandangkan bahawa pencirian rumput sambau di kedua-dua negara adalah lebih kurang sama. Pengaruh kepelbagaian ekotip rumput sambau ke atas tahap virulen kulat