Establishment of Regeneration and Transformation Systems for Some Sugarcane Cultivars

Thesis Submitted For Degree of
Doctor of Philosophy of Science
in Botany

By

Khaled Hashem Radwan Mohamed
B.Sc. (1988) - Botany
M. Sc. (2005) - Botany

Ain Shams University
Faculty of Science
Botany Department
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This thesis has not been previously submitted for any degree at this or any other university.

The reference in the text will show specifically the extent to which I have availed myself of the work of other authors.

Khaled Hashem Radwan Mohamed

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Dedication

To

The Soul of My Mother

A strong and gentle soul who taught me
to trust in Allah, believe in hard work
and that so much could be done with little.

The Soul of My Father

For earning an honest living for us,
supporting and encouraging
me to believe in myself.

My Brother, My Sisters

For being there when I most
needed them.

Abstract

Sugarcane cultivations are affected by a number of fungal diseases; among these is Pokkah boeng which is caused by a fungus; *Fusarium moniliformis var.* Plant defensins and chaitinases are produced by Plants to protect them against fungal infection. Defensins are small cysteine-rich peptides which belong to a group of pathogenasis-related defense mechanism proteins. These proteins inhibit the growth of a broad range of microbes and are highly stable under extreme environmental stresses. Chitinases are endoglucanase enzymes that cleave the internal β-1,4-N-acetyl-D-glucos-amine linkages in chitin polymers; the major component of the cell wall of fungi. The plant vector pMON22653 harboring defensin (*def*1) gene derived from Medicago sativa, under the control of the CaMV 35S promoter and the plant vector pGL2 that harbors a class I rice chitinase gene (*chi*11) under the control of the CaMV 35S promoter and a hygromycin resistant gene as a plant selectable marker were used to transform sugarcane explants to develop plants resistant to Pokkah boeng. The fusarium-susceptible sugarcane cultivars C9 and PH were used to establish a regeneration system for sugarcane. For its high regenerabilty; C9 was further selected to develop transgenic sugarcane. Leaf disks were used as explants and transformation was performed using the biolistic delivery system. Transformation and transcription of transgenes were confirmed by Southern hybridizations and reverse-transcription PCR (RT-PCR). Greenhouse bioassay was performed on the transgenic pants by challenging with a vigorous isolate of the fungal pathogen

Fusarium moniliformis var. The level of fungal infectivity was determined using RT-PCR with specific primers. Transgenic lines were more resistant to infection by fusarium than the control plants. Results indicated that plants overexpressing defensin conferring a better resistance to fungal pathogens than these overexpressing chitinase. Nevertheless, expression of both chitinase (*chi*11) and defensin (*def*1) genes into sugarcane plants acquired them antifungal resistance against fusarium infection.

تأسيس نظامي إعادة التمايز والتحول الوراثي لبعض أصناف نباتات قصب السكر

رسالة مقدمة
للحصول علي درجة دكتوراة الفلسفة في العلوم في النبات (فسيولوجيا النبات)

من
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