بسم الله الرحمن الرحيم

MONA MAGHRABY

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

MONA MAGHRABY

شبكة المعلومات الجامعية

التوثيق الإلكتروني والميكروفيلم

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

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MONA MAGHRABY

STUDIES ON *AGROBACTERIUM*-MEDIATED GENETIC TRANSFORMATION OF RICE USING *DREB2A* GENE

By

GEHAD MOHAMED AHMED MOHAMED

B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2015

THESIS

Submitted in Partial Fulfillment of the

Requirements for the Degree of

MASTER OF SCIENCE

In

Agricultural Sciences

(Genetics)

Department of Genetics

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Formate reviewer **Vice Dean of Graduates Student**

APPROVAL SHEET

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Date: / /

SUPERVISION SHEET

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Title of Thesis: Studies On *Agrobacterium*-Mediated Genetic Transformation of Rice Using *DREB2A* Gene
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Department: Genetics **Approval:** / /

ABSTRACT

Transgenic technology offers a solution for meeting the increasing commercial demand on economically important plants such as rice (*Oryza sativa*), a widespread dietary staple. However, significant genotype-specific morphogenetic responses constitute a considerable base on rice regeneration in plant biotechnology contexts. The primary aim of this study was to develop an efficient regeneration and transformation system for two Egyptian rice cultivars, namely; Sakha104 and Giza178. In addition, the *DREB2A* gene was used for transforming rice explants in an attempt to enhance their abiotic stress tolerance. A variety of factors were investigated for maximum efficiency of both regeneration and transformation. Casein hydrolysate proved to improve callus induction and shoot regeneration of both cultivars. Although tryptophan demonstrated a stimulatory effect on callus induction of Sakha104 cultivar, it showed no positive effect on callus initiation of Giza178 genotype and shoot regeneration of both cultivars. The results indicated that gellan gum (gelrite and phytagel) was superior to agar products (agar and bacto agar) for callus induction. By contrast, no significant differences were found between different gelling agents for shoot regeneration. Sakha104 cultivar showed higher regeneration frequency compared with Giza178 cultivar. Two- to three-week-old scutellum callus served as an excellent starting material for transformation. These explants were inoculated with *Agrobacterium* strain LBA4404 harboring binary vector pCAMBIA1301 which contained *DREB2A* , *GUS* and *hygromycin* resistance genes. Mild wounding with either syringe or scalpel and sonication treatment increased significantly the efficiency of transformation. Similar to regeneration experiments, Sakha104 cultivar showed higher transformation frequency than Giza178 cultivar. Polymerase chain reaction and GUS analysis confirmed the presence of the transgenes. Transgenic rice leaves overexpressing *DREB2A* gene demonstrated better survival performance and higher chlorophyll content in the recovery phase after exposure to different levels of drought, salinity and heat stresses compared with non-transgenic control ones. It would be rewarding if this optimized protocol paved the way toward the genetic improvement of these important two cultivars.

Key words: *Oryza sativa* – Callus induction – Regeneration – *Agrobacterium*-mediated transformation – Abiotic stress tolerance.

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