

انتاج نباتات أرز محولة وراثياً مقاومة لثاقبة الساق “*Chilo agamemnon*”

رسالة ماجستير
في العلوم الزراعية
(وراثة)

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للحصول على درجة

الماجستير

في

العلوم الزراعية
(وراثة)

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كلية الزراعة
جامعة القاهرة
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المستخلص العربي

تم تصميم هذه الدراسة لمقارنة القدرة الكامنة على الكشف لسبعة اصناف من الارز المتباينة وراثيا باستخدام الاجنة الناضجة كمستقطع نباتي. تم تكوين كالوس جنيني على المستقطعات النباتية في خلال ثلاثة اسابيع في بيئة نمو تحتوى 3 mg/l من 2,4-D ونشأت الاجنة الجسدية مباشرة من الكالوس الجنيني بنقله الى بيئة تحتوى على ال BA بنسبة 3 mg/l و قد اظهر الصنف جيزة ١٧٨ اعلى نسبة تكشف (٣٩%) مقارنة بباقي الاصناف. وأظهرت النتائج المتحصل عليها من اختبار ال RAPD ان عدد الواسمات الكلية المتحصل عليها هو ٤٥ بالنسبة لاصناف الارز السبعة منهم ٢٩ واسم متباين بنسبة تباين 64.4% . ستة عشر واسم من ال 29 واسم المتباين وجد أنها متخصصة للأطعم الوراثية المستخدمة. كما تم دراسة الثبات الوراثي بين النباتات الناتجة من زراعة الانسجة باستخدام طريقة ال RAPD و اوضحت النتائج ان نسبة التماثل الوراثي بين النبات الام و النباتات الناتجة من زراعة الانسجة للصنف جيزة ١٧٨ وصلت الى ٩١%. و تهدف هذه الدراسة الى انتاج نباتات ارز مهندسة وراثيا تشفر لجين *cry1Ia5* لمقاومة الحشرات و تم استخدام الناقل البلازميدي *BPI-121* المحتوى على جين *cry1Ia5* تحت السيطرة الجينية للمشغل 35S والناهي *nos* و تم التأكد من نجاح نقل و ادماج جين ال *cry1Ia5* داخل جينوم النباتات الناتجة باستخدام كل من الاختبارات التالية ال PCR و ال Southern blot و ال Dot blot. ثم اختبار ثلاثة خطوط مهندسة وراثيا تشفر ل *cry1Ia5* بالنسبة لمقاومتها ليرقات ثاقبات الساق *Chilo Agamemnon*. قد وصلت نسبة الموت الى ١٠٠% في حالة تغذية اليرقات على هذه النباتات لمدة اربعة ايام مقارنة بالكنترول

الكلمات الدالة: جين ال *Cry1Ia5* ، الثبات الوراثي فى الارز، المقاومة للحشرات، اعادة الكشف فى الأرز، التحول الوراثي.

**PRODUCTION OF TRANSGENIC RICE PLANTS
RESISTANT TO STEM BORER “*Chilo agamemnon*”**

By

ETR HUSSEIN KAMEL MOHAMMED

B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2006

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ABSTRACT

The present investigation was conducted to compare seven agronomically and genetically different rice (*Oryza sativa* L.) cultivars for their capacity for callus induction and plant regeneration from mature zygotic embryos. White embryonic calli were formed within three weeks in the presence of 3 mg/l 2,4, D . The somatic embryos directly originated from the embryogenic callus in the presence of 3 mg/ l BA. The cultivar Giza 178 showed high regeneration frequency (39%).The data obtained from the random amplified polymorphic DNA analysis showed that a total of 45 DNA markers were detected among the seven rice cultivars of which, 29 bands were polymorphic (64.4%) .

Sixteen out of the twenty-nine polymorphic RAPD markers generated were found to be genotype specific. Genetic stability in tissue cultured rice plants was examined by randomly amplified polymorphic DNA (RAPD) analysis. The genetic similarity between the mother and the regenerated plants was high for Giza 178 (91%). The main objective of the present work was to produce transgenic Egyptian rice plantlets expressing the *cry1Ia5* gene which renders plant insect tolerance. The *pBI-121* plasmid harboring the *cry1Ia5* gene under the control of 35-S promoter and NOS terminator was used. The stable integration of the *cry1Ia5* gene was confirmed using PCR, Southern blot and Dot blot analyses. Three transgenic lines expressing the *cry1Ia5* gene were bioassayed for resistance to neonate larvae of stem borer (*Chilo agamemnon*). Larval mortality of up to 100% was recorded after four days comparing with the control plants.

Key words: *cry1Ia5* gene, Genetic stability, Insect resistance, Rice regeneration, Transformation.

DEDICATION

I dedicate this work to whom my heart felt thanks: to my father, mother, sister and brother and to Professor Sawsan Samy Youssef and Dr. Rēda Elwany Moghaieb for all the support and encouragement they continually offered along the period of my post-graduate studies.

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INTRODUCTION

Rice provides staple diet for more than one third of world's population. It accounts for as much as 75% of the caloric intake of the 2 billion humans living in Asia and as much as 33% of the caloric intake of nearly one billion humans in Africa and Latin America. It is the most important food in that part of the world where population densities are high and overall dietary levels are least adequate (Kinoshita and Mori, 2001).

Rice (*Oryza sativa* L. $2n=2x=24$), is an annual grass which belongs to the family Gramineae (Sun *et al.*, 1990). There are two cultivated species of rice *Oryza sativa* and *Oryza glaberrima*. *O. glaberrima* is cultivated only in a limited scale in West Africa. *O. sativa* has great variation and distribution and has traditionally been divided into two subspecies, *Indica* and *Japonica* (Goff *et al.*, 2002 and Yu *et al.*, 2002). Rice production in over 1.5 billion hectares of land has an overall worldwide production of 596 million tons per annum (FAO, 1995). because rice occupies approximately 9% of the planet's arable land, it is also a key area of concern and of opportunity in environmental protection. Asia produces more than 90% of this amount, and Latin America produces 5% (Khush and Brar, 2002 and Joshi and Behera, 2006).

In Egypt rice is considered as the second cash-export crop after cotton. Some of the Egyptian rice varieties are susceptible to the rice blast fungal pathogen *Pyricularia grisea* and rice stem borers *Chilo agamemnon* Bleszynski. The rice cultivation area in Egypt is