MOLECULAR GENETIC STUDIES FOR CORN RESISTANCE TO SOME BORERS

By

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B.Sc., Agric. Sci. (Agronomy), King Saud Univ., 1989

M.Sc., Agric. Sci. (Genetics), ENSA-Rennes, France, 1996

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ABSTRACT

Abdussalam Ibrahim Ali Tayeb. Molecular Genetic Studies for Corn Resistance to Some Borers. Unpublished Doctor of Philosophy Dissertation, Department of Genetics, Faculty of Agriculture, Ain Shams University, 2003.

Eight maize genotypes were evaluated in the field for their resistance, through artificial infestation, against the corn borer *Sesamia cretica*. The genotypes were classified into tolerant (5 genotypes) and sensitive (3 genotypes). SDS-protein, five isozyme systems and eleven random arbitrary primers were used to detect biochemical and molecular markers for maize resistance against *Sesamia* insect. Protein and isozyme patterns revealed inconsistent changes in gene expression of the studied genotypes in response to the insect attack. No clear-cut markers were detected utilizing these two systems. The results of RAPD-PCR analysis indicated a high level of polymorphism among the studied genotypes. Among the 181 fragments, 178 were polymorphic among maize genotypes and three fragments were monomorphic. In this study, 28 RAPD-PCR markers for maize resistance to *Sesamia cretica* were detected. Eight were negative markers and 20 were positive ones. A chitinase gene (*chi*) responsible for insects and pathogens toxicity was isolated from an endogenous strain of *Serratia marcescens.* After identification, the isolated gene was introduced into a suitable vector containing the *bar* cassette as a reporter gene and the *chi* gene which was governed by the ubi promoter to be expressible in maize plant cells. A protocol for maize immature embryo transformation by biolistic (gene gun) and plant regeneration was adjusted using a DNA vector carrying the GUS gene was used for maize transformation with the *chi* gene. One of the studied maize genotypes, with moderate resistance to the corn borer, was used as a target for *chi* gene transformation. Transformed embryos were developed into plants *via* tissue culture regeneration. The PCR test on DNA extracted from regenerated plantlets and Basta leaf painting confirmed the integration of both of the reporter and the *chi* genes in some of the regenerated maize plantlets.

Key words: Maize (*Zea mays* L.), corn borers, resistance, RAPD-PCR, SDS-PAGE, Isozymes, molecular markers, chitinase gene, biolistic, GUS, transient expression, maize immature embryos, transformation.

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I- INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereal crops in Arab countries, *eg*., Egypt, Yemen, *etc*. Cultivated area for grain corn production is about 714400[1] hectares in Egypt and 40521[2] hectares in Yemen with production of 5.8 million and 50384 tons of corn grains in each country, respectively. The worldwide gap between cereal crops production and consumption is continually widening. A major shift in global cereal demand is underway. By year 2020, demand for maize will surpass the demand for both wheat and rice. This shift will be reflected in a 50% increase in global maize demand from its 1995 level of 558 million tons to 837 million tons by year 2020[3]. The ever increasing demand for maize presents an urgent challenge for maize breeders to achieve substantial increases in corn grain yield. Given the limited opportunities for augmenting maize area in most countries, future output growth must come by vertically intensifying production on current maize land. Moreover, even in modern farming systems where improved maize seed is used, the gap between potential and actual yields is quite large because of the various biological (biotic) and environmental/ physical (abiotic) stresses faced by farmers. Maize production may be affected by insects attacking the plant organs in the field at any stage of maize production or by serious damages to the grains during storage. Its severity depends on germplasm used, cultivation practices, levels of pest infestation, control strategies used, and climate. Corn borers especially the great Sugarcane worm or the pink borer (*Sesamia cretica*) is one of the most important insect pests in Egypt. Even the best genetic materials often do not possess the tolerance and resistance needed to overcome the damages and yield losses caused by this insect. This insect typically produces one to three generations per year. Chemical control is ineffective once the borers have tunneled into the plant due to the inaccessibility of the chemical to the insect pest.

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[1] Agriculture Economy Year Book 1996, Ministry of Agriculture, A.R.E.

[2] Statistical Year Book 1996, Ministry of Planning and development, R.O.Y.

[3] http://www.cimmyt.org/Research/Economics/map/facts_trends/maizeft9900/pdfs/maizeft9900_Part1a.pdf

The resistance genes transfer into elite cultivars *via* traditional breeding can take up to 15-20 years. This process is considerably accelerated, however, by using molecular markers generated *via* random fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) techniques. These techniques make it possible to screen segregating populations molecularly rather than for diseased phenotypes, which is time-consuming, environment-dependent and labor-intensive. Marker-assisted breeding programs have been estimated to reduce the time-to-market by 50-70% (**Tanksley *et al*., 1989**).

Plant genetic manipulation as part of a breeding program can make a significant contribution in the production of insect-resistant crops and offers advantages over using conventional plant breeding alone and enables the desired gene(s) to be transferred to the recipient plants without the co-transfer of undesirable characteristics, thereby greatly speeding up the development of new varieties, and also allows the transfer of genes across incompatibility barriers. With genetic engineering, genes can be introduced from sources which are wholly unavailable to conventional plant breeding. Worldwide, the beneficial economic impact of plant biotechnology has so far been almost exclusively on crops of high economic importance including maize. The development of corn transformation methodology **(Fromm *et al*., 1990)** created the opportunity to protect corn plants from insect feeding damage using genes isolated from different sources, *ex.* the bacterium *Bacillus thuringiensis*. Several laboratories have developed transgenic crop plants producing chitinase enzyme as a source of insects and pathogen resistance **(Cornelissen and Melchers, 1993; Chanprame and Widholm, 1996).** However, the proportion of transgenic crops grown in developing countries increased from 14% in 1997 to 24% in 2000 and transgenic maize crop grown worldwide is in an area of about 10.3 million hectares[4].

The objectives of this study are:

[4]http://www.cimmyt.org/Research/Economics/map/facts_trends/maizeft9900/pdfs/maizeft9900_Part1a.pdf

1- Detection of molecular markers for maize resistance to the corn borer *Sesamia cretica* in the studied genotypes using SDS-Protein, isozymes and RAPD-PCR.

2- Isolation of chitinaseB gene from the bacterium *Serratia marcescnce*. This gene was found to produce the chitinase enzyme which hydrolyses the chitin existing in a high concentration in the insect cuticle or the fungus cell wall. Maize plant transformation with this gene would enhance its resistance against the corn borers and the fungal diseases.

3- Identification and sequencing the isolated gene.

4- Incorporation of the isolated gene into an appropriate plasmid vector suitable for gene expression in maize.

5- Maize transformation with the obtained recombinant DNA construct.

6- Confirmation of gene integration by PCR and the Basta leaf painting.