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مركز الشبكات وتكنولوجيا المعلومات

قسم التوثيق الإلكتروني



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جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

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**LASER –MEDIATED GENE TRANSFER IN EGYPTIAN
WHEAT *Triticum aestivum* L.**

THESIS

B18439
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Submitted in Partial Fulfillment of the Requirements
for the Degree of

DOCTOR OF PHILOSOPHY

In

Laser in Biotechnology

By

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2006

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To My Father's Soul

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ABSTRACT

Wheat is one of the most important cereal crops worldwide, with the largest harvested area and production levels (occupies approximately 20% of the world's cultivated land). In Egypt, the cultivated area increased to 1.6 million feddans.

For thousands of years, the only method available for the introduction of new characteristics into plants has been conventional breeding involving sexual hybridization, followed by the selection of plants with the most desirable phenotypes. However, plant breeding is a lengthy process, taking ten to fifteen years to produce and to release a new variety. Plant genetic engineering not only accelerates the process of plant breeding, but enables the introduction of new genes, either by overcoming the barriers of sexual incompatibility through somatic hybridization or by the introduction of specific genes into plant cells using transformation methodologies Southgate *et al.* (1995).

As monocotyledonous plants, wheat has lagged behind dicotyledonous plants in ease and efficiency of genetic transformation. Thus, a reliable procedure of transformation and regeneration for wheat tissues are needed. Rice was the first major cereal crop transformed by the use of direct DNA delivery into regeneration-competent protoplasts Toriyama *et al.* (1988). In cereals other than rice, it has been very difficult to regenerate fertile plants from protoplasts. Additionally, it has been difficult to maintain regenerable long-term suspension cultures in cereal crops Redway *et al.* (1990); Vasil *et al.* (1992). The efficient production of fertile transgenic wheat was accomplished by using callus cultures as target tissues and microprojectile bombardment as the mechanism of DNA delivery Weeks *et al.* (1993); Bahieldin *et al.* (2000). This methodology opened the way to overcome many problems of wheat transformation.

Recently, laser technology also provides novel approaches to the genetic manipulation of plants Grishko *et al.* (1999). Laser microbeam was found to be a

tool in gene transfer in rice Guo *et al.* (1995), and *Brassica napus* L. Weber *et al.* (1990) and (1988).

The present work is concerned with:

I. Laser microbeam cell surgery as a new technology for gene transfer in Egyptian wheat *Triticum aestivum* L. Cv Giza 164.

II. Study the mutagenic effects of different laser types and doses on the regenerated callus and plants from irradiated immature embryos of Egyptian wheat *Triticum aestivum* L. Cv Giza 164, this study involved:

- 1- Molecular level (DNA and protein).
- 2- Morphological level.

Immature embryos of *Triticum aestivum* L. cv. Giza 164 were used in this experiment as the explant source for laser microbeam mediated transformation with the reporter genes GUS and *bar*.

Under aseptically condition the calli of immature embryos were arranged on a specific slide and the plasmid mixed with TW medium or osmotic medium was added and covered the slide, irradiated by the following setup: optical and mechanical setup similar to that used by Guo *et al.* (1995) was used with the following modification:

- I. The laser system used here was Lambda Physics Excimer Laser with wavelength 308, 6nm, 6ns pulse with energy 13 mj and repetition rate up to 200Hz..
- II. The mechanical system developed here using two Orile stepper motors in X-Y directions allowing lateral motion of 40 microns with a time interval matching the laser repetition rate.
- III. The whole system was computer controlled using our own software.

Using this home made setup for gene transfer in wheat proved to be a powerful technique as we could introduce genes into 1.5 million cells in about 2 hours only resulting in a homogenous distribution of punctured cells. This result was confirmed here by GUS histochemical assay of putative transgenic callus.

PCR reaction was conducted to ensure the presence of the genes of interest in the transformed and control calli. Moreover, the expression of *bar* gene was tested on

the RNA extracts of the treated calli with laser microbeam after 1 or 7 days from treatment when exogenous DNA dissolved in TW or osmotic media and control.

Finally, the Raman spectroscopic technique was used here for the first time to characterize the DNA extracted from the Egyptian wheat before and after gene transfer as well as after exposure to different types of laser irradiations, mainly: Ar⁺, Ho-Cd, He-Ne and diode laser.

Analysis of the results clearly showed that, different types of laser irradiation have affected the morphological parameters of the regenerated plants from the irradiated immature embryos; these could be summarized as follows:

[1] Most of the lasers used here, were found to cause an improvement (i.e., an increase) in the studied parameters. Some of these increases are highly significant, significant or not significant.

[2] Most of these results accorded well with the previously literatures.

The intensities of all recorded Raman bands showed a remarkable increase as compared with control samples.

Our results confirmed that the use of UV laser microbeam as a new technique for gene transfer did not affect physiochemical properties of DNA molecules.