Transgenic plant production using particle bombardment in Egyptian barley

(Received: 01. 09. 2006; Accepted: 10 .09.2006)

Mohamed Abdallah
Agricultural Genetic Engineering Research Institute, 9 Gamaa St. Giza, Egypt.

ABSTRACT

A system for Egyptian barley (Hordeum vulgare L. cv Giza 123) transformation via biolistic bombardment has been established. Immature embryos 1.0-1.5 mm in length were aseptically isolated from barley plants cultivated in the open field. Embryos were then cultured on callus induction medium with scutellum side up. Scutellum derived calli were bombarded with the plasmid pAHC25, containing the marker gene uidA as a reporter gene and bar gene as a selectable marker gene. Bombarded tissues were transferred into callus induction medium containing bialaphos for selection. Calli grown on selection medium were transferred into regeneration medium supplemented with bialaphos. Putative transgenic callus events produced putative transgenic plants. Integration of transgenes was confirmed by molecular analysis. The expression of uidA genes was studied by histochemical staining while the expression of bar gene was studied by leaf paining assay technique.

Key words: *Barley, uidA, bar, plasmid, transformation, regeneration, biolistic bombardment, PCR, scutellum, immature embryos.*

INTRODUCTION

Barley is one of the most important crop plants in the world and has also received considerable attention in tissue culture and genetic transformation research (Hagio *et al*., 1995). Regeneration of fertile plants from protoplast culture was reported by Jahne *et al*. (1991). Lazzeri *et al*. (1991) obtained stable transformed callus tissues using polyethylene glycol induced DNA uptake by barley protoplasts. By that time, most varieties of barley were still recalcitrant to protoplast cultures, with only a few genotypes being responsive to culture conditions (Hagio *et al*., 1995).

Transfer of DNA mediated by *Agrobacterium tumefaciens* provides a desirable alternative to protoplast-dependent system in many dicotyledonous species, but use of the bacterium for transforming important cereal crops has thus far been hindered by the limited host range of the bacterium (Fraley *et al*., 1986). For these reasons Sanford and coworkers (Sanford *et al*., 1987; Klein *et al*., 1987) have developed a method whereby substances can be delivered into cells of intact tissues *via* particle bombardment process. Small high-density particles (microprojectiles) are accelerated to high velocity by particle gun apparatus. These microprojectiles have sufficient momentum to penetrate plant cell walls and membranes and can carry DNA or other substances into the interior of bombarded cells. It has been demonstrated that such microprojectiles can enter cells without

causing death and they can effectively deliver foreign genes into intact epidermal tissue of *Allium cepa* (Klein *et al.*, 1987). The invention of particle bombardment has facilitated gene transfer to even the more recalcitrant monocotyledonous crop plants like maize (Gordon-Kamm *et al.*, 1990), rice (Christou *et al.* 1991), wheat (Vasil *et al.*, 1992) and oat (Somers *et al.*, 1992). It also eliminated the need for protoplasts which were required for gene uptake. Procedure using organized tissues such as immature embryos (Christou *et al.*, 1991) as target tissue for cereal transformation may make the system less genotype-dependent.

Through particle bombardment several groups obtained transient expression of reporter genes in barley cells and tissues (Kartha *et al.*, 1989; Mendel *et al.*, 1989, Creissen *et al.*, 1990). Stable transformed callus tissues were reported by Ritala *et al.* (1993). Shortly after that, good progress has been made in genetic transformation of barley where production of transgenic barley plants was reported by Wan and Lemaux (1994), Ritala *et al.* (1994) and Hagio *et al.* (1995). These reports indicated that immature embryos were used as a plant source in the transformation experiments.

Production of stable transformed fertile barley plants using immature embryos *via* particle bombardment was described in this report. Kaush *et al.* (1995) described the necessary prerequisites of a recipient cell system for useful genetic transformation of plants. The description included the following major considerations: (i) the target cells must be accessible to the delivery system or competent for DNA uptake; (ii) the recipient cells competent for integrative transformation, where aspects such as timing of introduction during the cell cycle and/or capability for recombination may be important; (iii) cells should be capable of sustained divisions after integrative transformation to allow the development of a transformed clonal sector that can be selected; and (iv) to be useful for gene-expression studies in whole plants, those clonal sectors must retain morphogenic competence for regeneration of fertile plants.

MATERIALS AND METHODS

Plasmid and DNA preparation for bombardment

The plasmid pAHC25 (Chrestensen and Quil, 1996) was used to transform barley in the present study. The plasmid has *uidA* gene expression cassette and *bar* gene expression cassette (Fig. 1), both genes are under the control of the maize ubiquitin promoter. The *E. coli* strain XL1-Blue (Stratagene, USA) containing the plasmid pAHC25, from frozen stock, was cultured on solid LB medium containing 50 µg/ml kanamycin and incubated at 37°C. One single colony was inoculated into 500 ml LB liquid medium with 50 µg/ml kanamycin and incubated in a shaker incubator at 37°C and 150 RPM. Cells were harvested and the plasmid DNA was isolated using Wizard plus Megapreps (Promega, USA) kit. DNA concentration was adjusted to 1.0 µg/µl.

Microprojectile preparation

Plasmid DNA was precipitated onto gold particles (1.0 µm in diameter) following protocol modified from the original Bio-Rad procedure as follows: the precipitation mixture comprised 2 mg of particles, 50 µl of 2.5 M $CaCl_2$, 20 µl of 100 mM spermidine free base and 10 µl DNA (1µg/µl). After microprojectile-DNA precipitation, the supernatant was discarded; particles were washed in 250 µl absolute ethanol and resuspended in 100 µl absolute ethanol. For each bombardment, 10 µl of microprojectile-DNA suspension was placed onto the macroprojectile then allowed to air dry in the laminar flow hood.

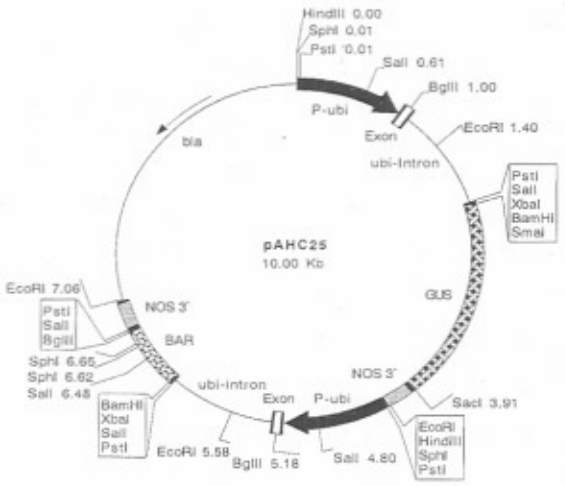

Fig. (1): Schematic representation of pAHC25 plasmid (10.0 kb) containing uidA (4.181 kb) and bar (2.871 kb) cassettes, both under control of maize ubi promoter.

Plant materials and bombardment

Plants of the barley (*Hordeum vulgare* L.) cultivar Giza 123 were grown in the open field. All inflorescences were tagged at the onset of anthesis and spikes were harvested when the immature embryos had grown to a size of 1.0-1.5 mm in length. Kernels were removed manually from the spikes, then surface sterilized by immersion for 10 minutes in 20% solution of commercial bleach (1.2% sodium hypochlorite). Kernels were subsequently rinsed several times with sterile double distilled water and embryos were isolated aseptically and placed on callus induction medium with scutellum side up. After 7 days, the germinated seedlings were removed and discarded and the scutellum derived calli were transferred into the center of the bombardment medium for four hours before bombardment; 25 calli per plate. Bombardments were carried out at a distance of 6 and 9 cm from the stopping screen using a PDS1000/He microprojectile gun (Bio-Rad) at three different helium pressures (650, 900 and 1100 psi), based on Barcelo *et al.* (1994). Also osmotic treatment to target tissues during bombardment was also considered (+/-).

Selection and regeneration

Bombarded tissues were incubated in dark at 23°C for two days. In transient expression experiments, calli were harvested and assayed for GUS activity as described later. In stable transformation experiments, calli were distributed over the surface of callus induction medium and reincubated in the dark for one week. Calli were then transferred into a selection medium and incubated in the dark for 3 weeks, and transferred onto fresh selection medium every 3 weeks until the control (non-bombarded) tissues were died. Callus colonies developed on selection medium were transferred into a regeneration medium and cultured under 16 hours light (250 $\mu Es^{-1}m^{-2}$, from cool white fluorescent tubes) at 25°C for 2-3 weeks rounds. All regenerated shoots were transferred into rooting medium for elongation and root formation.

Culture media

The media used in regeneration was based on Cho *et al.* (1998). The callus induction medium contained MS salts (Murashige and Skoog, 1962) supplemented with 30.0 g/l maltose, 1.0 mg/l thiamine HCl, 0.25 g/l myo-inositol, 1.0 g/l casein

hydrolysate, 0.65 g/l L-proline, 5.0 µM cupric sulfate, 2.5 mg/l 2,4-D, and 0.05 mg/l BAP, pH 5.8 and 3.5 g/l phytagel. The composition of osmotic treatment medium was similar to the composition of callus induction medium with substituting 0.2 M manitol and 0.2 M sorbitol instead of maltose. The composition of selection medium is a callus induction medium supplemented with 3.0 mg/l bialaphos. Plant regeneration medium contained selection medium with 2.5 mg/l BA instead of 2,4-D. Rooting medium contained a callus induction medium without growth regulators.

Analysis of marker gene activity

Transient gene expression of GUS activity was assayed 2 days after bombardment by incubating bombarded tissues in X-Gluc buffer, as described by Jefferson, (1987). Blue spots were developed after overnight incubation at 37°C. Same protocol was followed to assay GUS expression in the tissues of putative transgenic T_0 plants with an extra step in which chlorophyll was extracted after overnight incubation before examining the expression of GUS gene (in case of leaf tissues). To determine herbicide sensitivity of T_0 plants, a section of leaf blade was painted once, using a cotton swap with 0.25% (v/v) Basata solution plus 0.1% Tween 20 (Cho *et al*., 1998). Resistance to the herbicide solution was examined one week after application by scoring tissue necrosis.

Molecular analysis

DNA of putative transgenic T_0 plants was isolated using DNeasy plant mini kit (Qiagen, Germany). PCR mixture contained 50-200 ng barley DNA or 10 ng pAHC25 plasmid DNA, 0.4 µM of each oligonucleitide primer, 200 µM of each dNTP, 1.5 U HotStartTaq DNA polymerase (QIAGEN, Germany) and 1X buffer, supplied by the enzyme manufacturer, containing 1.5 mM $MgCl_2$ in a total volume 25 µl. Thirty five cycles were performed for all PCR reactions. The annealing temperature of *uidA* gene was 62°C and 57°C for *bar* gene. Primers were designed to amplify a 5` sequence of *uidA* and *bar* genes. For *uidA* gene the forward primer used was 5`-GTGTACGTATCACCGTTTGGTGTGAAC-3` and the reverse primer 5`-ATCGCCGCTTTGGACATACCATCCGTA-3`. For *bar* gene, the forward primer used was 5`-GTCTGCACCATCGTCAACC-3` and the reverse primer 5`-GAAGTCCAGCTGCCAGAAAC-3`. The expected amplified PCR product for *uidA* gene was 1050 bp and 443 bp for *bar* gene. The amplified samples were analyzed by electrophoresis in 1.2% (w/v) agarose gels. Integration of transgenes was confirmed by Dot-Blot hybridization analysis of DNA isolated from T_0 plants using DNA labeling and detection kit (Roche, Germany).

RESULTS AND DISCUSSION

In our laboratory there is concern to transform high-molecular-weight (HMW) gluten subunit genes into wheat, maize and barley to study the expression of those genes in such crops. We successfully transformed Dy10 glutenin subunit gene into maize (Abdallah *et al*., 2004) and wheat (unpublished data). Dy10 gene has not been transformed into barley because the transformation system has not been established yet. For these reason, the aim of this work was to establish a transformation system in barley and hence allow transforming HMW and other agronomically important genes into barley. In this report biolistic bombardment was used to establish a transformation system in barley.

The strategy to develop transgenic barley plants using biolistic bombardment is based on three major steps:(i) establishment of an efficient regeneration system in barley from

immature embryos, (ii) establishment of a reasonable transient expression of the reporter gene using biolistic bombardment, and (iii) using such regeneration and transient expression systems together to develop transgenic barley plants.

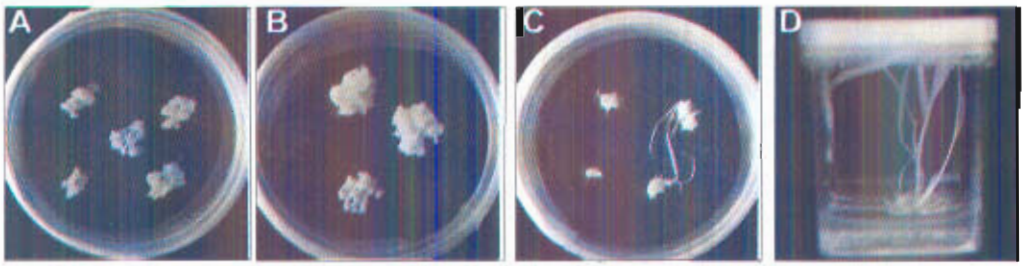

Fig.: (2):Different stages of regeneration of barley plants (T_0). (A) Non-bombarded scutellum derived calli on selection medium, after 3 months, showing growth inhibition. (B) Putative transgenic scutellum derived calli recovered on selection medium, after 3 months, showing healthy growth (after trimming out all dead tissues). (C) Shoot regeneration on regeneration medium with selection. (D) Shoot elongation and root formation on rooting medium.

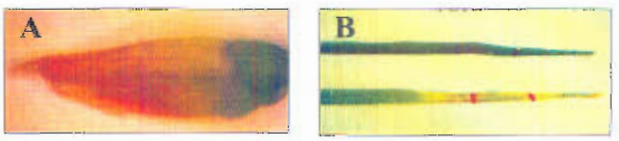

Fig. (3): Expression of transgenes. (A) Histochemical staining for uidA gene activity. Tassel tissue showing GUS activity (blue color) in the lower part (right) while the rest of tissue did not show GUS activity. (B) Leaf painting assay for bar gene: leaf of transgenic barley plant (top) and leaf of control plant (bottom) painted with glufosinate in the marked area. Transgenic plant showed resistance to glufosinate while control plant showed necrosis.

A number of regeneration experiments using the protocol developed by Cho *et al.*, (1998) were conducted on the barley cultivar Giza 123 with the exception that immature embryos were placed scutellum side up. The efficiency of regeneration ranged from 90-95%. This high regeneration efficiency was sufficient and promising to start the transformation experiments. Immature embryos were used as a plant source for regeneration experiments, where Wan and Lemaux (1994), Ritala *et al.* (1994), Hagio *et al.* (1995) and Cho *et al.* (1998) successfully used this explant to produce fertile transgenic barley plants. This organized explant was also used successfully to transform some other cereal crops such as maize (Gordon-Kamm *et al.*, 1990), rice (Christou *et al.* 1991), wheat (Vasil *et al.*, 1992) and oat (Somers *et al.*, 1992).

For transient GUS expression, some factors were studied to get the most suitable transient expression as mentioned in materials and methods. The factors under investigation were studied in factorial experiments. The negative control was bombarded tissues without DNA. Expression of transient activity of *uidA* gene was examined histochemically (Jeferson, 1987). Blue spots of various sizes were observed and each blue spot (whether an

aggregate or a single spot) was considered as one expression unit (Klein *et al.*, 1988).

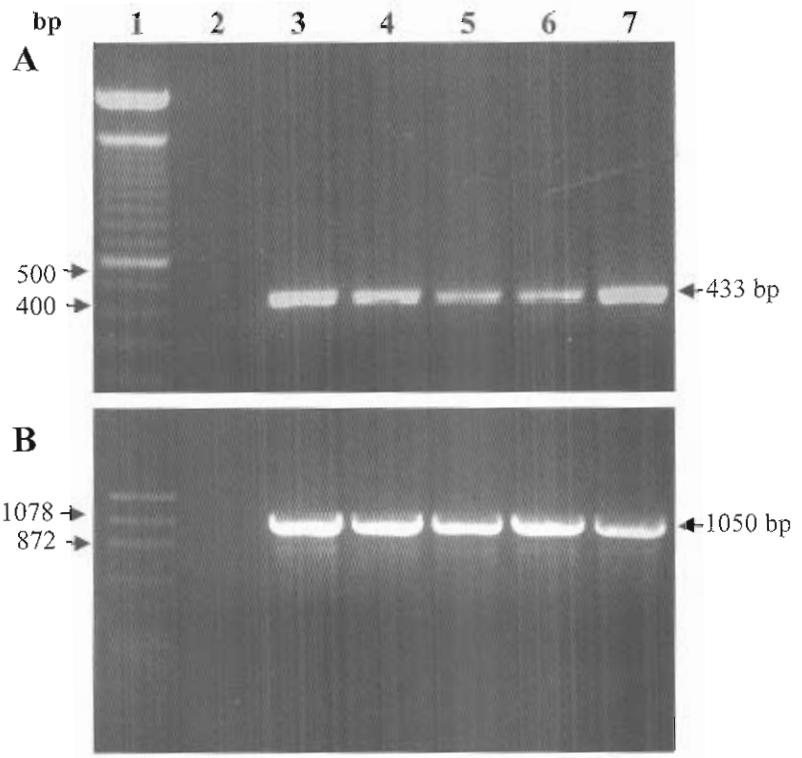

Fig. (4): PCR analysis of transgenic barley plants (To). The amplified products are 433 bp fragment from bar gene (A) and 1050 bp fragment from uidA gene (B). Lane 1 is DNA markers, 100 bp Ladder (A) and ΦX174/HaeIII (B). Lane 2 is negative control (non-transformed barley). Lane 3 is positive control (pAHC25 plasmid). Lanes 4-7 are transgenic barley plants.

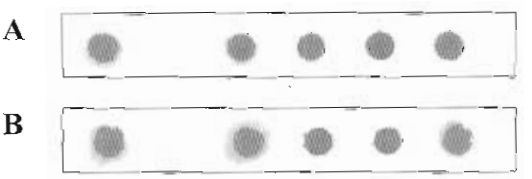

Fig. (5): Dot-Blot hybridization analysis for transgenic barley plants with uidA (A) and bar (B) genes. Dot 1 is pAHC25 plasmid control (positive control). Dot 2 is non-transformed barley (negative control). Dots 3-6 are transgenic barley plants (To).

Results of transient expression experiments (data not shown) indicated that the treatment in which osmotic treatment, 900 psi helium pressure and 6 cm bombarding distance gave the highest level of transient expression of *uidA* gene. These conditions were followed in a stable transformation experiments (Hagio *et al.*, 1995). It was noticed that all osmotically treated calli showed higher GUS expression than non-treated calli. Transient expression enhancement by the osmotic treatment could be due to the partial plasmolysis of the target cells, because under those conditions cells may be less likely to lose cell sap as a consequence of membrane damage by the penetrating particles (Vain *et al.*, 1993 and Melchiorre *et al.*, 2002). This result disagrees with the results obtained by Klein *et al.* (1988) because they observed that the osmotically adjusted

cells are exhibited a substantially lower level of the transgene activity. The reason could be the type of recipient cells where they got their results in maize transformation experiments or the type of osmotic agent used where in this study 0.2 M manitol and 0.2 M sorbitol used while the other authors used 0.4 manitol. Another possibility could be the type of transgene used in both studies, where *uidA* gene was used while the other authors got their results using *CAT* (chloramphenicol acetyltransferase) gene. The interaction between the type of recipient cells and type of transgene could be another reason for the differences in the two reports.

In stable transformation experiments, bombarded calli were maintained under osmotic treatment for two days, then transferred to the callus induction medium for one week for recovery. Calli were transferred into callus induction medium supplemented with 3 mg/l bialaphos and reincubated in the dark. Cultures were subcultured into fresh media every three weeks. It took about three months until all non-bombarded calli (controls) completely died i.e. showed visible growth inhibition or browning (Fig. 2). Four putative transgenic callus colonies recovered from 1000 bombarded tissues (0.4 %). Putative transgenic callus events were transferred to regeneration medium supplemented with 3 mg/l bialaphos and incubated in the light and subcultured for 2-3 weeks. Regenerated shoots were transferred into rooting medium with no selection. Stages of barley regeneration are shown in Fig. 2. The well developed shoots with healthy roots were transferred to pots with sterile soil in the growth chamber under the same conditions of donor plants for acclimatization. The plants were fertilized once weekly until maturation and setting seeds; the four plants set seeds (Table 1).

While plasmid pAHC25 carries *uidA* gene that was functional in assays for transient expression, no GUS activity was observed in leaf samples taken from these putative transformed plants and treated with X-Gluc in histochemical stain. A similar result was obtained by Witrzens *et al.* (1998). *uidA* gene appears to be prone to inactivate in some cereal cultures (Lambe *et al.*, 1995) and plants (Finnegan and McElory1995), and thus it is noteworthy that from our transgenic barley plants none of them showed GUS expression in leaf and root tissues. The only visible expression was observed in spike tissues and grains (Fig. 3a). A similar result was obtained in our laboratory in some transformation experiments when the same plasmid was used to transform wheat (unpublished data). The same plasmid was used to transform other barley cultivars, and the transgenic barley plants showed GUS activity in histochemical staining (Cho *et al.*, 1998). It means that the genotype could be an effective factor for GUS activity or *uidA* gene was inserted in a certain location in the genome that makes it active only in a certain developmental stage. Functional expression of *bar* gene was assayed in T_0 plants using leaf painting assay. Basta solution (0.25%) was applied to marked area of leaf blades in T_0 and non-transgenic plants (controls). After one week, all T_0 plants did not show necrosis while negative control showed necrosis (Fig. 3b). Resistance of T_0 plants to herbicide indicates that all plants are transgenic and functionally expressed *bar*.

Putative transgenic T_0 plants were analyzed by PCR to confirm the integration of both transgenes (*bar* and *uidA*) in their genomes. The analysis indicated that PCR products for both transgenes and the corresponding positive controls were in the expected size, 443 bp for *bar* gene and 1050 bp for *uidA* gene. Negative controls (non-transgenic plants) did not show any PCR product as shown in Fig. (4). PCR results indicated that all transgenic barley plants were

positive for both transgenes. Results of PCR analysis are shown in Table (1).

Table (1): Analysis of transgenic barley plants.

Line no.	PCR analysis		Dot blot analysis		Transgene expression		Fertility
	uidA gene	*bar* gene	*uidA* gene	*bar* gene	GUS stain	Basta resistance	
1	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+
3	+	+	+	+	-	+	+
4	+	+	+	+	-	+	+

In order to confirm the integration of transgenes in T_0 plants, Dot-Blot hybridization analysis was performed. DNA isolated from leaf tissues and transferred to nylon membrane in a Dot-Blot apparatus. Hybridization was performed with Digoxygenin labeled probe for both genes. The positive controls (plasmid DNA) exhibited hybridization, while the negative controls (DNA from non-transformed plants) did not exhibit any hybridization and all T_0 plants exhibited hybridization with the probes of both genes (Fig. 5). These results confirmed the stable integration of both genes in T_0 plants. Dot-Blot analysis results are shown in Table (1).

Data of the analysis of T_0 plants indicated that there is no escapes that reflect the efficiency of selection followed in this work, where 3 mg/l bialaphos was used from the first round of selection to the stage of regeneration. Other authors eliminate the selection agent from the second month of culture (Ortiz *et al.*, 1996) or use lower concentration in the beginning of selection step (Rasco-Gaunt *et al.*, 2001). These strategies can enhance the escape events (Altpeter *et al.*, 1996). Some authors use selection agent only in rooting culture (Ritala *et al.*, 1994); this strategy consumes more effort and labor and the majority of the regenerated plants are not transgenic.

The results of these study indicated that this protocol could be used as an adequate strategy to obtain genetically modified barley plants. The barley cultivar used in this study can be transformed with foreign DNA. The transferred DNA will integrate stably into the barley genome and will be expressed without affecting the fertility or general performance of the plant. It was noticed that during the whole culture period, no albino plants were regenerated in this study. It is known that genetic factors (Foroughi-Wehr *et al.*, 1982) and culture conditions (Ziauddin and Kasha 1990) influence albinism. Our culture and selection conditions may provide useful information to overcome the problem of albinism.

In conclusion, microprojectile bombardment has proven to produce transgenic Egyptian barley plants with selectable marker and reporter genes. HMW gluten subunit genes or other agronomically important genes could be transformed into barley using the protocol used in this study. Also some factors could be studied to enhance the frequency of transformation.

ACKNOWLEDGMENTS

The author thanks Dr. Khaled Abdalla and Dr. Ashraf Fahmy for critical reading of the manuscript and helpful discussions. The author also thanks Department of Barley, Field Crop Research Institute, Agricultural Research Center for providing barley germplasm used in this study.

REFERENCES

Abdallah, M.; Fahmy, A.H.; Abdalla, K.S. and Maaty, W.S. (2004). Transformation of high-molecular-weight (HMW) glutenin subunit Dy10 gene into maize. Arab J. Biotech., 7(2): 165-172.

Altpeter, F.; Vasil, V.; Srivastava, V.; Stgoger, E. and Vasil, V. (1996). Accelerated production of transgenic wheat (*Triticum aesativum* L.) Plant Cell Reports, 16: 12-17.

Barcelo, P.; Hagel, V.; Becker, D.; Martin, A. and Lorz, H. (1994). Transgenic cereal (Tritordeum) plants obtained at high efficiency by microprojectile bombardment of inflorescence tissue. The Plant Journal, 4(4): 583-592.

Cho, M.; Jiang, B.; and Lemaux, G. (1998). Transformation of recalcitrant barley cultivars through improvement of regenerability and decreased albinism. Plant Sciences, 138: 229-244.

Christou, P.; Ford, T.L. and Kofron, M. (1991). Production of transgenic rice (*Oryza sativa* L.) plants from agronomically important *indica* and *japonica* varieties *via* electric discharge particle acceleration of exogenous DNA into immature zygotic embryos. Biotechnology, 9: 957-962.

Creissen, G.; Smith, C.; Francis, R.; Reynolds, H. and Mullineaux, P. (1990). *Agrobacterium* and microprojectile viral DNA delivery into barley microspores derived cultures. Plant Cell Reports, 8: 680-683.

Cristensen, A.A. and Qwail, P.H. (1996). Ubiquitin promoter based vectors for high level expression of selectable and/or screenable marker genes into mono and dicotyledonouos plants. Transgenic research, 5: 213-218 .

Finnegan, J. and McElory, D. (1995). Transgene inactivation: plant fight back. Biotechnology, 12: 883-888.

Foroughi-Wehr, B.; Fridet, W. and Wanzel, G. (1982). On the genetic improvement of androgenetic haploid formation in *Hordeum vulgare* L.. Theo. Appl. Genet., 62: 233-239.

Fraley, R.T.; Rogers, R.B.; and Horsch, R.B. (1968). Genetic transformation in higher plants. Crit. Rev. Plant Sci. 4:1-6.

Gordon-Kamm, W.J.; Spancer, T.M.; Mangano, M.L.; Adam, T.R.; Daines, R.J.; Start, W.J.; O`Brien, J.V.; Chambers, S.A.; Adams Jr; W.R.; Willetts, N.G.; Rice, T.B.; Mackey, C.J.; Krueger, R.W.; Kausch, A.P. and Lemaux, P.G. (1990). Transformation of maize cells and regeneration of fertile transgenic plants. Plant Cell, 2: 603-618.

Hagio, T.; Hirabayashi, T.; Machii, H. and Tomotsune, H. (1995). Production of fertile transgenic barley (*Hordeum vulgare* L.) plants using the hygromycin-resistance marker. Plant Cell Reports, 14: 329-334.

Jahne, A.; Lazzeri, P.A. and Lorz, H. (1991). Regeneration of fertile plants from protoplasts derived from embrypgenic cell suspensions of barley. Plant Cell Reports, 10: 1-6

Jefferson, R.A. (1987). Assaying chimeric genes in plants. The GUS gene fusion system. Plant Molecular Biology Reporter, 5:387-405.

Kartha, K.K.; Chibbar, R.N.; Georges, F.; Leung, N.; Caswell, K.; Kandall, E. and Qureshi, J. (1989). Transient expression of chloramphenicol acetyltransferase (CAT) gene in barley cell cultures and immature embryos through microprojectile bombardment. Plant Cell Reports, 8:429-432

Kaush, A. P.; Adams, T.R.; Mangano, M.; Zachweija, S. J.; Gordon-Kamm, W.; Willetts, N.G.; Chambers, S.A.; Adam Jr., W.; Anderson, A.; Williams, G. and

Haines, G. (1995). Effects of micropr-ojectile bombardment on embryogenic suspension cell cultures of maize (*Zea mays* L.) used for genetic transformation. Planta, 196: 501-509.

Klein, T.M.; Graddziel, T.; From, M.E. and Sanford, J.C. (1988). Factors influencing gene delivery into *Zea mays* cells by high-velocity microprojectiles Biotechnology, 6:559-563.

Klein, T.M.; Wolf, E.D.; Wu, R. and Sanford, J.C. (1987). High-velocity microprojectiles for delivering nucleic acids into living cells. Nature (London), 327:70-73.

Lambe, P.; Dinant, M. and Matagne, R.F. (1995). Differential long-term expression and methylation of the hygromycin phosphotransferase and β-glucuronidase (GUS) genes in transgenic pearl millet (*Pennisetum glaucum*) callus. Plant Science, 108:149-164.

Lazzeri, P.A.; Brettschneidr, R.; Luhrs, R. and Lortz, H. (1991). Stable transformation of barley *via* PEG-induced DNA uptake ino protoplasts. Theor. Appl. Genet., 81:437-444.

Melchiorre, M.N.; Lascano, R.H. and Tripp, V.S. (2002). Transgenic wheat plants resistant to herbicide BASTA obtained by microprojectile bombardment. BioCell, 26(2): 217-223

Mendel, R.R.; Muller, B.; Scholze, J.; Kolesnikov, V. and Zelinin, A. (1989). Delivery of foreign genes to intact barley cells by high velocity microprojectile. Theor. Appl. Genet., 78:31-34.

Murashige, T. and Skoog, F. (1962). A revised medium for growth and bioassays with tobacco tissue culture. Physiol. Plant., 15:437-497

Ortiz, J.P.; Reggiardo, M.I.; Ravizzeni, R.A.; Altabe, S.G.; Servigni, D.L.; Spitelar, M.A.; Morata, M.M.; Eliase, F.E. and Vellejos, R.A. (1996). Hygromycin resistance as an efficient selectable marker for wheat stable transformation. Plant Cell Reports, 15: 877-881.

Rasco-Guant, S.; Riley, A.; Cannel, M.; Barcelo, P. and Lazzeri, P. (2001). Procedures allowing the transformation of a range of European elite (*Triticum aesativum* L.) varieties *via* particle bombardment. Journal of Experimental Botany. 52: 865-874.

Ritala, A.; Mannonem, L.; Aspegren, K.; Salmenkallio-Martilla, M.; Kurten, U.; Hannus, R.; Lozano, J.M.; Teeri, T.H. and Kauppinjen, V. (1993). Stable transformation of barley tissue culture by particle bombardment. Plant Cell Reports, 12:435-440.

Ritala, A.; Aspegren, K.; Kurten, U.; Salmenkallio-Martilla, M.; Mannonen, L., Hannus, R.; Kaupennin, R.; Terri, H. and Enari, T. (1994). Fertile transgenic barley by particle bombardment of immature embryos. Plant Molecular Biology, 24:317-325.

Sanford, J.C.; Klein, T.M.; Wolf, E.D. and Allen, N. (1987). Delivery of substances into cells and tissues using a particle bombardment process. Particular Science & Technology, 5, 27-37.

Somers, D.A.; Rines, H.W.; Kaeppler, H.F. and Bushnell, W.R. (1992). Fertile transgenic oat plants. Biotechnology, 10: 1589-1594.

Vain, P.; mcmullen, M.D. and Finer, J.J. (1993). Osmotic treatment enhances particle bombardment-mediated transient and stable transformation of maize. Plant Cell Reports, 12: 84-88.

Vasil, V.; Castyillo, A.M.; Fromm, M.E. and Vasil, I.K. (1992). Herbicide resistant fertile transgenic wheat plants obtained by microprojectile bombardment of regenerable

embryogenic callus. Biotechnology, 10:667-674.

Wan, Y. and Lemaux, P.G. (1994). Generation of large numbers of independent transformed fertile barley plants. Plant Physiology, 104:37-38

Witrzens, B.; Brettell, R.; Murray, F.; McElory, D. and Li, Z. (1998). Comparison of three selectable marker genes for transformation of wheat by microprojectile bombardment. Aust. J. Plant Physiol, 25: 399-44.

Ziauddin, A. and Kasha, K.J. (1990). Long term callus cultures of diploid barley (*Hordeum vulgare*). II effect of auxins on chromosome status of cultures and regeneration of plants. Euphytica, 48:279-286.

الملخص العربي

انتاج نباتات شعير مصرى محورة التركيب الوراثى باستخدام تقنية الدفع الجينى

محمـــد عبـــد اللـــه

معهد بحوث الهندسة الوراثية الزراعية – مركز البحوث الزراعية
٩ ش الجامعة – الجيزة ١٢٦١٩– جمهورية مصر العربية

تم تشييد نظام للنقل الجينى للشعير المصرى صنف جيزة ١٢٣ و ذلك باستخدام تقنية الدفع الجينى هذا و قد تـــم عـــزل أجنة شعير غير ناضجة ذات أطوال تتراوح بين ما ١–1,5 مم تحت ظروف معقمة، و ذلك من نباتات شعير مزروعـــة قـــى الحقل. و قد زرعت تلك الأجنة على بيئة تكوين الكالوس لمدة اسبوع بعدها نقلت للبيئة ذات الضغط الأسموزى العـــالى حيـــث تمت عملية الدفع الجينى و ذلك باستخدام بلازميد pAHC25، و هذا البلازميد يحتوى على جين *uidA* القياسى و جـــين *bar* الانتخابى و قد تم نقل تلك الانسجة لبيئة انتخاب الأنسجة المحورة وراثيا دون غيرها، و قد تم نقل تلك الأنسجة لبيئة التكشف و اعادة الاستيلاد نتج عنها الحصول على نباتات شعير تم نقلها للتربة للأقلمة. و قد تم التأكد من وجود جينـــات التحـــور فـــى المحتوى الوراثى لتلك النباتات باستخدام التحليلات الجزيئية كما تم دراسة التعبير الجينى لجين *uidA* باستخدام تقنيـــة الـــصبغ النسيجى الكيميائى، و أيضا التعبير الجينى لجين *bar* باستخدام تقنية الدهان الورقى.