The competence of maize shoot meristems for transformation of Egyptian maize inbred lines

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Ebtissam H. A. Hussein[*,], Shireen K. Assem[*], Hanaiya A. El-Itriby[*], Fathy M. Abdel-Galil[***] and Magdy A. Madkour[*]**

* Agricultural Genetic Engineering Research Institute (AGERI), ARC, Giza, Egypt.
** Department of Genetics, Faculty of Agriculture, Cairo University, Giza, Egypt.
*** Department of Chemistry, Faculty of Science, Cairo University, Egypt.

ABSTRACT

A regeneration system was established for elite Egyptian maize inbred lines using shoot meristems as explants. This system was previously reported as genotype non-specific. Line Gz643 was identified as the best line revealing the highest regeneration frequency. Addition of BA and 2,4-D greatly enhanced the formation of multiple shoot clumps and the regenerability of some of the tested lines. Transformation was performed with the particle delivery system using the GUS and Bar genes in a single plasmid (pAB-6) or two plasmids (pAct1-F and pTW-a). Different transformation parameters were tested i.e. acceleration pressure and number of shots. The use of acceleration pressure 1550 psi. and four shots per plate (for transformation with pAB-6 or with pAct1-F and pTW-a) gave the best results as expressed by the number of blue spots in the GUS assay . Stable transformation was confirmed in Ro transformed plants by means of histochemical GUS assay. Moreover, PCR and Southern blot analysis proved the integration of the full-length genes in some of the transgenic plants.

Key words: *Maize, shoot meristem, transformation.*

INTRODUCTION

The successful use of molecular and cellular genetic technologies in plant improvement programs depends primarily on the development of a reliable regeneration system. These regenerable cultures can then be used for gene transfer. The efficient regeneration of normal and fertile plants from single cells, a basic prerequisite for the genetic improvement of plants, proved to be rather difficult for gramineous species because of their extreme recalcitrance to manipulation *in vitro* (Vasil, 1994). Efforts have been made by researchers to overcome these constraints through the culture of immature and undifferentiated tissue and organ explants at defined stages of development on nutrient media containing high concentrations of strong synthetic auxins, (Vasil and Vasil, 1984, 1992; Bohorova *et al.*,1995 and Carvalho *et al.*, 1997).

The shoot tip, or shoot apex, consists of the shoot apical meristem, a region in which lateral organ primordia form a subapical region of cell enlargement and several leaf primordia. The meristem region contains apical initial cells and subepidermal cells from which the gametes are derived.

Research on maize (*Zea mays* L.) morphogenesis demonstrated that the maize meristem is morphogenetically plastic and can be manipulated to produce multiple shoots, somatic embryos, tassels, or ears in a relatively genotype-independent manner by simple variation of *in vitro* culture conditions (Zhong *et al.*, 1992a and b). Zhong *et al.* (1992a) developed *in vitro* methods to regenerate clumps of multiple shoots and somatic embryos at high frequency from shoot tips of aseptically-grown seedlings as well as from shoot apices of precociously-germinated immature zygotic embryos of corn (*Zea mays* L.). Zhong *et al.* (1992b) studied the *in vitro* morphogenic pattern of corn (*Zea mays* L.) shoot tips excised from aseptically-grown seedlings, and of explants of axillary shoot buds, immature tassels and ears (staminate and pistillate inflorescences) obtained from greenhouse-grown corn plants.

Theoretically, there are two possibilities for recovering transgenic plants *via* transfer of DNA into the shoot apical meristem. One possibility is that transgenic progeny may be directly produced *via* transformation of the subepidermal germline cells followed by the development of a partially transgenic reproductive organ. In this case, the primary transformants will always be chimeric. An alternative possibility is to multiply transgenic apical meristem cells and/or germ-line cells, which can be reprogrammed in the developmental direction under *in vitro* conditions. Transgenic plants can be regenerated from cells with or without selection (Zhong *et al.*, 1992a and b). Bilang *et al.* (1993) established a method that allows the target delivery of DNA-carrying gold particles to vegetative shoot apical meristems of cereal species. Lowe *et al.* (1995) investigated whether cells in the developing shoot meristem of immature zygotic embryos might provide a genotype independent, more universal target for production of transformed maize plants. They pointed out that this method has been used successfully with genotypes that include a sweet corn hybrid and an elite field corn inbred. Similarly, Sautter *et al.* (1995) used shoot apical meristems as explants since they provide a tissue which regenerates *in situ* fertile plants for most given genotypes or species. Zhong *et al.* (1996) developed a novel and reproducible system for recovery of fertile transgenic maize (*Zea mays* L.) plants. The transformation was performed using microprojectile bombardment of cultured shoot apices of maize with a plasmid carrying two linked genes, the *Streptomyces hygroscopicus* phosphinothricin acetyl transferase gene (*Bar*) and the potato proteinase inhibitor II gene, either alone or in combination with another plasmid containing the 5′ region of the rice actin 1 gene fused to the *Escherichia coli* (*GUS*) gene.

The present investigation was an attempt to establish efficient regeneration and transformation systems for elite Egyptian maize inbred lines using the shoot apical meristem as an explant. The effect of medium composition on the formation of multiple shoot clumps was investigated. The transformation conditions were optimized for multiple shoot clumps using the biolistic particle delivery system and plasmid-DNA containing the *GUS* reporter gene and the herbicide resistant *Bar* gene. The efficiency of transient transformation was determined in transformed shoot clumps with a single plasmid carrying both *GUS* and *Bar* genes in comparison to co-transformation where each gene is borne on a different plasmid.

MATERIALS AND METHODS

Plant Material

Ten elite genotypes of maize (*Zea mays* L.) inbred lines with different or similar

genetic background were used in this investigation, i.e., Sd 63, Sd 62, G 221D, G 336, Rg 15, 93/24/91y, Sd 7, Gz 643 y, Gz 624 wy, and Gz 630, (Table 1). Maize seeds were provided by the Maize Department, Field Crops Research Institute, ARC, Giza, Egypt.

Culture Initiation and Maintenance

Mature seeds of the ten corn (*Zea mays* L.) genotypes were surface sterilized first in 70% ethanol for 10 min, washed once with sterile distilled water and then soaked for 30 min in 2.6% Sodium hypochlorite (prepared from commercial bleach) containing 0.1% Tween 20 (polyoxyethylene sorbitan monolaurate). The seeds were then washed three times with distilled water before germination on MS medium (Murashige and Skoog , 1962) containing 3% sucrose and solidified with 3g/l phytagel in Sigma culture tubes. The tubes were maintained in darkness at 24±1°C. The seedlings length reached about 3-5 cm within a week of sowing the seeds; the position of the shoot tip of the seedling inside the coleoptile could be determined by the localized enlargement of the seedling at the junction of mesocotyl and coleoptile. Sections about 5 mm long of seedlings containing a shoot tip, three to five leaf primordia, and a portion of young leaf and stem immediately below the leaf primordia were excised and cultured on one of three shoot multiplication medium. Five shoot tips were cultured in each Petri dish on 20 ml of medium, with a total of 30-50 shoot tips for each genotype. The explants were laid horizontally and partly buried in the medium.

Table (1): Pedigree of the ten maize inbred lines.

Code #	Inbred Line	Pedigree
1	Sd 63	Tepalcinco No.5
2	Sd 62	Tepalcinco No.5
3	G 221D	Early White Composite
4	G 336	H 3091469, Mexico
5	Rg 15	(Synthetic Laposta x Ci 64) (SC 14)
6	93/24/91 y	Eto x CBC
7	Sd 7	(American Early Dent x Composite A4)
8	Gz 643 y	B73 x Sd 62 (S_8)
9	Gz 624 wy	B73 x Sd 62 (S_8)
10	Gz 630	B73 x Sd 62 (BC_1 Sd 62) S_7

Shoot Multiplication

To test the response of different maize genotypes in producing multiple shoot clumps, a shoot multiplication medium (SM2) consisting of MS basal medium supplemented with 500 mg/l CH, 0.5 mg/l 2,4-D and 2.0 mg/l BA was chosen according to Zhong *et al.*, (1992a). Two other media, SM1 and SM3, in addition to SM2 medium were tried with the inbred line Gz 643 to determine which of the three media would give the highest number of adventitious shoots per shoot-tip explant. The components of the three media are presented in Table (2). Subsequent subcultures were performed with a four weeks interval between each culture.

Table (2): Components of media used for shoot multiplication

Medium	Basal Medium	CH (mg/l)	2,4-D (mg/l)	BA (mg/l)	Sucrose (%)
SM1	MS	500	-	2	3
SM2	MS	500	0.5	2	3
SM3	MS	500	0.5	1	3

The multiple shoot clumps were subcultured on shoot multiplication media for four to six months. The number of multiplied meristems was counted under a Zeiss SV6 binocular stereomicroscope, during the first three subcultures, to determine the ability of the maize shoot tips from different maize genotypes to multiply and form clusters of multiple shoot clumps. Shoot tips from the multiplied clumps at the end of each subculture (4 weeks) usually have several germinated leaves.

Regeneration of Multiple Shoot Clumps

Green corn shoots were obtained from the multiple shoot clumps four to six months after culture initiation by transferring the multiple shoot clumps to shooting medium (M1). Developed shoots were rooted on (M2) medium. Table (3) illustrates the components

Table (3): Components of media used in regeneration of multiple shoot clumps.

Medium	Basal Medium	BA (mg/l)	IBA (mg/l)	Sucrose (%)
M1	MS	0.5	0.5	3
M2	MS	-	1.0	3

of the two regeneration media M1 and M2.

Regeneration of shoot clumps was also tested on MS medium free of hormones. The regenerated plantlets were acclimatized in the greenhouse, adjusted at 28°C with a 16-h photoperiod, by transferring them to an aquarium containing a modified-Hoagland solution, (Johnson *et al.*, 1957).

When an extensive root system had formed, plantlets were transferred to 15-cm pots containing 1:1 mixture of sterile peatmoss: soil, in the containment greenhouse adjusted at 28°C with a 16-h photoperiod, and 90% humidity.

Plasmid DNAs

The plasmids pAct1-F and pTW-a were provided by Dr.M.B.Sticklen (Michigan State University), while pAB-6 was kindly supplied by Dr.A.Bahieldein (Ain Shams University and AGERI). pAct1-F (McElroy *et al.*. 1990) includes the *GUS* coding region controlled by the 1.3-actin1 gene (*Act1*). pTW-a contains the selectable marker gene *Bar* (phosphinothricin acetyl transferase) driven by CaMV-35S promoter. pAB-6 contains the *Bar* gene driven by the CaMV 35S promoter and the *GUS* gene driven by the rice *Act1* promoter. Recombinant plasmids were amplified in liquid cultures of *Escherichia coli*, and prepared according to a modified Sambrook *et al.* (1989) procedure.

Particle Bombardment

Plasmid DNA was precipitated onto tungsten particles 1.1 μm in diameter (M17, Bio-Rad) following a modification of the original protocol for the Biolistic PDS-

1000/He Particle Delivery System (Bio-Rad) (Zhong *et al*,1996).

All the transformation experiments were performed using shoot clumps of the inbred line Gz 643. Four to six months old shoot clumps were used as a target for microprojectile bombardment. For each bombardment, 10 µl of the particle-DNA suspension (75µg of particles per shot) was pipetted onto the center of the macrocarriers.

Eight experiments (four plates each) were carried out on the shoot clumps of inbred line Gz643, with a total of 224 shoot clumps, to compare the effect of using two different pressures (1550 or 1800 psi) with a single plasmid (pAB-6) or with a combination of the two plasmids (pTW-a and pAct1-F) at a ratio of (1:1). Two or four shots were applied per plate.

Selection and Regeneration of Transformed Shoot Clumps

The active ingredient, PPT (Glufosinate ammonium (ammonium-DL-homoalanin-4-methylphosphinate, Riedel-de Haen-Germany) was used for the selection of herbicide resistant shoot clumps.

The bombarded shoot clumps were immediately transferred to fresh multiplication medium without selection and incubated for 4 weeks under continuous light (60 µmol(quanta^{-1}).m.s) and 25°C. The clumps were then divided and subcultured on shoot multiplication medium SM2 containing 3 mg/l PPT for 4 weeks (Zhong *et al*.1996). Subsequent subcultures at 4-week intervals were carried out by selecting, dividing and culturing green clumps on multiplication medium with 3 mg/l PPT.

Histochemical *GUS* Assay

Histochemical localization of the *GUS* activity in the bombarded tissues was performed according to the method described by Zhong *et al.* (1996) on two shoot clumps selected at random from each bombarded plate.

Molecular Analysis of Transgenes

Extraction and purification of genomic DNA

Genomic DNA was isolated from leaf tissues using the CTAB method (Rogers and Bendich, 1985).

PCR analysis

Two sets of primers were used to detect the *GUS* and *Bar* genes by PCR analysis, i.e., (GUS-1): CTC GAC GGC CTG TGG GCA TTC AGT C, (GUS-2): TAG ATA TCA CAC TCT GTC TGG CTT TTG G; (Bar-1): TGC CAC CGA GGC GGA CAT GCC GGC, and (Bar-2): CCT GAA GTC GGA CGG CCA TGG CGG

Amplification of the DNA was performed in a Perkin Elmer thermal cycler 2400 programmed to fulfill 35 cycles. The temperature profiles in the different cycles was as follows: An initial strand separation cycle at 94°C for 5 min. This was followed by 33 cycles including a denaturation step at 95°C for 1 min, an annealing step at 55°C for 2 min and a polymerization step at 72°C for 2 min. The final cycle was a polymerization cycle performed at 72°C for 8 min. Amplified DNA was analyzed on a 1.0% agarose gel stained with ethidium bromide.

Southern Hybridization

Southern blot hybridization analysis was carried out on the PCR amplified DNA using the Digoxigenin non-radioactive labeling and detection system from Boehringer Mannheim. To liberate the *Bar* and *GUS* genes, the recombinant plasmids (pTW-a and pAct1-F) were cut with the restriction enzymes *SmaI* and (*BamHI* and *SacI*), respectively. The

reaction was electrophoresed to separate the insert. The insert was eluted from the gel using dialysis tubing. These DNA inserts were labeled with the random priming DNA labeling and detection kit and used as probes.

Statistical Analysis

Statistical analysis was performed according to Steel and Torrie (1980) using the SAS computer software (version 5) with associated least significant differences (LSD) function.

RESULTS AND DISCUSSION

Shoot apical meristems represent an excellent tissue for plant regeneration particularly in species for which an *in vitro* tissue culture regeneration system is not established and thus it possesses a considerable potential for genotype independent gene transfer to recalcitrant species, (Sautter, 1993).

Shoot Multiplication and Regeneration

Corn-shoot meristem was shown by Zhong *et al.*, (1992a) to be committed to form either clumps of multiple shoots or somatic embryos *in vitro* by manipulating the concentration of BA and 2,4-D in the culture medium. This supported the concept of McDaniel and Poething (1988) that organ formation from apical meristem cells in corn is determined just before the organ begins to be initiated.

In the present study, ten elite Egyptian maize (*Zea mays* L.) inbred lines were evaluated for their relative efficiency in the proliferation of multiple shoot clumps from shoot tip cultures. The tissue containing the meristem, identified by the swelling that occurs at the junction of the mesocotyl and coleoptile, was excised and culured on medium SM2 that was designated previously by Zhong *et al.*(1992a) as a corn-shoot-multiplication medium. Shoot tips began to produce multiple shoot clumps within two weeks of culture. The multiple shoot clumps arising from these explants were divided and subcultured at 4 weeks intervals, after removal of any elongating leaves and necrotic tissues, on a fresh medium of the same composition and incubated in continuous light at 25°C (Fig.1 A, B and C).

For each inbred line, 4-5 experiments were conducted, with a total number of 120-200 shoot tips per line. The number of shoot tips that showed capacity to proliferate and multiply was determined after 2 weeks from shoot tip culture. The shoot multiplication potentiality for each line was calculated as the average number of multiplied shoot tips. The relative efficiency of shoot multiplication in seedling shoot-tip cultures of the genotypes was evaluated by counting the number of shoots (meristems) per shoot tip after one, two and three months of culture, then calculating the average number of shoots per shoot tip culture. The number of shoots or meristems per multiple shoot clump was counted under a binocular stereo-microscope, (Fig.1 E and F).

The results in Table (4) showed a variation in the ability of shoot tips from different genotypes to multiply and to form multiple shoot clumps. Therefore, the ability to induce shoot multiplication at high frequencies from excised meristems is genotype dependent. These findings are in close agreement with the results of Lowe *et al.*(1995). On the other hand, these results contradict the concept of Zhong *et al.* (1992a) and Zhong *et al.*(1996) that this system is relatively genotype independent and that shoot proliferation can occur in a wide range of genotypes by manipulating the concentration of BA and 2,4-D in the culture medium. In this context, Hu and Wang (1983) stated that cytokinins, particularly benzyladenine, are

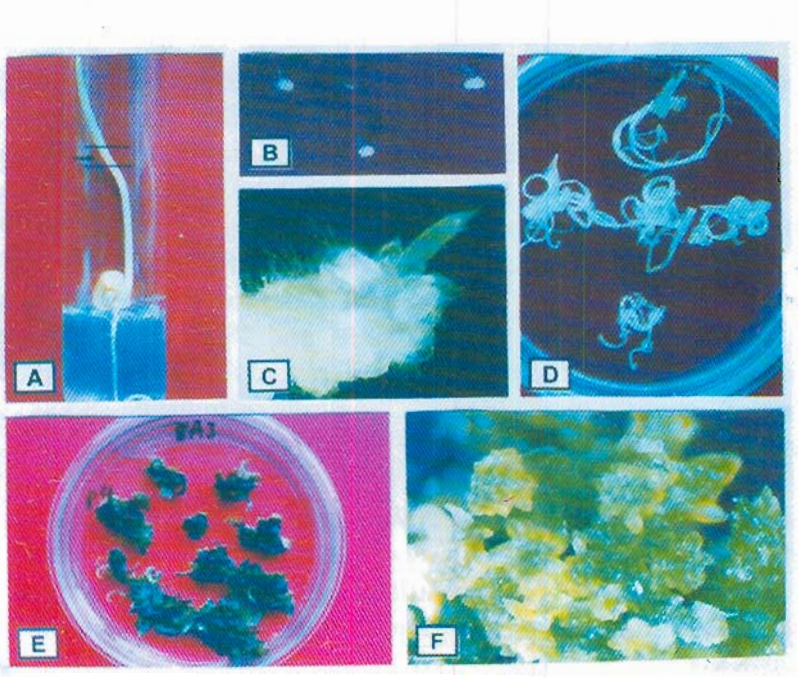

Fig. (1): Steps of shoot multiplication. (A): Maize seedling germinated on MS medium. (B): 5 mm long sections of maize seedling containing the meristems. (C): Multiplied meristem after 2 weeks of culture on multiplication medium. (D): Multiplied shoot tips. (E) and (F): Multiplied shoot tips after 3 months of culture.

Table (4): Mean values for shoot multiplication of the ten Egyptian inbred lines.

Inbred Line	No. Multiple Shoots	No. Meristems after one Month	No. Meristems after two months	No. Meristems after three months
Sd 63	43.000^{bc}	0.995^{cd}	1.680^{cd}	1.690^{cd}
Sd 62	30.225^{cde}	1.330^{c}	2.379^{bc}	2.535^{c}
G 221D	32.820^{cd}	0.903^{d}	1.138^{de}	1.463^{cde}
G 336	7.667^{g}	0.222^{f}	0.307^{e}	0.338^{e}
Rg 15	13.500^{fg}	0.332^{ef}	0.490^{e}	0.583^{de}
93/24/91 y	16.333^{efg}	0.465^{ef}	0.590^{de}	0.745^{de}
Sd 7	22.167^{defg}	0.726^{de}	0.947^{de}	1.043^{de}
Gz 643 y	62.787^{a}	3.127^{a}	6.167^{a}	10.025^{a}
Gz 624 cr	50.109^{ab}	2.134^{b}	3.015^{b}	4.794^{b}
Gz 630	23.167^{def}	0.583^{def}	0.760^{de}	0.895^{de}

Means with the same letter are not significantly different.

widely used to obtain proliferation of multiple shoots from shoot-tip cultures of many plants.

In order to prove the importance of BA (cytokinin) and 2,4-D (auxin) in the induction of multiple shoot clumps, another experiment was carried out using the inbred line Gz 643 which performed better than the other nine lines on the multiplication medium SM2 that contains 0.5 mg/l 2,4-D and 2 mg/l BA. In this experiment, the shoot-tip explants from *in-*

vitro-germinated seedlings of Gz 643 were cultured on another two different media containing different amounts of 2,4-D and BA. Four replicates were carried out with a total of 80 shoot tips cultured on each medium.

As shown in Table (5) the number of adventitious shoots that were produced per shoot-tip explant was considerably high (10.0) when the culture was on medium SM2 containing a combination of 2,4-D and BA. Moreover, adventitious shoots that were produced in the presence of 2,4-D and BA remained smaller, and leaf elongation was minimal as compared to the media deficient in 2,4-D or BA. Therefore, the determination of the optimum concentration of BA is very critical to obtain a large number of multiple shoot tips. These results agree with the findings of Zhong *et al.* (1992a). They showed that in corn, manipulating the concentration of BA and 2,4-D in the culture medium of shoot meristems could lead to the formation of either clumps of multiple shoots or somatic embryos *in vitro*.

Table (5): Shoot multiplication frequency and the average number of meristems of inbred line Gz643 after 3 months on three different shoot multiplication media

Medium (2,4-D , BA) mg/l	Shoot Multiplication Frequency (%)	No. of meristems after 3 months
SM1 (0.0 , 2.0)	6.25	0.7
SM2 (0.5 , 2.0)	63.9	10.0
SM3 (0.5 , 1.0)	21.25	1.3

The ability of multiplied shoot tips of the inbred line Gz 643 to regenerate into plantlets was tested by transfering the shoot clumps to the M1 shoot elongation medium containing 0.5 mg/l BA and 0.5 mg/l IBA, and then after 4 weeks the developed shoots were transferred to the rooting medium M2 containing 1 mg/l IBA.

The regenerated plantlets were acclimatized and transplanted into pots. Only 70% of these shoots developed into normal plants. The failure of the remaining shoot tips to develop into plantlets could be attributed to the nutritional limitations imposed by the crowded shoots.

Genetic Transformation of Shoot Apical Meristems

In the present study, the results of the shoot tip multiplication ability revealed that the genotype Gz 643 was the best line since it could easily proliferate multiple shoots and it could regenerate normal fertile plants. Therefore, all shoot-tip transformation experiments were conducted on this inbred line. The shoot clumps used in all transformation experiments were more than 2-month-old that were subcultured every 4 weeks as recommended by Zhong *et al.* (1996).

Although successful transformation of maize shoot meristems has been reported using microprojectile bombardment (Zhong *et al.*, 1996), the efficiency of transformation is highly dependent upon the transformation conditions. Therefore, in the present investigation, different bombardment parametrs were tested, including the plasmid DNA used, acceleration pressure and the number of shots per plate.

Eight independent experiments with different bombardment parameters were carried out on a total number of 224 shoot clumps of the inbred line Gz 643, each shoot clump contained 10 to 20 meristems.

Transient Expression of *GUS* Gene in Transformed Shoot Clumps

In order to determine the level of expression of the *GUS* gene, the histochemical *GUS* assay was performed two days after bombardment on two shoot clumps from each bombarded plate with a total of 64 shoot clumps chosen at random. The number of blue foci (blue spots) was counted under the binocular stereomicroscope and each blue spot was considered as a *GUS* expressing unit (Figs.2&3).

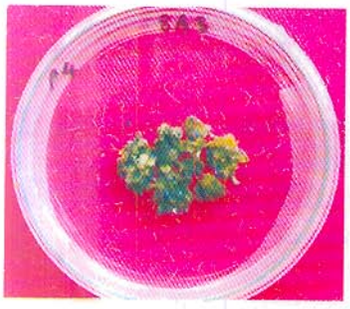

Fig.(2): Shoot clumps prepared for bombardment.

Fig.(3): The transient GUS blue expression in transformed shoot clumps.

Results presented in Table (6) indicated that, increasing the bombardment pressure from 1550 psi to 1800 psi. resulted in a significant decrease in the number of *GUS* expression units. While, increasing the number of applied shots per plate from 2 to 4 increased the number of blue foci, significantly. These findings are in agreement with those of Zhong *et al.* (1996). They found that the optimal delivery of DNA into subepidermal cells of shoot tips was achieved by the bombardment of more than 2-month-old shoot -tip clumps, that were subcultured every 2 weeks, with a low density (75 µg/shot) of 1.0 µm gold or tungsten particles with multiple shots (up to four) and 1550 psi acceleration pressure.

Table (6): Mean number of GUS expression units (blue spots) counted in the transformation experiments of shoot clumps using different parameters.

Plasmid	Pressure	No. of Shots	No. of Blue Spots
pAB-6	1550	2	136.000
	1550	4	326.250
	1800	2	63.000
	1800	4	125.500
Co-Transf.	1550	2	81.500
	1550	4	363.750
	1800	2	51.750
	1800	4	101.250

It could be suggested that the decrease in the number of *GUS* expression units when using a high velocity (1800 psi), resulted from a higher penetration of the DNA coated particles which damaged a large number of cells. While, increasing the number of shots led to the incorporation of the plasmid DNA into a large number of cells which was expressed into a higher level of transient *GUS* expression.

From the present results it could be concluded that the presence of *GUS* gene in two different constructs i.e., pAB-6 or pAct1-F under the control of the same promoter (rice actin-1) did not greatly affect the transient

GUS expression level. While the choice of acceleration pressure and the number of shots per plate is critical for the transformation and co-transformation experiments. The two plasmids pAB-6 and pAct1-F gave comparable results when used to transform multiple shoot clumps under the same conditions of pressure (velocity of particles), number of shots, particle density and particle size.

Molecular Analysis

A number of regenerated plants resulting from the transformation experiments were subjected to molecular analysis to confirm the integration of the transgenes (*Bar* and *GUS* genes) in the genome of these plants.

PCR Analysis

Total genomic DNA from leaf samples of putatively transgenic plants resulting from transformation and co-transformation experiments of shoot-tip clumps were analyzed by PCR using primers specific to the coding region of the *GUS* gene and to the coding region of the *Bar* gene. The expected amplification product was 750 bp for the *GUS* gene and 484 bp for the *Bar* gene. The results of the PCR analysis are given in Table (7).

Table (7): Summary of the results of PCR analysis.

Plasmid	No. of positive PCR events	
	GUS	*Bar*
PAB-6	7	7
pTW-a + pAct1-F	13	13

Amplified DNA from seven and thirteen putatively transgenic plants resulting from shoot clumps transformation and co-transformation experiments, respectively revealed the presence of the *GUS*-DNA fragment and the *Bar* DNA fragment at the expected molecular weight. This suggests that, whether the *GUS* and *Bar* genes were linked in the pAB-6 or unlinked in the two plasmids (pAct1-F and pTW-a), they had been cointegrated into the genome of the putatively transgenic plants. In this context, Zhong *et al.* (1996) reported that co-expression of the *Bar* and *GUS* genes was 57% when shoot meristems were bombarded with two different plasmids, one carrying the *Bar* and the other carrying the *GUS*.

Southern Blot Analysis

The results of the PCR analyses were further confirmed by Southern blot hybridization of the amplified DNA using the *GUS* and *Bar* coding regions as probes. The PCR amplified DNA of five selected PCR positive (*GUS* and *Bar*) samples is shown in Fig.(4) and the corresponding Southern blots are presented in Fig.(5). The results of Southern blotting of the PCR amplified DNA confirmed the presence of *GUS* (750 bp) and *Bar* (484 bp) DNA fragments in the tested samples (Lanes no. 1-5) and in the corresponding plasmid DNA and their absence in the nontransformed samples. Similarly, Zhong *et al.* (1993) analyzed 15 putatively transgenic turfgrass plants by PCR analysis using primers specific for the coding region of the *GUS* gene, the amplified DNA from four of these sample showed the presence of a 750 bp *GUS* DNA fragment which was further confirmed by Southern blot hybridization of the amplified DNA using the *GUS* coding region as a probe. These results indicated that the *GUS* and *Bar* genes are integrated into the genome of the positive samples.

Thus, from the present work it could be concluded that, a reproducible regeneration and transformation system for Egyptian maize inbred lines was successfully developed using the shoot apical meristems as explants. Among the studied lines, Gz 643 was identified as the best line for regeneration in this system. However, screening of more Egyptian

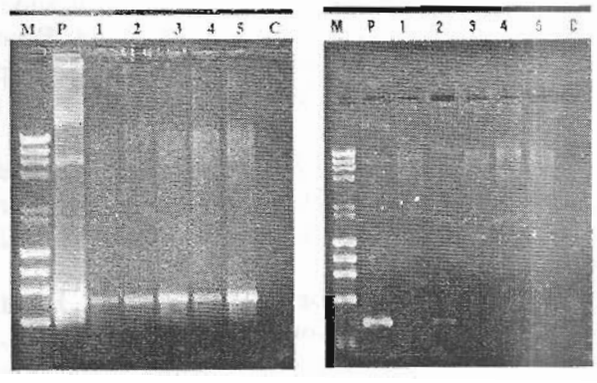

Fig.(4): PCR amplified DNA from untransformed and putatively transformed maize plants. Lane 1 to 5: Amplified DNA from 5 putatively transformed maize plants. P: Amplified plasmid DNA. C: Amplified genomic DNA from untransformed maize plants. M: DNA molecular weight size marker. (A) using pAct1-F and (B) using pTW-a.

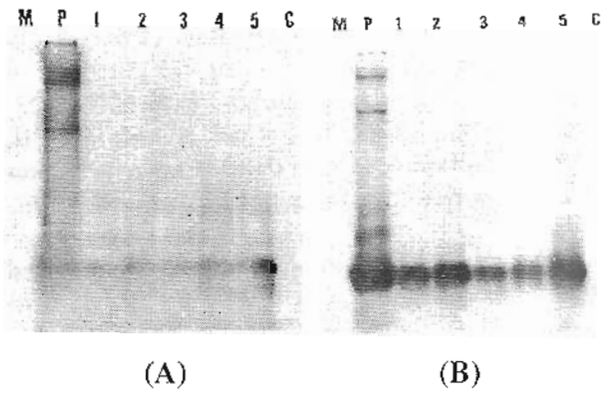

Fig.(5): Autoradiogram of Southern blot of PCR amplified DNA from untransformed and putatively transformed maize plants. Lane 1 to 5: Amplified DNA from 5 putatively transformed maize plants, P: Amplified plasmid DNA, C: Amplified genomic DNA from untransformed maize plants, M: DNA molecular weight size marker. (a) using pAct1-F and (B) using pTW-a.

genotypes is needed to identify the most economically important lines that could be regenerated.

This result could facilitate the introduction of foreign genes conferring resistance to insects, fungal diseases, environmental stresses and other commercially important genes into regenerable inbred lines. Consequently, this will lead to the improvement of the productivity and nutritional value of this important crop and to minimize the use of pesticides and environmental pollution.

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الملخص العربي

التباين في كفاءة التحول الوراثي للقمم النامية في سلالات من الذرة الشامية المصرية

إبتسام حسين على حسين*'، شيرين كمال عاصم*، هنيه عباس الإتربى*، فتحى محمد عبد الجليل*** ، مجدى أحمد مدكور***

* معهد بحوث الهندسة الوراثية الزراعية، مركز البحوث الزراعية، الجيزة ، جمهورية مصر العربية.

** قسم الوراثة ، كلية الزراعة ، جامعة القاهرة ، جمهورية مصر العربية.

*** قسم الكيمياء ، كلية العلوم ، جامعة القاهرة ، جمهورية مصر العربية.

تـم التوصـل الى طـريقة مثلى لإعادة التمايز في سلالات الذرة الشامية المصرية وذلك بزراعة القمم النامية في الأنبوب. وقـد كـان من المعتقد أن معدل إعادة التمايز بهذه الطريقة لا يختلف باختلاف التركيب الوراثي. وقد أظهرت السلالة جيزة- ٦٤٣ أعـلى معـدل لإعـادة الـتمايز. كما أوضحت النتائج أن إضافة مادتي الـ 6-BA و2,4-D أدت إلى تشجيع تكوين المجموعات المـتكاثرة مـن القمـم الـنامية وكذلـك الى زيـادة معدل التمايز لبعض السلالات المختبرة. وقد تم إجراء التحول الوراثي لهذه السـلالة (جيـزة-٦٤٣) باسـتخدام جهـاز الدفع المباشر لنقل جيني ال *GUS* و*Bar* الى مجموعات القمم النامية وذلك إما عن طـريق تحميـلها فـي بلازميـد واحـد (pAB-6) أو بلازميدين منفصلين (pTW-a & pAct1-F) وذلك تحت ظروف مختلفة مـن الضـغط وعـدد مـرات القـذف. وقد أظهرت النتائج أن أكبر عدد من البقع الزرقاء التى تمثل التعبير الجيني المؤقت لجين الـ *GUS* قـد أمكـن الحصـول عليه عند استخدام قوة ضغط تعادل ١٥٥٠ و ٤ قذفات لكل طبق. وقدتم التأكد من دخول جينات الـ *Bar*و*GUS* فـي جيـنوم بعض النباتات المحولة عن طريق تقنية التفاعل المتسلسل للبوليميرات (PCR) باستخدام بادئات خاصة لجيني ال *Bar*و*GUS* وكذلك عن طريق تقنية الـ Southern blotting التي أثبتت وجود الحزم الخاصة بالجينات المنقولة.