Transformation of the salt-tolerance gene *BI-GST* into Egyptian maize inbred lines

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ABSTRACT

Maize yield is severely affected by soil salinity. In an effort to engineer maize for improved salt tolerance, immature embryo derived calli of four selected Egyptian maize inbred lines (Gz 639, Gz 649, Sd 34 and Sd 62) and the American inbred 188, were bombarded with a plasmid containing the Bax-Inhibiting glutathione S-transferase (BI-GST) gene for abiotic stress tolerance and the bar gene for herbicide resistance. Thirty independent transgenic events were obtained from different experiments. The transformation efficiency varied among the different genotypes. The highest transformation efficiency has been obtained by A 188 in comparison to the other genotypes. While, the transformation efficiency of Gz 639 revealed the highest transformation efficiency among the Egyptian lines. Putative transgenic events have been tested by herbicide application through leaf painting, which revealed different levels of tolerance to the herbicide Basta. For molecular confirmation of putative transgenic plants, PCR analysis has been carried out and revealed the presence of both of the BI-GST and the bar genes in the DNA of the putatively transgenic plants. Southern blot hybridization confirmed the integration of the gene of interest (BI-GST) into the genome of the maize transgenic plants. Moreover, enzyme assays for GST and peroxidase revealed different levels of catalytic activities among the different transgenic maize lines and within lines. The GST activity ranged from 11.6 u/gm for Sd 34 to 1.2 u/gm for Gz 639 (19B). While, the highest peroxidase activity was 13.6 min^{-1} gm^{-1} ml^{-1} for A 188 (27) and the lowest was 0.9 min^{-1} gm^{-1} ml^{-1} for Gz 639(15). These results demonstrate that the BI-GST gene has been successfully transferred into maize lines and the salt tolerance may be improved in BI-GST transgenic plants.

***Key Words**: transformation, transgenic maize, immature embryos, B1-GST gene, salt–tolerance.*

INTRODUCTION

Abiotic stresses, such as drought, salinity, extreme temperatures, chemical toxicity and oxidative stress are serious threats to agriculture and the natural status of the environment (Munns, 2002 and Wang *et al.*, 2003). Environmental stress presents a major challenge in our quest for sustainable food production as it reduces the potential yields as high as 70% in crop plants (Pradeep *et al.*, 2006). Salinity is one of the major abiotic stresses that adversely affect crop productivity and quality. About 20% of irrigated agricultural land is adversely affected by salinity. The problem of soil salinity is further increasing because of the use of poor quality water for irrigation and poor drainage

(Chinnusamy *et al.,* 2005). Increased salinization of arable land is expected to have devastating global effects, resulting in 30% land loss within the next 25 years, and up to 50% by the year 2050 (Munns, 2002 and Wang *et al.,* 2003). In Egypt, there is a remarkable and continuous growth in population and land scarcity as well. Therefore, the challenge for agricultural researchers is to produce high-yielding crop varieties. The effort exerted in this context is interrupted by two major limiting factors, i.e. drought and salinity. They are considered as major constraints and have adverse impacts on crop production, food security and socioeconomic aspects in the Middle East. About 40% of all arable lands cannot be used because of the potential salinity problems. High salinity causes ion imbalance due to elevated toxic levels of the cytoplasmic sodium and drought stress (Town and Mahamed, 2008). In general, salinity can inhibit plant growth by three major ways (Greenway and Munns, 1980): I) Water deficit arising from the more negative water potential (elevated osmotic pressure) of the soil solution; II) Specific ion toxicity usually associated with either excessive chloride or sodium uptake; and III) Nutrient ion imbalance when the excess of Na^+ or Cl^- leads to a diminished uptake of K^+, $Ca2^+$, $NO3^-$ or P, or to impaired internal distribution of one or another of these ions. Moreover, under different abiotic and biotic stresses, plants accumulate active oxygen species (ROS)(Shou *et al.,* 2004).

Maize is an important crop not only is one of the most abundant sources of food and feed for people and livestock allover the world but also is an important component of many industrial products. Maize byproducts are present in, for example, glue, paint, insecticides, toothpaste, rubber tires, rayon, and molded plastics, among others. Maize is also currently a major source of ethanol, a major biofuel that is more environmentally friendly than gasoline and that may be a more economical fuel alternative in the long run (Lawrence and Walbot, 2007). Like other cereal crops, the yield of maize (*Zea mays* L.) is affected by several biotic and abiotic factors. Drought and salt cause 24 million tons of yield loss in maize annually (Halsey *et al.,* 2005). GSTs are a family of multifunctional enzymes that play critical roles in the detoxification of xenobiotics. The protection of tissues against oxidative damage is accomplished by quenching reactive molecules with the addition of reduced glutathione (GSH) (Chen *et al.,* 1996). Direct proof of the importance of GSTs in protection from xenobiotics was that expression of the maize GSTIV in tobacco provided protection from metolachlor (Jepson *et al.,* 1997). Moreover, Ulmasov *et al.* (1995) demonstrated the role of GSTs in stress tolerance .They stated that expression of GSTs in plants is highly responsive to biotic and abiotic stress and to a wide variety of stress-associated chemicals, including 2, 3-dichlorophenoxyacetic acid and other synthetic and natural auxins, salicylic acid, methyl jasmonate, abscisic acid, and H_2O_2. Dixon *et al.* (2002) reported that, in plants, all the GSTs are dimmers composed of 25KDa subunits, and on the basis of sequences similarity and gene organization, they appear to have evolved from a common ancestral GST into four distinct classes, namely the Phi, Tau, Zeta, and Theta GSTs. The two largest classes are the plant-specific Phi and Tau GSTs. Both classes have major roles in herbicide detoxification. In addition, these GSTs have less well characterized roles in endogenous metabolism including functioning as glutathione peroxidases counteracting oxidative stress and also acting as flavonoid-binding proteins, stress signaling proteins and regulators of

apoptosis. Atanassova *et al.* (2003) reported that the plant tau-class glutathione S-transferase BI-GST (Bax-Inhibiting glutathione S-transferase) was identified as a potent inhibitor of Bax lethality in yeast. Bax is a phenotype associated with oxidative stress and disruption of mitochondrial functions. They added that BI-GST restores glutathione levels to wild type levels, preserves the mitochondrial membrane potential in Bax expressing yeast cells. Additionally, it was shown that expression of BI-GST in yeast enhances cell viability upon treatment with pro-oxidant compounds. In the present investigation four Egyptian maize inbred lines and an American inbred were transformed using the biolistic particle delivery system and a plasmid containing the *BI-GST* gene for abiotic stress tolerance and the *bar* gene as a selectable marker. The integration and expression of the transgenes have been demonstrated by subjecting the putatively transgenic plants to leaf painting, enzymes assay, PCR and Southern blot analyses.

MATERIALS AND METHODS

Plant material

Four elite Egyptian maize (*Zea mays* L.) inbred lines, namely Sd 62, Sd 34, Gz 639 and Gz 649, in addition to an American inbred (A188) were used in this investigation. Seeds were sown in the field during the maize season, plants were self pollinated and ears were harvested 10-15 days post pollination. Immature embryos were excised to be used as explants in transformation experiments.

Plasmid construct

The plasmid BI-GST/bar has been used for the transformation of maize tissues. This plasmid contains the *BI-GST* (Bax-Inhibing Glutathione S-Transferase) and the *bar* (bialaphos resistance) genes (Fig.1).Both genes were driven by the CaMV-35S promoter and terminated by the *nos* terminator. The plasmid DNA was propagated in *E.coli.* DH5α, isolated on a large scale according to Promega kit (Cat# A7270).

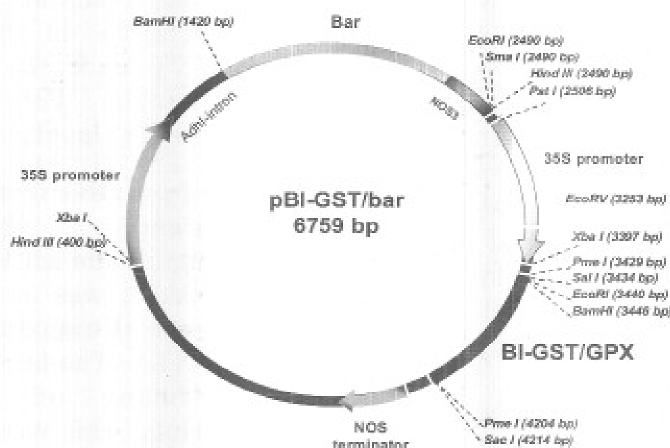

Fig. (1): Schematic representation of plasmid BI-GST/bar.

Explantation and osmotic treatment

Immature embryos (1.0 – 1.5 mm) were aseptically excised from the kernels and cultured scutellum up on callus induction medium (Carvalho *et al.*, 1997). This medium was composed of N6 basal salt and vitamins,

supplemented with 2 mg/l 2,4-D, 2.88 gm/l L-proline, 8.5 mg/l silver nitrate, 3% sucrose and solidified with 3 gm /l phytagel. After 4-7 days, the calli were exposed to an osmoticum treatment as described by Vain *et al.* (1993) and El-Itriby *et al.* (2003). This was performed by placing the calli on callus induction medium supplemented with 45.4 gm/l sorbitol and 45.4 gm/l mannitol. Calli were subjected to osmoticum treatment 4h prior to bombardment and continued for 16h after bombardment.

Particle bombardment and selection of transformants

Plasmid DNA was precipitated onto gold particles (Bio-Rad 1.0µm in diameter) following a modification of the original protocol for the Bio-Rad Biolistic PDS-1000/He Particle Delivery System. Briefly, 50 mg of gold particles were prepared in 0.5ml sterile water. Each 50 µl aliquot of the gold particles was mixed with 5 µl plasmid DNA (1 µg/µl), 50µl $CaCl_2$ (5M) and 20µl spermidine (0.1M) with continuous vortexing. The mixture was vortexed for 3min, spun down for 10 sec and the supernatant was removed. The gold particles were resuspended in absolute alcohol (250 µl) and vortexed, then spun down and the supernatant was discarded. Particles were resuspended in 70 µl of absolute ethanol. The bombardment parameters used in the transformation experiments were 900 psi pressure and one shot per plate. After bombardment the plates were transferred to callus induction medium for one week in darkness as a recovery period to allow healing of the transformed cells. Selection of the transformed events for the *bar* gene (herbicide resistance) has been performed by transferring the transformed calli to N6-based callus-induction medium containing 1.5 mg/l bialaphos for two weeks. The concentration of the selective agent was then increased to 3.0 mg/l in the subsequent subcultures.

Regeneration of putatively-transgenic plantlets

For regeneration, the bialaphos-resistant calli, which grew uniformly on the selection medium, were transferred to the shooting medium (MS based medium containing 0.25 mg/l NAA and 1g/l myo-inositol) supplemented with 3 mg/l bialaphos. The selected shooted calli were transferred to the rooting medium (MS containing 0.5 g/l myo-inositol) supplemented with 3mg/l bialaphos.

Acclimatization of putative transgenic plantlets

The developed putatively transgenic plantlets were transferred to an aquarium containinga Hoagland solution. Then, healthy rooted plantlets were transferred to pots containing a mixture of peat moss: soil: sand (1:1:1) in the greenhouse adjusted at 28°C under 16 hr photoperiod and 50% humidity. Pots were watered using half strength Hoagland solution.

Evaluation of putative transgenics

PAT activity assay

To determine the activity of PAT, the *bar* gene product, was assayed indirectly by the resistance of transgenic plants to the herbicide BASTA. The herbicide was used for leaf painting of the putatively transgenic plants. This was carried out by painting approximately 5-10 cm leaf sectors near the tip of the youngest fully-extended leaf at the four-leaf stage with a solution of the herbicide containing 0.1 % (v/v) Tween 20.

Peroxidase and glutathione S-transferase enzymes activity assays

For enzyme assay, maize leaves (0.25 g) were homogenized at 4°C with 3.0 ml, 100 mM potassium phosphate buffer (pH 7.0) which contained 1mM EDTA and 1%(w/v) polyvinyl pyrrolidone (PVP). The homogenate was filtered through four layers of cheesecloth and centrifuged at 15000 x g for 20 min.

Peroxidase activity

Peroxidase activity was measured by following the change of absorption at 470 nm due to guaiacol oxidation by spectrophotometer (*Smartspec 3000*) according to the procedure described by Yordanova *et al.* (2004). The activity was assayed for 10 min in a reaction solution (3 ml total volume) composed of 100 mM potassium phosphate buffer (pH 7.0), 20 mM guaiacol, 10 mM H_2O_2 and 50µl of crude extract.

Glutathione *S*-transferase activity

GST was measured according to the procedure described by Lim *et al.* (2005) by following the change in absorption at 340 nm due to 1-chloro-2,4-dinitrobenzene (CDNB) by spectrophotometer (*Smartspec 3000*) . The activity was assayed for 10 min in a reaction solution (1.5 ml total volume) composed of 100 mM potassium phosphate buffer (pH 7.0), 20 mM reduced GSH, 20 mM CDNB and 50µl of crude extract.

PCR screening for transgenic plants

Genomic DNA was extracted from putatively transgenic plantlets and non-transformed plants as control using the CTAB protocol described by Murray and Thompson (1980). Two sets of primers were used to detect the *bar* and the *BI-GST* genes. The nucleotides sequence of the specific primers for the *bar* gene: Pbar1 (5' TGC CAC CGA GGG GAC ATG CCG GC 3') and P*bar*2 (5' CCT GAA GTG GAG GCC A TG GGG 3') were used to amplify a 484 bp fragment of DNA containing the *bar* gene. While, primers P*gst*1 (5' TGT TGG ATT TTT GGC CTA GC3') and P*gst*2 (5' TGC TCT CCT CCC TTT GTT GT3') were used to amplify a fragment of 343 bp for the *BI-GST* gene. The PCR reactions were carried out in a volume of 25µl containing 50 ng of genomic DNA template, 1X PCR buffer, 1µM forward and reverse primer, 200 µM dNTPs, 0.5 unit of Taq polymerase. The PCR temperature profile included an initial DNA denaturation for 3 min at 95°C followed by 40 cycles of (95°C, for 1 min; 55°C (for the *gst* gene) or 60°C (for the *bar* gene) for 1 min and 72°C for 1 min) and a final extension step at 72°C for 7 min.

Genomic southern analysis

Ten micrograms of genomic DNA from transformed and control plants were digested with the restriction enzymes *BamH1* and *Sac1* in order to detect the integration of the *BI-GST* gene in the regenerated transformed plants according to Kreike *et al.* (1990). The digested DNA was electrophoretically separated on a 0.8% agarose gel, transferred to a positively charged nylon membrane (Boehringer Mannheim) and then cross linked to the membrane by UV irradiation. The hybridization probe was prepared by digesting the pBI-GST with *BamH1* and *Sac1* to liberate the BI-GST DNA. After separation by electrophoresis, the desired insert was excised using the Agarose Gel DNA Extraction kit (Qiagen kit) and labeled using a random priming DNA labeling and detection kit (Roche Cat. No.(11093657910). the labeled probe was

incubated with the membrane at 65°C for 16-18 hr and the hybridization signals were detected by the colorimetric method.

Statistical analysis

Statistical analysis has been performed according to Steel and Torrie (1980) using the SAS computer software (version 5) with associated least significant differences (LSR) module. Experiments were designed as factorial experiments in a completely randomized design.

RESULTS AND DISCUSSION

Maize Transformation

Immature zygotic embryos have become the explant of choice in cereals (Ward and Jordan, 2001; El-Itriby *et al.*, 2003 and Oduor *et al.*, 2006). In the present study, the biolistic particle delivery system was used for the transformation of maize immature embryos (Fig. 2).

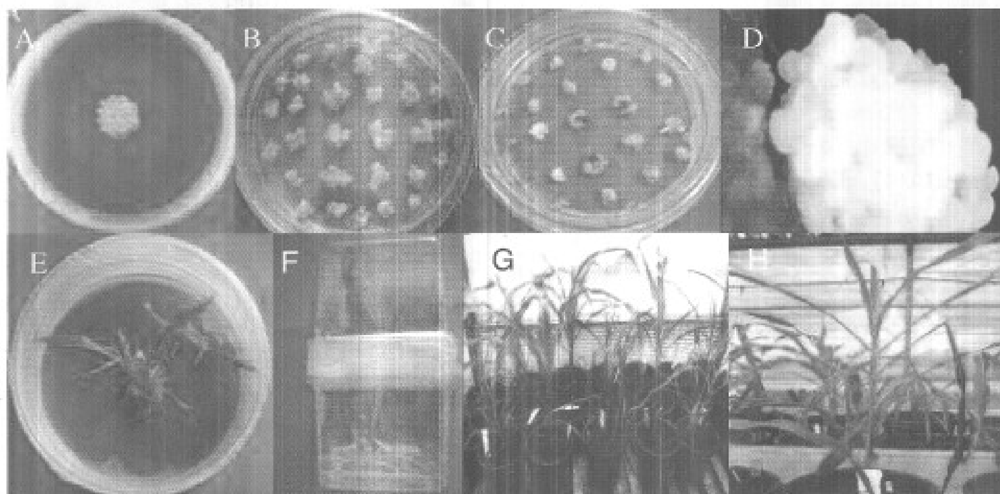

Fig. (2): Maize transformation: (A) Immature embryos after bombardment. (B and C) Callus on selection medium containing 1.5 mg/l bialaphos and 3 mg/l bialaphos, respectively. (D) Embryogenic calli . (E) Shoot formation on regeneration medium. (F) Regenerated maize plantlet. (G&H) Regenerated maize plants.

Transformation experiments were performed on four Egyptian maize inbred lines, i.e., Sd 34, Sd 62, Gz639, Gz 649 and one American inbred (A188). Thirty immature embryo derived calli were bombarded per plate and the osmotic treatment has been applied to all of the bombarded plates (Fig 2A). Osmotic treatment has been reported by Vain *et al.* (1993) and El-Itriby *et al.* (2003) to increase the transformation efficiency. In total, 7067 explants were bombarded using the gene gun transformation method. Putative transgenic lines have been obtained from all transformation experiments. Most of putative transgenic lines were developed into morphologically normal plants. Transformation efficiency has been calculated as the number of regenerated plantlets/ number of bombarded embryos x (100). The mean number of bialaphos survived calli varied among the different genotypes which was reflected on the mean number of regenerated

plantlets and the mean number of cell lines obtained (Table 1). Results revealed that A188 revealed the highest significant transformation efficiency among all genotypes used in transformation experiments (10.80). While, Gz 639 revealed the highest significant transformation efficiency (6.79) among the Egyptian lines. However the number of Gz 639 transgenic plants reaching maturity in the greenhouse was higher than that of A188. On the other hand, the lowest transformation frequency (1.12%) has been expressed by Sd62 which was not significant than that of Sd 34 (1.30%) (Table 1 and Fig. 3). In this respect Binott *et al.* (2008) reported that the success of regeneration procedures is affected predominantly by genotype, the type of explant material employed and medium composition. Genotype specificity of somatic embryogenesis and regeneration in maize has been reported previously by Hodges *et al.* (1986), Willman *et al.* (1989) and El-Itriby *et al.* (2003). In the present study, the American line A188 and the Egyptian line Gz 639 were able to produce higher percentage of type II callus compared to the other three Egyptian lines, and this was reflected on the number of regenerated transgenic plants recovered from each genotype.

Table (1): Means of number of surviving calli, number of plantlets and number of cell lines and transformation frequency for the five maize inbred lines.

Genotype	No. of bombarded explants.	Mean No. of survived calli	Mean No. of plantlets	Mean No. of cell lines	Transformation frequency %
A188	1452	18.20_a	6.22_a	3.16_a	10.80_a
G639	1072	12.94_b	2.36_b	2.03_b	6.79_b
G649	1719	12.24_b	1.60_c	1.29_c	4.34_c
Sd34	1531	8.55_c	0.39_d	0.39_d	1.30_d
Sd62	1293	5.98_d	0.34_d	0.34_d	1.12_d

Means in the same column followed by the same letters are not significantly different at 5% level of probability.

Herbicide resistance of putative transgenic plants was tested by painting the upper and lower sufaces of the middle part of a fully extended green plant's leaf with 1g/l basta. Herbicide painting of putative transgenic and non-transgenic plants showed different levels of tolerance of transgenic plants to the herbicide in comparison to non-transgenic plants which showed wilting at the painted areas. The transgenic plant leaves were resistant to the herbicide (stay-green), while non-transgenic leaves turned yellow within 7 days of herbicide application (Fig.4 and Table 2). Resistance of putative transgenics to basta herbicide verify *bar* gene expression in transgenic maize plants.

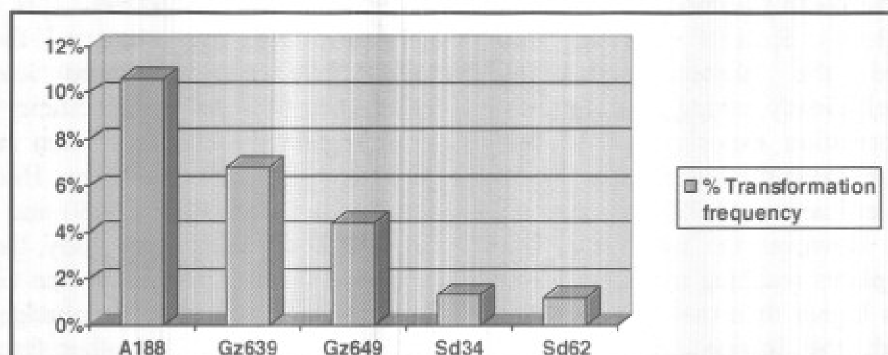

Fig. (3): Histogram illustrating the transformation frequency for the five maize inbred lines. Evaluation of the putative transgenic plants with the herbicide Basta.

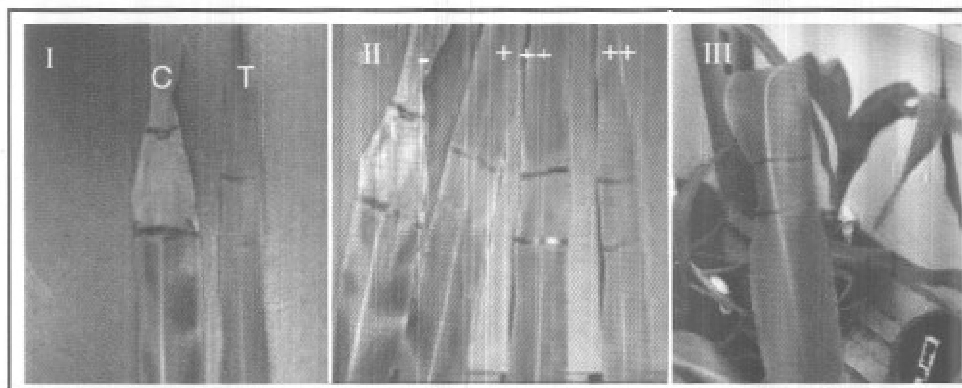

Fig. (4): Leaf painting with the herbicide Basta (I) Basta herbicide painting of transgenic (T) and control (C) plants.(II) Different levels of tolerance to the herbicide Basta.(III)High expression of tolerance to the herbicide Basta.

Molecular analysis

The introduction of the *BI-GST* gene into maize plants was demonstrated by PCR analysis. DNA amplification of all plants regenerated in the presence of bialophos was analysed by PCR for the co-integration of the *BI-GST* and *bar* genes. After regeneration under selection pressure, PCR analysis has been carried out on DNA extracted from putatively transformed plants using primers specific to the coding regions of the *BI-GST* and *bar* genes. A total of thirty independent lines (i.e. one line of Sd 62, one line of Sd 34, two lines of Gz 649, fourteen lines of Gz 639 and twelve lines of A188) of the survived putatively transgenic lines analyzed could amplify a 343 bp fragment of *BI-GST* and the *bar* amplification fragment at 484 bp (Fig.5_A and 5_B, respectively). These bands were also amplified by DNA of the positive control (plasmid BI-GST/bar), while were absent in the DNA of the non-transgenic plants. PCR analysis with BI-GST and bar primers confirmed that the targeted genes were introduced into the transgenic plant's genome.

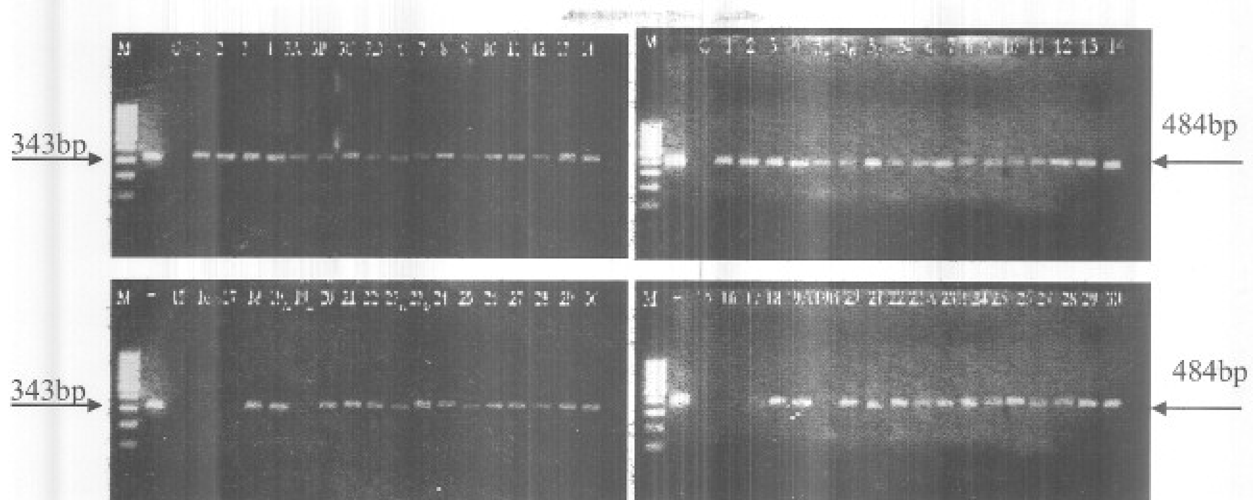

Fig.(5): PCR amplified DNA using the BI-GST gene specific primers (A)and the bar gene primer (B) from BI-GST/bar plasmid (+), untransformed (C) and putatively transformed maize plants (1-30), (M) 100 bp ladder DNA molecular weight size marker.

Southern blot hybridization of T_0 plants was performed to further confirm the results of leaf painting and PCR analysis. It was carried out using a *BI-GST* specific probe.Results of southern blot analysis are shown in Fig.(6). Digestion of 10 µg of genomic DNA samples with *BamH1 and Sac1*, resulted in the detection of a clear hybridizing band at 768 bp in the genome of transgenic plants, which corresponds to the *BI-GST* (lanes 1 to 23B) while there was no hybridyzing band in the control wild type plants (lane C). These results confirmed that the *BI-GST* gene had been integrated into the genome of the transgenic plants.

PCR and southern blot analyses have been used by several authors to confirm the introduction and integration of the transgenes in transgenic plant genomes. Milligan *et al.* (2001) proved the introduction and integration of the *GST-27* and *pat* genes into the genome of six independent T_0 lines of wheat out of 1010 explants co-bombarded with plasmids containing the *GST-27* and *pat* genes. Moreover, Lim *et al.* (2005) used PCR analysis to confirm the introduction of the NT107 gene (a partial GST cDNA fragment) into the genome of 35 transformed *D. superbus* plants, Southern blot analysis proved the integration of the transgenes in the genome of the analyzed plants. Hantao *et al.* (2004) transformed the salt-tolerance gene, *CSRG1* into tobacco genome by the ameliorated leaf discs method of *Agrobacterium*-mediated transformation. Thirteen stable resistant lines were obtained under selection pressure of 50 mg/l hygromycin and 150 mg/l kanamycin. Assessment of PCR amplification and Southern blot analysis showed that *CSRG1* gene has been integrated into the genome of eleven transgenic lines.

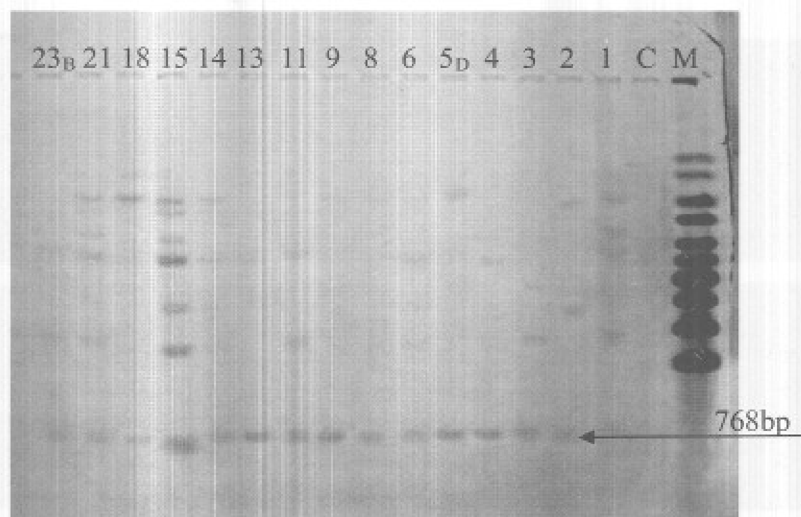

Fig. (6): Southern analysis of some T_0 putatively transgenic plants of maize. (M) Molecular weight size marker; (C) negative control; (1-23_B) represent 15 samples of transgenic plants.

Enzyme assays

Levels of total GST activity and peroxidase activity were assayed in transgenic and wild-type plants using CDNB and guaiacol as a substrate, respectively. GST and peroxidase expressing transgenic maize plants had an approximately more than two fold higher activity in leaf extracts compared to the control plants (Table 2). Results from these assays revealed different levels of catalytic activities among the different lines and within the same line .The highest activity of GST was 11.2 u/g, which has been obtained by the transgenic line No.2 of Sd 34 and its peroxidase activity was 8.1 min^{-1} gm^{-1} ml^{-1}. Moreover, transgenic line No. 5_D of Gz 639 gave a relatively high GST activity (8.6 u/g) and its peroxidase activity was considerably high (12 min^{-1} gm^{-1} ml^{-1}). The highest peroxidase activity was achieved by the A188 transgenic line no.27 which gave 13.6 min^{-1} gm^{-1} ml^{-1} and its GST activity was 5.5 u/g. The means of enzymes' activity are presented in Fig. (7) showing that the mean GST activity of all transgenic lines was higher than that of the control for these lines. The highest means for GST have been obtained by Sd 34 (11.2) and the highest mean for peroxidase enzyme activity has been revealed by A 188 as well as Sd 34 (8.14 and 8.10, respectively). Enzyme assays results were in consistent with the herbicide painting, PCR and Southern blot analysis. These results confirmed that *BI-GST* transgenes encode an active GST and peroxidase in transgenic maize plants. In this context, Lim *et al.* (2005) determined the levels of total GST activity in transgenic and wild type *Dianthus superbus* plants using CDNB as a substrate. GST-expressing transgenic plants showed approximately two fold higher activity in leaf extracts compared to control plants. Kis *et al.* (2004) demonstrated that the transgenic tobacco plants can exhibit a ten-fold increase in peroxidase activity compared with wild-type tobacco levels.

It is commonly considered that salt tolerance of plants is an integration of many physiological salt-tolerant characters, which are controlled by many genes in different chromosomes (Hantao *et al.*, 2004). GSTs and specially BI-GST may be one of the key genes

of salt tolerance which can protect transgenic plants under salt stress conditions.

Table (2): Data of PCR, leaf painting and enzyme assay for the transformation events from the five maize inbred lines.

Plant #		PCR		Leaf painting	GST u/g	Peroxidase ($min^{-1}\ gm^{-1}\ ml^{-1}$)
		Bar	GST			
Sd62	Cont.	-	-	-	1.15	1.0
	1	+	+	++	4.60	5.9
Sd34	Cont.	-	-	-	1.14	0.6
	2	+	+	+++	11.2	8.1
Gz 649	Cont.	-	-	-	1.32	0.94
	3	+	+	+	3.2	8.9
	4	+	+	++	4.8	3.6
Gz 639	Cont.	-	-	-	1.1	0.94
	5A	+	+	++	2.4	10.4
	5B	+	+	+	1.6	5.8
	5C	+	+	+	1.2	1.8
	5D	+	+	+++	8.6	12
	6	+	+	++	4.8	3.5
	7	+	+	++	3.8	2.6
	8	+	+	+++	6.2	5.0
	9	+	+	+++	7.9	4.5
	10	+	+	+	4.6	1.9
	11	+	+	+++	6.7	5.36
	12	+	+	+	3.7	2.0
	13	+	+	+	2.7	1.8
	14	+	+	++	2.9	4.6
	15	-	-	-	1.5	0.9
	16	-	-	-	1.2	1.0
	17	-	-	-	1.4	1.1
	18	+	+	-	2.13	1.6
A188	Cont.	-	-	-	1.18	0.56
	19A	+	+	+	2.5	3.2
	19B	-	-	-	1.2	0.8
	20	+	+	+	4.47	7.2
	21	+	+	+++	7.2	12.0
	22	+	+	+	5.6	8.0
	23A	+	+	++	5.2	11.1
	23B	+	+	+++	5.4	9.6
	24	+	+	++	4.5	6.5
	25	+	+	+++	7.0	11.2
	26	+	+	+++	4.5	5.0
	27	+	+	+++	5.5	13.6
	28	+	+	+	6.0	8.8
	29	+	+	+	4.8	6.4
	30	+	+	+	6.5	10.5

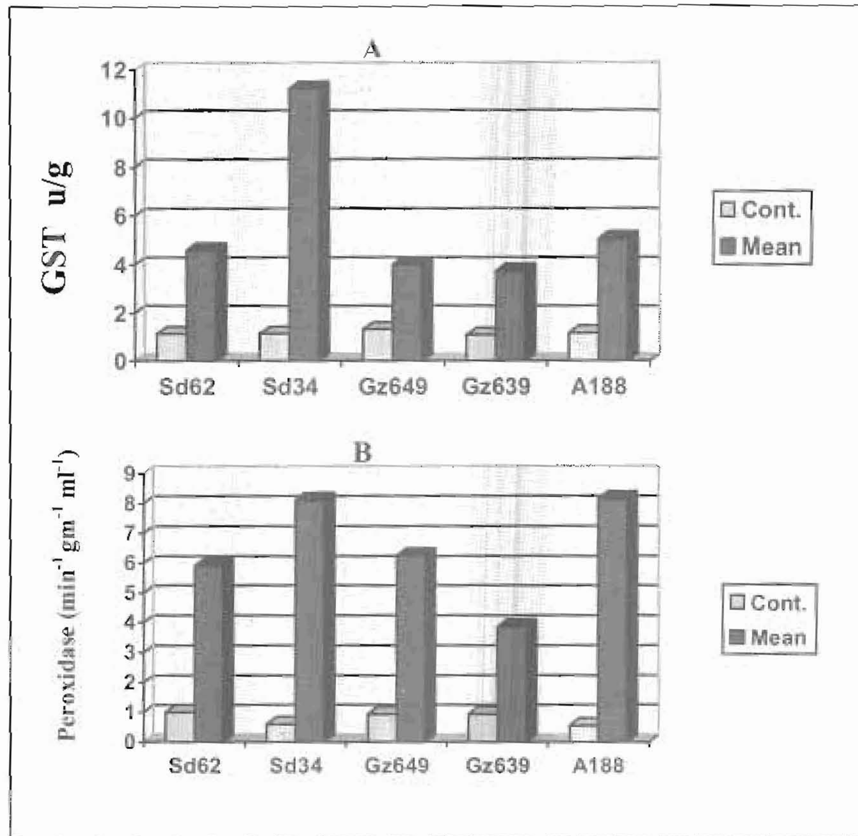

Fig. (7): Histogram revealing the means of GST (A) and peroxidase (B) enzyme activity for transgenic maize lines as compared to the control genotypes.

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الملخص العربي

التحول الوراثي لسلالات مصرية من الذرة الشامية بجين *BI-Gst* لتحمل الملوحة

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يتأثر محصول الذرة الشامية تأثراً شديداً من ملوحة التربة. و في محاولة منا لهندسة الذرة وراثياً لزيادة قدرتها على تحمل زيادة الملوحة و الإجهاد البيئي، تم اجراء عملية التحول الوراثي لأربعة سلالات تربية داخلية مصرية من الذرة الشامية هي جيزة 639، جيزة 649، سدس 34 و سدس 62 بالإضافة إلى السلالة الامريكية A188 باستخدام طريقة القذف المباشر لنقل جين *BI-GST* لتحمل الإجهاد البيئي و جين الإنتخاب *bar* لمقاومة مبيدات الحشائش إلى الكالس الناتج من الأجنة غير الناضجة للخمس سلالات من الذرة الشامية. وقد امكن الحصول على عدد ثلاثون نباتاً محولاً وراثياً من تجارب التحول الوراثي المختلفة. وقد تفاوتت كفاءة التحول الوراثي بين السلالات المختلفة. حيث كانت أعلى كفاءة للتحول الوراثي من قبل سلالة A188 مقارنة مع السلالات الأخرى. في حين أن كفاءة التحول الوراثي للسلاله جيزة 639 كشفت عن أعلى نسبة للتحول الوراثي بين السلالات المصرية. و قد تم اختبار النباتات المعدلة وراثياً باستخدام مبيد الحشائش "باستا" حيث اظهرت هذه النباتات مستويات مختلفة من المقاومة لهذا المبيد. و قد تم التحليل الجزيئي للنباتات المحولة وراثياً باستخدام تقنية تفاعل البلمرة المتسلسل PCR الذي أثبت وجود الجينات المنقولة إليها. و قد أكد تحليل Southern blot وجود الجين المنقولة في جينوم نباتات الذرة المعدلة وراثياً. و علاوةً على ذلك، فقد كشف تحليل النشاط الإنزيمي لإنزيمي GST, Peroxidase عن مستويات مختلفة من النشاط الإنزيمي لكل منهما في السلالات المحوله المختلفة و داخل كل سلالة. و قد تراوح النشاط الإنزيمي للـ GST في السلالات المعدلة وراثياً بين 11.6 u/gm للسلالة سدس 34 إلى 1.2 u/gm للنبات رقم (16) من السلالة جيزة 639 ونبات رقم (19_B) من سلالة A188. في حين أن أعلى نشاط لإنزيم Peroxidase كان $13.6\ min^{-1}\ gm^{-1}\ ml^{-1}$ الذي تم الحصول عليه من النبات رقم (27) للسلالة A188 . و قد كان أقل نشاط إنزيمي للPeroxidase هو $0.9\ min^{-1}\ gm^{-1}\ ml^{-1}$ الذي تم الحصول عليه من النبات (15) للسلالة جيزة 639 . هذه النتائج تدل على أنه قد تم نقل جين BI-GST بنجاح إلى سلالات الذرة الشامية و أن وجود هذا الجين في النباتات المعدلة وراثياً قد يحسن من قدرة هذه النباتات على تحمل الإجهاد البيئي مثل زيادة ملوحة التربة.