A rapid and efficient tomato regeneration and transformation system

Paper presented at the workshops "Better Plants for Better Life" conducted during the German/Egyptian Year of Science and Technology 2007 at ARC in Cairo/Egypt and FAL in Braunschweig/Germany

Ghada A. Abu-El-Heba[1], Gihan M. Hussein[1] and Naglaa A. Abdalla[1,2]

Abstract

Tomatoes have great economic importance in greenhouse and field production in Egypt. Tissue culture and molecular engineering have provided rapid modes to develop desirable varieties of cultivated plant species. Genetic engineering will allow adding new fruit quality genes, one at a time, with simultaneous avoiding introduction of the rouge characteristics. In this work, rapid regeneration and transformation systems of tomato cultivar CastleRock has been developed using hypocotyl with a part of cotyledon (hypocotyledonary), after removal of primary meristem, as an explant. The explants were cultured on ten different combinations of hormones and vitamins. The highest frequency and earliest regeneration were obtained on medium X1N which composed of MS medium supplemented with 1 mg/l BAP, 1 mg/l Zeatin ripozide, 5mg/l $AgNO_3$ and Nitch&Nitch vitamin. Shoots were developed after only 10 - 12 days with a percentage of 92 %. In addition, transformation system has been established with two different methods, *Agrobacterium* mediated transformation and Biolistic gun. In the case of *Agrobacterium* transformation, the binary vector pISV2678 which contains the *gus*-intron and *bar* genes was used. While, the plant vector pMON-RTG harboring the *gus* gene was used with biolistic gun transformation. Putative transgenic shoots were regenerated on the selected regeneration medium and developed shoots were transferred to rooting media. T_0 plants were analyzed using histochemical *gus* assay and the PCR.

Keywords: Agrobacterium mediated transformation, biolistic gun

Zusammenfassung

Ein schnelles und effizientes Sytem zur Regeneration und Transformation von Tomaten

Die Arbeit beschreibt ein Verfahren zur schnellen und effizienten Regeneration und genetischen Transformation der in Ägypten weitverbreiteten Tomatensorte "Castle Rock". Ausgangsmaterial ist das Hypocotyl der Pflanze mit einem Teil des Keimblattes nach Entfernen des primären Meristems. Die besten Regenerationsraten wurden auf einem MS Medium mit Zusatz von 1 mg/l BAP, 1 mg/l Zeatin ripozide, 5 mg/l $AgNO_3$ und Nitch&Nitch Vitamin erzielt. 92 % der Ansätze entwickelten bereits nach 10 - 12 Tagen Sprösslinge. Zur genetischen Transformation wurden *Agrobacterium* und eine Gen-Kanone eingesetzt.

Schlüsselwörter: Agrobacterium vermittelte Transformation, Gen-Kanone

1 Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC), 12619 Giza/Egypt; Email: gghosny@gmail.com

2 Departments of Genetics, Faculty of Agriculture, Cairo University, 12619 Giza/Egypt

1 Introduction

Tomato is a major vegetable crop that has achieved tremendous popularity over the last century and it is grown in almost every country of the world. Tomatoes breeding programs can highly benefit of biotechnological tools, such as gene transfer technology, which allows the introduction of foreign genes into a germplasm, without modifying the genetic background of elite varieties. However, a breeding program associated to biotechnological tools depends upon the development of an efficient *in vitro* plant regeneration system. *In vitro* plant regeneration of tomatoes using protocols for adventitious shoot regeneration from cotyledon segments has been reported (vanRoekel et al., 1993). The system is based on three culture steps (Dong & Jia, 1991): a bud induction phase, culturing the explants in medium supplemented with cytokinin (Compton and Gray, 1993); an elongation phase, transferring the shoot buds to medium with a lower concentration of cytokinin (Dong and Jia, 1991); and a rooting phase, using a culture medium supplemented with auxin (Compton and Gray, 1994; Abu El-Heba, 2004).

The hormones auxin and cytokinin control plant development through a multitude of complex interactions. The balance between auxins and cytokinins controls the formation of roots, shoots, and callus tissue *in vitro* (DeRopp, 1954 and Skoog and Miller, 1957). The mode of interaction between auxins and cytokinins can therefore be synergistic, antagonistic, or additive and is dependent on the type of tissue and on the plant species in which the interaction occurs. Although the molecular mechanisms underlying most of these auxin-cytokinin interactions are unknown, they are thought to include mutual control of auxin and cytokinin metabolism, interactions in the control of gene expression, and posttranscriptional interactions (Coenen and Lomax, 1997).

In vitro regeneration through organogenesis and somatic embryogenesis can be used for multiplication of genetically identical clones and it is an integral part of genetic transformation procedures. The *in vitro* morphogenetic responses of cultured plants are affected by different components of the culture media and it is important to evaluate their effect on plant regeneration. Although advances are being made toward better understanding of metabolic processes correlated with regeneration (Lambe' et al., 1997; Canirney et al., 2000), determining of the conditions for *in vitro* plant regeneration is still largely an empirical process. Thus, *in vitro* regeneration can be difficult to achieve for some plant species or particular genotypes within a species.

Development of protocols for *in vitro* selection can provide new advances for the production of stress tolerant cultivars (Bhatia et al., 2004). The use of a combination of molecular and conventional breeding techniques could be the option for the development of cultivars resistant to biotic and abiotic stresses. Transformation frequency is one of the most important limiting factors in obtaining transgenic plants. In addition, a reliable regeneration protocol for the target plant material is another important parameter. Therefore, prior the transformation work, plant regeneration conditions have to be optimized for a given plant species and type of explant (Potrykus et al., 1995). Somatic embryogenesis in tomato is still at its infancy, and efficient procedures for large-scale production *via* somatic embryogenesis are yet to be developed (Bhatia et al., 2004). Tomato regeneration has been previously reported *via* organogenesis in several articles using different explants, such as, leaf (McCormic et al., 1986 and Gaffer, 1997 and Öktem et al., 1999) and cotyledon (vanRoekel et al., 1993). In addition, Pozueta-Romero, et al. (2001) regenerated shoots of three tomato cultivars after 14 days from the hypocotyl after removing the primary and axillary meristems. This paper attempts to develop efficient protocols for tomato transformation and *in vitro* regeneration with a high and rapid regenerative ability using the proximal part of hypocotyl as explants.

2 Materials and methods

2.1 Agrobacterium strain and plant vector

A. tumefaciens strain LBA4404 (Horsch et al., 1985) containing a disarmed Ti plasmid was transformed with the binary plasmid pISV2678, which has *gus*-intron under the control of e35S promoter and *nos* terminator as well as *bar* gene under the control of *nos* promoter, AMV leader and pAg7 terminator within the T-DNA region. The binary vector was kindly provided by Dr. P. Ratet, Institut des Sciences Vegetables (ISV), Centre National de la Recherche Scientifique (CNRS), Gif-Sur-Yvette, France.

The plasmid pMONRTG harboring the *gus* gene under the control of enhanced cauliflower *mosaic virus* (e35S CaMV) promoter and NOS terminator was used to adapt plant transformation with biolistic gunr.

2.2 Tomato regeneration

All cultures of regeneration and transformation treatments were carried out on MS salts supplemented with sucrose (3 %), phytagel (0.28 %) and the pH was adjusted to 5.8 before autoclaving (121°C, 20 min). *In vitro* cultured plant materials were incubated in a controlled growth chamber at 25 °C ± 2 and 8/16 hr (dark/light) photoperiod.

2.3 Explant preparation

Tomato (*Lycopersicon esculentum* Mill.) cv. CastleRock seeds were used as explant sources. Seeds were surface sterilized for 25 min, in commercial bleach solution (2.5 % NaClO) and rinsed three times in distilled water. The seeds were germinated on hormone free MS medium solidified with 0.6 % (w/v) agar and incubated at 27°C in the dark for three days then they were maintained under photoperiod of 16 h illumination for another 5 days. The hypocotyl explants of *in vitro* grown seedlings were excised from the 7-day-old seedling and the cotyledons were cut into two parts followed by removing the bud from the proximal part by a sharp scalpel.

2.4 Shoot regeneration

Hypocotyl explants from 7 days old seedlings were incubated on different regeneration media to identify the best formulation for inducing shoot organogenesis. The compositions of these different media are listed in Table 1. A total of 10 explants per plate were cultured, ten plates per treatment were used in a completely randomized block design, and the whole experiment was repeated three times. The efficiency of shoot regeneration was based on the number of regenerated explants having shoots. The mean of the shoots numbers per explants in each treatment was counted.

The elongated shoots were excised individually and transferred onto half MS medium supplemented with 0.1 mg/l IAA for rooting (Lee et al., 1997). Three weeks later, the shoots which failed to form roots were trimmed and replanted on the fresh rooting medium. Subsequently, rooted plantlets were acclimatized in pots containing soil composed of peat-moose: sand: clay (1:1:1, v:v:v), then covered with plastic bags to increase the humidity and grown under controlled greenhouse with a photoperiod of 16/8 h (light/dark)conditions. Plants were hardened by removing the plastic bags gradually after 7 - 10 days.

2.5 Transformation via biolistic gun

The biolistic gun and plasmid pMONRTG have been used for transferring the *gus* gene. Gold particles were prepared and coated with plasmid pMONRTG DNA according to BioRad protocol. Explants were prepared and cultured in the middle of Petri dishes contained the suitable shoot formation medium then they were shooted with the pMONRTG DNA coated gold particles. These explants were shooted twice under pressure of 1350 psi once at sample plate distance 6 cm and the second at 9 cm as recommended by Abu El-Heba (2004). Thereafter, bombarded explants were cultured on shoot formation medium and each experiment was performed through three replicas. The number of explants in each replica was 60 with a total number of 180. The data was recorded based on the mean of this experiment.

2.6 Agrobacterium mediate transformation

Suspension of *A. tumefaciens* LBA4404-pISV2678 was prepared as follows: two ml Luria-Bertani (LB) broth that contained 100 mg/L kanamycin and 50 µM 3', 5-Dimethoxy-4'-hydroxyacetophenone (acetosyringone) were inoculated with *Agrobacterium* and grown 16 h at 28 °C with vigorous shaking. An aliquot of 100 µl from the culture was used to inoculate 10 ml of a LB broth that contained 100 mg/L kanamycin and incubated at 28 °C with vigorous shaking until the absorbance at 550 nm was between 0.4 - 0.8 (equivalent to early log phase for most of the *Agrobacterium* strains). The cells were then pelleted and resuspended in an MS basal liquid medium. The hypocotyls were inoculated by dunking in an *Agrobacterium* suspension for 10 - 15 min. After inoculation in this suspension, the explants were blotted dry on sterile filter paper, replated onto the suitable shoot formation medium and cocultivated for 3 days under optimized growth conditions. After cocultivation, the explants were transferred to a shoot induction media that contained 250 µg/L

Table 1:
Composition of shoot formation media used for induction of shoot organogenesis from tomato hypocotyls

Media	MS	XM	X1B	X1N	X2B	X2N	X3B	X3N	X4B	X4N
MS vitamin	+	+	–	–	–	–	–	–	–	–
B5 vitamin	–		+	–	+	–	+	–	+	–
Nitch&Nitch vitamin	–	–	–	+	–	+	–	+	–	+
BA (mg/l)	–	1	1	1	–	–	–	–	–	–
2iP (mg/l)	–	–	–	–	–	–	1	1	–	–
Ziatin R (mg/l)	–	–	1	1	–	–	–	–	–	–
ABA (mg/l)	–	–	–	–	–	–	0.25	0.25	0.25	0.25
Silver Nitrate 5mg/l	–	–	5	5	5	5	5	5	5	5

bialaphos and 300 mg/L carbinicillin (both antibiotics were added as filter sterilized solutions after autoclaving) to select transformed shoots and prevent bacterial growth. After an additional 2 weeks of incubation in culture, the number of elongated shoots was counted. The elongated shoots were excised individually from the explants and subcultured on rooting media that containing 250 µg/L bialaphos. The experiment had three replica 50, 110 and 90 explants with a total number of 250 explants.

2.7 Regeneration of transformed explants

Transformed explants of both methods were then transferred to suitable shoot formation medium, the developed shoots were transferred to the rooting medium, and finally the plantlets were acclimatized in the greenhouse. The established putatively transgenic tomato plants were designated as T0-generation plants. A set of the untransformed explants were also regenerated as negative controls.

2.8 PCR assay

The genomic DNA of tomato was extracted from young leaves of putative transgenic plants by the method of Delaporta et al. (1983) and used in PCR analyzes. Two pairs of specific primers were used to detect the transgenes in putative transformed plants. The *bar* specific primers (namely P1, 5'AAA AGC TTC CAC CAT GAG CCC AGA ACG ACG3' and P2, 5'AAG GAT CCT CAG ATC TCG GTG ACG G') was designed to amplify 540 bp of the *bar* coding sequence, while the *gus* gene specific primers (namely, P3, 5'CCA GAT CTA ACA ATG CGC GGT GGT CAG TCC C3' and P4, 5'CCA GAT CTA TTC ATT GTT TGC CTC CCT GCT GC3') were designed to amplify the whole open reading frame (with or without the intron). The PCR reactions were carried out in a total volume of 25 ml, containing 1 µl DNA, 20 pmol of each primer, 200 µ M each dNTP, 0.5 units *Taq* DNA polymerase and 3 µl 10 PCR buffer. The PCR temperature profile was as follows: initial denaturation of DNA at 94 °C for 5 min, 35 cycles comprised of 1 min denaturation at 94 °C, 1 min annealing at 55°C for *gus* gene or 60 °C for *bar* gene, 1 min elongation step at 72 °C followed by a final extension step at 72 °C for 7 min. Amplification products were analyzed by electrophoresis on 1 % agarose gels and detected straining with ethidium bromide.

2.9 Histochemical GUS assay

Histochemical analysis of GUS activity in transformed explants, young leaves, roots and stem of transformed tomato plants using 5-bromo-4-chloro-3-indolyl-b-d-glucuronide (X-Glu) as a substrate was carried out as described by Jefferson (1987). Following tissue-staining with X-Glu overnight at 37 °C, chlorophyll from leaves and pigments from fruits were removed by soaking these tissues in a mixture of 70 % ethanol and 10 % commercial bleach Clorox for 4 ± 6 h. The ethanol bleach mixture was replaced three or four times then washed with double distilled H_2O.

2.10 Herbicide tolerance in transgenic plants

The upper surfaces of five fully expanded leaflets of plants grown in the greenhouse were thoroughly wetted by painting with Basta (200 g/L of glufosinate ammonium; Hoechst) solution containing 2 mg/ L of PPT. Tolerance to PPT was scored 7 day after leaf painting.

3 Results and discussion

The *in vitro* morphogenic responses of cultured plant tissues are affected by the different components of the culture media, especially by concentration of growth hormones, and it is therefore important to evaluate their effects on plant regeneration. Tomato is one of the most studied higher plants because of its importance as a crop species, and of several advantages for genetic, molecular and physiological studies (Mccormick et al., 1986).

Development of an efficient protocol for tomato transformation and its subsequent regeneration is a prerequest for the production of transgenic plants. Previous data for tomato regeneration have been reported using cotyledon explants (Raj et al., 2005 and (vanRoekel et al., 1993) and leaf explants (McCormic et al., 1986 and Gaffer, 1997 and Öktem et al., 1999). In this study, a rapid shoot regeneration system of tomato cv. CastleRock *via* direct shoot organogenesis using the proximal zone of the hypocotyl was established. In addition, transformation using biolistic and *Agrobacterium*-systems were adopted.

3.1 Effect of culture media on shoot organogenesis

It is important to optimize the regeneration frequency of tomato to increase the likelihood of recovery of transformants, therefore 10 media (Table 1) were evaluated for their regeneration ability of the hypocotyl explants of cv. CastleRock. The explants derived from hypocotyls, were isolated from seedlings of CastleRock tomato cultivar. Hundred explants were cultured on each type of MS medium supplemented with growth regulators. Previous studies demonstrated that 8 to 10 day old cotyledons of tomato were superior to other sources of explants, including hypocotyls, stems and leaves for promoting shoot organogenesis of tomato (Hamza and Chupeau, 1993; VanRoekel et al., 1993; Ling et al., 1998). In our experiments 8-day-old *in vitro* seedlings were used as source of explants.

The different formulations were designed based on a general survey of the most likely growth regulators and their concentrations that have been reported to promote shoots from hypocotyl tissues of tomatoes. The frequency of adventitious shoot regeneration differed depending on the type and concentration of growth regulators added to the regeneration medium. Since all media formulations induced shoot in all hypocotyl explants (Figure 1), the evaluation of shoot induction was based on the percentage of the regenerated shoots in each treatment and number of shoots per explants. Result represented in Table 2 shows that the three media X1N, X1B and XM which contained 1mg/l BA and 5mg/l silver nitrate but X1N and X1B contained 1mg/l Ziatin ripozide, induced the highest (3.5) mean shoots per explant compared to other media (1.5). Obtained results revealed that the regeneration frequency varied from 40 - 92 %. The highest regeneration frequency was obtained with medium X1N as it gave a percentage of 92 % followed by both media X1B and XM (90 % for both). It was also observed that these three media (X1N, X1B and XM) were the earliest among the other media in producing shoots as the shoots started to differentiate during 7 - 10 days while the others needed about 3 weeks. Furthermore, produced shoots on these three media were developed within 2 - 3 weeks compared to the other seven media, which had to be re-cultured in a fresh medium to develop. The number of shoots/explant

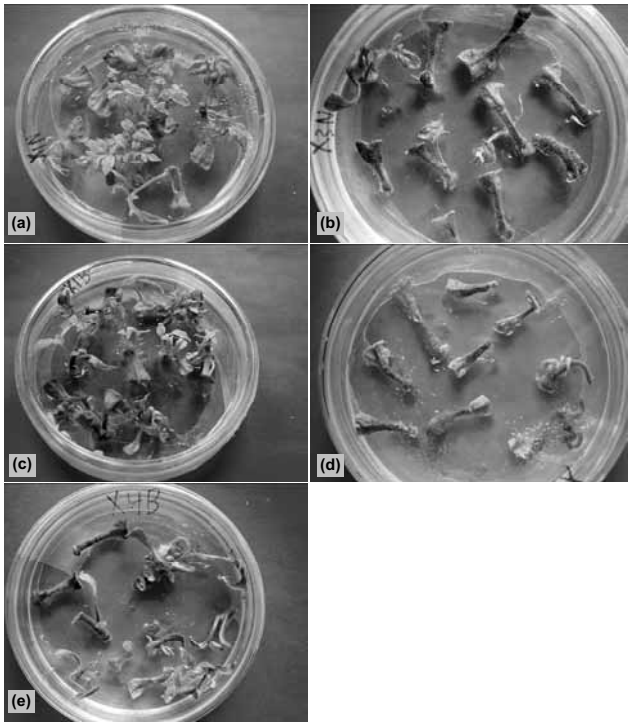

Figure 1:

Regeneration performance of cultivar tomato CastleRock using the proximal zone of hypocotyl as an explant on different shoot formation media

Table 2:

Regeneration frequency of tomato hypocotyls on ten different regeneration media

Media	% of no. of explant with shoots	No. of shoots/ explants	Time of shoot differentiation
MS	40	1	6 weeks
XM	90	3 - 4	15 days
X1B	90	3 - 4	15 days
X1N	92	3 - 4	10 - 12 days
X2B	60	1	2 - 3 weeks
X2N	62	1	2 - 3 weeks
X3B	45	1	3 weeks
X3N	43	1	3 weeks
X4B	65	2	3 weeks
X4N	60	2	3 weeks

were also considered as they were 3 - 4 shoots/explant for these three media compared to 1 - 2 shoots/explant for the other media. It was also recorded that there was no difference among the media containing B5 vitamins and that containing Nitch and Nitch vitamins. Therefore, the X1N medium was selected to use in the transformation routine work. This finding suggested presence of the cytokinins BA and zeatin in the culture medium had an invigorating effect on cell differentiation in tomato hypocotyl. The presence of BA in the culture medium has long been reported to promote shoot organogenesis in a large number

Figure 2:

(a) Putatively transformed shoot, b) Rooted plants after 3 to 4 weeks on rooting media, and c) Acclimatized putatively transgenic plants under the biocontainment greenhouse.

of plant species (Huetteman and Preece, 1993). Nogueira et al. (2001) observed high regeneration frequency 92 % or 85 % on cotyledonary explants of the tomato genotype Santa Clara or its natural mutant Firme, respectively.

Our experiments supported the results of other authors (Ichimura and Oda 1995; Nogueria et al., 2001) who found that the most efficient medium for *in vitro* regeneration of tomato being induction medium supplemented with a cytokinin zeatin.

The developed shoots excited and transferred to the rooting media for 2 - 3 weeks. Only 75 % of shoots were successfully formed roots at the first 3 weeks. However, reculturing the unrooted shoots on the free medium which enhanced them to form roots after 2 - 3 weeks. Tomato regeneration of proximal zone of the hypocotyl explants performing direct organogenesis is illustrated in Figure 2.

3.2 Tomato transformation

Two methods (biolistic bombardment and *Agrobacterium* mediated systems) were evaluated for their efficiency in the tomato transformation using the hypocotyl explants. The use of biolistics has the advantage of allowing a fast analysis, but it has to be adapted to the specific characteristics of the plant tissue.

In the case of biolistic bombardment, hypocotyls were bombarded with pMONRTG coated gold particles, containing the eCaMV 35S promoter was fused to the *gus* gene, to optimize the transformation assay. This promoter has been shown to be functional in transgenic strawberry fruits (El Mansouri et al., 1996; Jiménez-Bermúdez et al., 2002 and Dessoky et al., 2006). The main parameters that can be optimized in the biolistic transformation of plant tissue are the size of the gold particle, the distance between the sample and the macro-carrier, the amount of DNA used in each bombardment, as well as the number of shots per sample (Sanford et al., 1993). The distances between the sample and the macro-carrier as well as the number of shots per sample were optimized for tomato transformation. The addition of an osmoticum to the bombardment medium has been shown to increase the efficiency of transformation in a number of tissues, since it provides osmotic support for the tissue and minimizes cell damage (Baum et al., 1997; Sanford et al., 1993). Transformation system was carried out as previously established by the author (Abu El-Heba, 2004) using cotyledons as explant source. It was clearly observed that 13500 psi bombardment pressure with two shots and 6 cm distances between the sample and the macro-carrier were needed to detect activity of the reporter gene over the background level. The shoot regeneration frequency of tomato hypocotyl explants decline after the bombardment. The explants having shoots varied among the replica from 38 - 45 explants with a total number of 124 explants, representing 68.9 % (Table 3). The low regeneration efficiency obtained after the bombardment treatment might be due to harmfull effects of this technique on the plant tissues, as the explants did not culture on the selection medium. Therefore the transformation efficiency was calculated based on the PCR analysis. Transformation of intact plant organs by particle bombardment has proved to be a useful approach to study the promoter activity of organ-specific genes, as the transformed cell can be monitored in its native organ environment (Goff et al., 1990; Potrykus, 1991).

Table 3:

Regeneration frequency of tomato hypocotyl explants after bombarded with the pMONRTG coated gold particles

Replicates	Number of explants	Surviving explant	
		No.	%
R1	60	45	**75**
R2	60	38	**63.3**
R3	60	41	**68.3**
Total	**180**	**124**	**68.8**

In the case of the second method, hypocotyl explants were wounded and infected with *A. tumeficiense* strain LBA4404 harboring the binary vector pISV2678. In tomato, the effect of the *Agrobacterium* concentration for co-cultivating cotyledon explants was previously described by Fillatti et al. (1987). They found that when the concentration of the bacteria increased or decreased from 5.108 bacteria per ml, the rate of transformation was reduced by at least 20 %. The hypocotyl explants were co-cultivated with selected *Agrobacterium* conjugant. These explants,\ were cultured on selection medium and incubated until the putative transformed shoots were formed. It was important to subculture the explants onto a fresh selection medium at least once after 10 days to promote faster shoot formation, and to prevent *Agrobacterium* regrowth. Results showed that the survivenging explants on bialaphos medium ranged among the replica from 25 - 32 explants with a total number of 250, representing 34 % transformation efficiency (Table 4).

Endogenous GUS activity was detected in transformed explants, leaves, stems, and roots of transgenic plants explants. Bialaphos-resistant shoots produced *via Agrobacterium* mediated transformation strategy were randomly selected and subjected to a histochmical assay and visually compared with non-transformed plant materials (Figure 5). A precentage of about 85 % of the tested plant materials developed blue color whereas, there was no GUS expression was observed in untransformed plants.In addition, transient *gus* gene expression was detected of ten explants of each bombarded treatment were examined.

Table 4:

Transformation frequency of the tomato hypocotyl explants produced *via Agrobacterium* on selection medium

Replicates	Number of explants	Surviving explant	
		No.	%
R1	50	25	**50**
R2	110	32	**29**
R3	90	28	**31.1**
Total	**250**	**85**	**34**

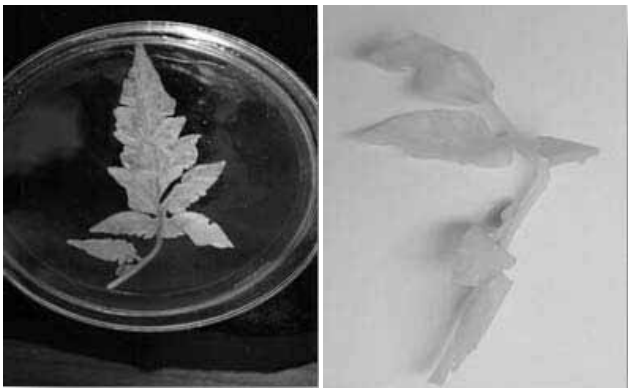

Figure 5:

Histochemical *gus* assay for transgenic and non-transgenic tomato plant using *Agrobacterium* transformation

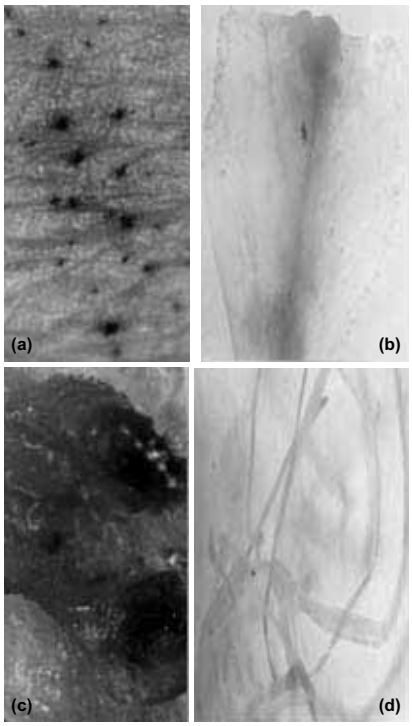

Figure 6:

Histochemical GUS assay for different transgenic tomato parts
(a) Blue spots after 48 hr of explant shooting, (b) Tomato leaf, (c) Tomato new bud and (d) Tomato root.

Results showed that all of those shoots obtained varied number of blue spots which have been observed under the binocular microscope (Figure 6 a). Furthermore, GUS expression has been also performed to all parts of developed plants grown in the greenhouse (Figure 6 b, c and d).

Varying levels of GUS activity, independent of a stable or transient expression of a reporter, were detected in leaf tissues of CaMV 35S transgenic plants. This observed difference in tissue specificity might be attributed to post-transcriptional regulation of GUS expression, as has been previously reported (D'Aoust et al., 1999).

Putative transformed shoots obtained from both treatments were transferred to soil for acclimatization. The survived tomato transformants were then transferred to the greenhouse, and allowed to grow until fruiting. From each transformation experiment, putative transgenic plants were analyzed for the presence of the chimeric *uid*A gene for both methods and the *bar* gene for the *Agrobacterium* transformation using PCR. The putative transgenic plants of T0-generation when screened by PCR using specific primers of transgenes. Although this plants gave an expected PCR amplicon no such amplicon was observed in untransformed (negative control) plants. Transgenic plants produced from direct transformation with the plasmid pMONRTG were able to amplify a fragment of ~1.8kb (Figure 3) while transformed plants with the binary vector pISV2678 amplified ~2kb for *gus*-intron gene and 540 bp (Figure 4) for *bar* gene transformed using specific primers for each gene. It was recorded that only 65 out of 150 (putatively transformed plants with pISV2678) and 53 out of 200 (putatively transformed plants with pMONRTG) plants were confirmed to be transgenic, representing a percentage of 30 and 26.5, respectively. Seven transgenic plants from each group (transformed either with pMONRTG or pISV2678) were used for *uid*A gene expression studies.

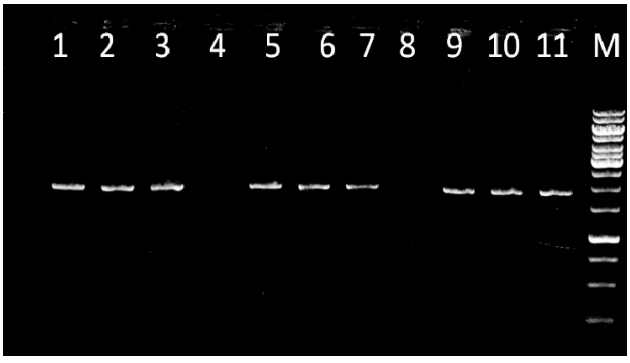

Figure 3:

PCR screening for the presence of *gus* gene in putative transgenic tomato plants using biolistic gun

Figure 4:

PCR screening for the presence of *gus* – intron (lane 1-9) & *bar* (11-19) genes in putative transgenic tomato plants using *Agrobacterium* transformation

References

Abu El-Heba (2004) Evaluation of some promoter's activity of some DNA plant viruses. Ain Shams : Univ, PhD thesis

Bhatia P, Ashwath N, Senaratna T, Midmore D (2004) Tissue culture studies of tomato (*Lycopersicon esculentum*). Plant Cell Tissue Organ Cult 78(1):1-21

Coenen C, Lomax TL (1997) Auxin-cytokinin interactions in higher plants : old problems and new tools. Trends Plant Sci 2(9):351-6

Compton ME, Gray DJ (1993) Shoot organogenesis and regeneration from diploid, triploid and tetraploid watermelon. J Amer Soc Hort Sci 118:151-175

Compton ME, Gray DJ (1994) Adventitious shoot organogenesis and plant regeneration from cotyledons of tetraploid watermelon. HortScience 29(3):211-213

D'Aoust MA, Nguyen-Quoc B, Yelle S (1999) Upstream regulatory regions from the maize Sh1 promoter confer tissuespecific expression of the GUS gene in tomato. Plant Cell Rep 18:803–808

Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA minipreparation : version III. Plant Mol Biol 1:19-21

DeRopp R (1954) Kinetin and auxin activity. Plant Physiol 31:253–254

Dessoky ES, Hussein G, Sadik AS, Abdallah NA (2006) Efficient regeneration and *Agrobacterium*-mediated transformation systems in melon (*Cuccmis melo* L.). Egypt J Genet Cyt 35:35-74

Dong JZ, Jia SR (1991) High efficiency plant regeneration from cotyledons of watermelon (*Citrullus vulgaris Schrad.*). Plant Cell Rep 9:559-562

El-Mansouri I, Mercado JA, Valpuesta V, Lopez-Aranda JM, Pliego F, Quesada MA (1996) Shoot regeneration and *Agrobacterium*- mediated transformation of Fragaria vesca. Plant Cell Rep 15:642–646

Fillatti JJ, Kiser J, Rose R, Comai L (1987) Efficient transfer of a glyphosate tolerance gene into tomato using a binary *Agrobacterium* tumefaciens vector. BioTechnology 5:726–730

Gaffer J, Tiznado ME, Handa AK (1997) Characterization and functional expression of a ubiquitously expressed tomato pectin methylesterase. Plant Phys 114:1547-1556

Goff SA, Klein TM, Roth BA, Fromm ME, Cone KC, Radicella JP, Chandler VL (1990) Transactivation of anthocyanins biosynthetic genes following transfer of B regulatory genes in maize tissue. EMBO J 9:2517–2522

Öktem HA, Mahmoudian M, Eyidofan F, Yücel M (1999) Gus gene delivery and expression in lentil cotyledonary nodes using particle bombardment, Lens Newslett 26:3–6

Hamza S, Chupeau Y (1993) Re-evaluation of conditions for plant regeneration and *Agrobacterium*-mediated transformation from tomato (*Lycopersicon esculentum*). J Exp Bot 44:1837–1845

Horsch RB, Fry JE, Hoffmann N, Wallroth M, Eichholtz D, Rogers SG, Fraley RT (1985) A simple and general method for transferring genes to plants. Science 227:1229–1231

Huetteman CA, Preece JE (1993) Thidiazuron : a potent cytokinin for woody plant tissue culture. Plant Cell Tiss Org Cult 33:105-119

Öktem HA, Bülbül Y, Öktem E, Yücel M (1999) Regeneration and *Agrobacterium*-mediated transformation studies in tomato (*Lycopersicon esculentum* Miller). Turkish J Botany 23:345-348

Ichimura K, Oda M (1995) Stimulation of root growth of lettuce by agar and its extract. Acta Hort (ISHS) 393:127-134

Jefferson RA, Kavanagh TA, Bevan MW (1987) GUS fusion : fl glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J 6:3901-3907

Jiménez-Bermúdez S, Redondo-Nevado J, Muñoz-Blanco J, Caballero JL, López-Aranda JM, Valpuesta V, Pliego-Alfaro F, Quesada MA, Mercado JA (2002) Manipulation of strawberry fruit softening by antisense expression of a pecate lyase gene. Plant Physiol 128:751–759

Lee KS, Schottler F, Collins JL, Lanzino G, Couture D, Rao A, Hiramatsu KI, Goto Y, Hong S-C, Caner H, Yamamoto H, Chen Z-F, Bertram E, Berr S, Omary R, Scrable H, Jackson T, Goble J, Eisenman L (1997) A genetic animal model of human neocortical heterotopia associated with seizures. J Neurosci 17:6236-6242

Ling HQ, Kriseleit D, Ganal MW (1998) Effect of ticarcillin/potassium clavulanate on callus growth and shoot regenerationin *Agrobacterium*-mediated transformation of tomato (*Lycopersicon esculentum* Mill.). Plant Cell Rep 17:843–847

McCormic S, Niedermeyer J, Fry B, Barnason A, Horch R, Farley R (1986) Leaf disk transformation of cultivated tomato (L. esculentum) using *Agrobacterium* tumefaciens.Plant Cell Rep 5:81-84

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol Plant 15:473-497

Myers KA, Lambe K, Aldridge TC, Macdonald N, Tugwood JD (1997) Amino acids in both the DNA binding and ligand binding domains influence the transcriptional activity of the human peroxisome proliferator acivated receptor alpha. Biomed Biophys Res Commun 239:522–526

Nogueira RMR, Miagostovich MP, Filippis AMB, Pereira MAS, Schatzmayr HG (2001) Dengue virus type 3 in Rio de Janeiro, Brazil. Mem Inst Oswaldo Cruz 96:925-926

Potrykus I (1991) Gene transfer to plants : assessment of published approaches and results. Annu Rev Plant Physiol 42:205–225

Potrykus I, Spangenberg G (eds) (1995) Gene transfer to plants. Berlin : Springer, 361 p

Pozueta-Romero J, Houlne G, Canas L, Schantz R, Chamarro J (2001) Enhanced regeneration of tomato and pepper seedling explants for *Agrobacterium*-mediated transformation. Plant cell tissue organ cult 67(2):173-180

Raj SK, Rachana S, Pandey SK, Singh BP (2005) *Agrobacterium*-medtaited tomato transformation and regeneration of transgenic lines expressing Tomato leaf curl virus coat protein gene for resistance against TLCV infection. Curr Sci 88:10-25

Sachs T, Thimann KV (1967) The role of auxins and cytokinins in the release of buds from dominance. Am J Bot 54:136–144

Sanford J, Smith F, Russell A (1993) Optimizing the biolistic process for different biological applications. Methods Enzymol 217:483–509

Skoog F, Miller CO (1957) Chemical regulation of growth and organ formation in plant tissue cultures *in vitro*. Symp Soc Exp Biol 11:118–131

vanRoekel JSC, Damm B, Melchers LS, Hoekema A (1993) Factors influencing transformation frequency of tomato. Plant Cell Rep 12:644-647

Yuan J, Henry R, McCaffery M, Cline L (1994) SecA homologue in protein transport within chloroplasts : evidence for edosymbiont-derived sorting. Science 266:796-798

Zhong S, Warkentin D, Sun B, Zhong H, Sticklen MB (1996) Variation in the inheritance of expression among subclones for unselected (*uid*A) and selected (Bar) transgenes in maize (Zea mays L.). Theor Appl Genet 92:752-761