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High Efficiency Plant Regeneration and Transformation of Watermelon (*Citrulus lanatus* cv. Giza1)

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Abstract: Transformation of watermelon using *Agrobacterium tumefacien* was studied. Plantlets regeneration were achieved in callus culture of cotyledon and leaf segments from 5-6 days old *in vitro* grown seedlings of *Citrulus lanatus*. Callus induction and plant regeneration at various frequencies were observed using different concentrations and combinations of growth regulators. The highest percentage of callus induction was observed in MS medium supplemented with 0.5 or 1.00 mg/l 2, 4-D when using cotyledons or leaf. Callus derived from cotyledons was differentiated to adventitious shoots on MS medium supplemented with 0.50 mg/l or 1.00 mg/l BA. No survived (100% mortality) explants were observed when 100 mg/I kanamycin was used in all types of explants and it chosen to selected of transformed explants. Cotyledon, leaf, shoot-tip and callus explants of Giza 1' were co- cultured with *Agrobacterium tumefaciens* strain EHA 105 for 12, 24, 48 and 72 hr. on LB medium. After co-cultivation periods of culture, explants were transferred to MS medium with 2 mg/l BA, 250 mg/l carbenicillin and 100 mg/l kanamycin for transformation selection of explants. GUS histochemical assay, gave the highest number and percentage of transformation frequency were recorded when using cotyledons after co-cultivation for 72 hour with *Agrobacterium tumefaciens* (9.66 and 96.7% respectively). PCR was utilized in this investigation for the rapid screening of GUS gene explants. For screening total genomic DNA was isolated from explants of several transformants. Using primer specific to GUS gene (F & R) a PCR product with a size of about 200bp was amplified when the total nucleic acid extracts prepared from the transformants were used as templates. The transformed explants were grown to maturity.

Key words: *In vitro*, Watermelon, Kanamycin, Transformation, *Agrobacterium tumefaciens*.

INTRODUCTION

Watermelon (*Citrulus lanatus* Thumb.) is an economically important crop and a valuable alternative source of water in desert areas. It is widely grown in the tropics and subtropics, most part of South East Asia, Africa, the Caribbean and the southern part of United States. The soluble fiber in watermelon may help to reduce cholesterol and risk of heart diseases. It is a good source of fiber, which is important for keeping digestive tract operating properly by preventing constipation, hemorrhoids and diverticular disease. It is an excellent source of important minerals. It is also rich in vitamin C and potassium. In propagation the introduction of new characters into watermelon by means of genetic manipulation is of great potential value, especially of the traits that would confer resistance to diseases and pests[1]. Ahad *et al*.[2] reported that establishment of an efficient protocol for plant regeneration from its immature and mature embryo axis explants of watermelon. Callus induction and regeneration at various frequencies were observed in different concentrations and combinations of growth regulators[3]. Cotyledonary explants of *Citrullus lanatus* Thumb (cv. Daesan) were co-cultivated with *Agrobacterium* strains (LBA4404, GV3101, EHA101) containing pPTN289 carrying with bar gene and pPTN290 carrying with nptII gene, respectively. There was a significant difference in the transformation frequency between bacteria strains and selective markers in watermelon plants[4]. Bakhsh *et al*.[5] demonstrated that technology development is innovative to many aspects of basic and applied plant transgenic science. Plant genetic engineering has opened new avenues to modify crops, and provided new solutions to solve specific needs. Development of procedures in cell biology to regenerate plants from single cells or organized tissue, and the discovery of novel techniques to transfer genes to plant cells provided the prerequisite for the practical use of genetic engineering in crop modification and improvement. Plant transformation technology has become an adaptable platform for cultivar improvement as well as for studying gene

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function in plants. This paper describes a transformation system for watermelon by co culturing cotyledons, shoot tips, leaf and callus explants with *Agrobacterium* harboring the pBI121 binary vector carrying GUS gene of E. coli as a reporter gene.

MATERIALS AND METHODS

Plant Material: Watermelon (*Citrullus lantus* cv.Giza1) mature seeds were used as explant source and obtained from Horticultural Research Institute in Agriculture Research Center. Seeds of watermelon cultivar Giza 1, was procured and used for organogenesis and transformation experiments. The seeds were sterilized by washing in 30% ethanol for 5 minutes followed by 10%sodium hypochlorite, for 10 min., rinsed with distilled water three times as the methods described by Vasudevan *et al*.[6]. Sterilized seeds were placed in one-half strength MS medium[7] to germinate in the dark at 25±2°C. Explants were prepared from cotyledons and leaves of *in vitro* grown seedlings taken as a source of experiments and described by Kose and Koç[8].

Callus Formation of Watermelon: The cotyledon segments and leaves were used as explants for callus formation and cultured on MS medium supplemented with various concentrations of 2,4-D (2,4 dichloro-phenoxy acetic acid) (0.0, 0.50, 1.00, 2.00, 2.50, 3.00, 3.5 or 4.00 mg/l) with 30 g/l sucrose and 7g/l agar. The culture medium of each treatment was distributed in culture jars (250 ml) each containing 50 ml. of MS basal medium. Each treatment contained 10 replicates (10 jars) and each replicate contained five explants. The pH of the media was adjusted to 5.7±0.1 before addition of agar and sterilized by autoclaving for 20 min. at 1.1 Kg/cm^2 pressure at 121°C. The cultures were maintained at 25±2ºC under dark.

Organogenesis of Watermelon Using BA and NAA: Ten weeks old callus (callus from cotyledon or callus from leaf) were subcultured on MS medium containing different combinations of BA (benzyl adenine), NAA (naphthalene acetic acid) with GA_3 at different concentration for organogenesis as shown in Table(3). Each treatment contained 20 replicates and each replicate include one explant. The organogenic calli formation were subcultured at monthly intervals on same medium for development and elongation of complete plantlets. The cultures were maintained at 25±2ºC under the cool white fluorescent lights for 16 hour photoperiod with a photon flux density of 3000 lux in growth chamber.

Establishment of Transformation System of Watermelon:

Sensitivity of Different non Transformed Explants (Cotyledon, Leaf, Shoot-tip and Callus) to Kanamycin: The sensitivity test of kanamycin was carried out in order to find the inhibitory concentration which arrests cotyledon, leaf, shoot-tip and callus growth. The sensitivity of cotyledon, leaf, shoot-tip and callus explants to kanamycin was determined by culturing the explants in shoot induction MS medium supplemented with 2.0 mg/l BA with different kanamycin concentrations (0, 25, 50, 75, 100, 125 and 150mg/L). Kanamycin was sterilized by filtration through 0.22 um disposable filter and incorporated into pre-cooled (45-50 ºC) autoclaved medium. The percentage of survival explants was recorded after 6 weeks from culture. Each treatment contained 3 replicates and each replicate consisted of 50 explants. The minimum inhibitory concentration of the selection marker was used throughout the selection procedure of selection transformed explants.

***Agrobacterium* Strain and Plasmid Vector:** pBI121, a binary vector carrying the CaMV 35S promoter-GUS gene-NOS terminator fusion and NOS promoter-neomycin phosphotransferase gene-NOS terminator fusion was transformed into *Agrobacterium tumefacien* strain EHA 105.

Transformation of Different Explants of Watermelon: *Agrobacterium tumefaciens* grown in LB liquid medium as a gene delivery system has made extensive use of both NPT II and Uid-A (GUS) genes as marker to monitor transformation. Explants (cotyledon, leaves, shoot-tip and callus) of Giza 1' were inoculated for 12, 24, 48 or 72 hr. with *Agrobacterium*. After inoculated with *Agrobacterium* the explants were blotted with sterile Whatman filter paper and placed onto MS medium with 2 mg/1 BA, 250 mg/1 carbenicillin, and 100 mg/l kanamycin, and cultured in the light.(The antibiotics were added to medium after autoclaving). Twenty five jars of callus, 150 of each leaf, cotyledon and shoot-tip were used per treatment, the control of both explants were not treated.

Culture Selection and Explant Regeneration: After co-cultivation with *Agrobacterium*, the explants were washed three times with sterile distilled water containing filter sterilized carbincillin (250mg/l), blotted dry and were subjected to selection. The cultures were maintained under 16 hr. photoperiod (3000 lux) at 25 ± 2 ºC. The explants were transferred to MS medium supplemented with BA (2.0mg/l), carbincillin (250mg/l) along with kanamycin (100mg/l) for tested transformed

explants. Two subcultures were done on the same medium. The medium of the same composition was changed once in 10 days. Each treatment consisted of a total of 150 explants.

Histochemical GUS Assay: The present study was investigated for the GUS expression of explants. Five Petri dishes were tested per treatment (explant pre-culture from 0 to 72 hour) with 10 explants per dish and (GUS) activity in the explants were performed according to Jefferson *et al*.[9]. For this aim after *Agrobacterium* infection of explants transformed and putative transgenic explants were incubated in X-gluc (5- bromo-4-chloro 3-indolyl -β-D glucuronide) solution at 37°C for 24 hr.

Isolation of DNA and PCR Analysis: The presence or absence of transformed (Uid-A) in control (nontransformed) and selected explants were confirmed by Polymerase Chain Reaction[10] for Uid-A gene. Genomic DNA was isolated from explants of control and transformed explants using the method described by Bernatsky and Tanksley[11] using Kit (Qigen Germany). Specific primer was designed based on the nucleotide sequence of GUS gene β-glucronidase obtained from (metabion GmbH Germany). The nucleotide sequence of these primer was, F, 5'-GGT GGG AAA GCC GTT ACA A-3' and R, 5'- GTT TAC GCG TTG TTC CGC CA-3'.The PCR reaction was conducted in a 0.5ml PCR tube containing 5µL of 10x-PCR buffer, 8µL dNTPs(dATP, dCTP, dGTP, dTTP) (1.25mM), 4µL primer F.(5pmol/µL), 4µL primer R.(5pmol/µL).1 µLDNA template, 100ng Taq DNA polymerase (2units/µL) and 27 µL d.H_2O. This mixture was covered with 50 µL mineral oil and then preheated for 4 min. at 94° C and subjected to 35 cycles each of 94°C/1 min, 55°C /2 min and 72°C/1 min. The reaction was then exposed to 72°C/5 min for one cycle. The PCR production was detected in the presence of standard DNA marker by electrophoresis in 1% agarose gel for 1 hr. at 60 volt and the bands were visualized by staining the gel with ethidium bromide and exposing to UV-transilluminator and the photos were taken with a digital camera.

Statistical Analysis: Data were analyzed as a Completely Randomized Design and means were separated by L.S.D test at 5%[12]. The percentage of GUS expression was calculated by the number of explants showing GUS positive divided by the number of explants subjected to co-cultivation and multiplied by 100[13].

RESULTS AND DISCUSSION

Effect of 2,4-D on Callus Formation of Watermelon: Cotyledon segments were cultured on MS medium with various levels of 2,4-D for callus induction. After four weeks of culture incubation callus mass further increased. Morphogenic potentialities of the explant was found to differ depending up to concentration of 2.4-D as shown in Table (1) and Fig. (1). Among the different concentrations tried, 2,4-D at 0.5mg/l was found highly effective for induction of callus. It was mostly soft, faster growing, light creamy-white and compact. The highest frequency of callus induction (100%) and 95% was recorded at the end of ten weeks on medium containing 0.5 and 2.5mg/l 2,4-D respectively and induced the highest fresh weight than other treatments. Results under discussion are in line with Mathukumar *et al.* [14] and Baskaran and Jayabalan[15].

Leaf segments were cultured on MS medium with various levels of 2,4-D for callus induction for one month. The data obtained showed that callus formation occurred with higher efficiency. Among the different concentrations tried, 2, 4-D at 1.00 and 2.00mg/l was found highly effective for induction of callus from leaf and produced highest callus formation (100%) (Table 2). It was mostly soft, faster growing, light creamy-white and friable. Similar observation were made earlier, Thiruvengadam *et al*.[16] found that MS medium containing 1.0mg/l 2,4-D approximately 90% of leaf explant of *Momordica charantia* L. gave rise to a well organized friable calli. At different concentration of BA and Kin green, compact and hard calluses produced. These calluses turned to be embryogenic under the stress of PGR.

Effect of BA and NAA on Formation of Organogenesis from Callus of Watermelon: All these studies indicated that watermelon explant requires small contractions of 2,4-D for callus induction. Shooting response is dependent upon concentration of cytokinin supplemented in the medium. Cytokinin work as signaling molecules that activate totipotent cells of callus for shoot organogenesis where as in the case of direct organogenesis, these molecules activate preexisting machinery in the case of somatic cells (from leaf and cotyledon). Various concentrations of BA and NAA were used in different combinations for shoot regeneration from callus derived from cotyledon. During this investigation shoot formation was highly influenced by concentrations and type of the growth regulators used in the experiment Fig. (2) and Table (3). Among the different concentrations and

combinations of BA and NAA for shoot multiplication, best performance was showed in MS medium supplemented callus produced shoots was 75%. The number of shoots and average shoot length per culture were 6.4 and 2.5 respectively. The results of the present investigation agree with the findings of Wehner and Lockly (17) achieved adventitious shoot formation from the callus of cotyledon culture of *Cucumis sativas*. Halder and Gadgril (18) obtained callus and shoot but development when cotyledons were cultured in MS medium supplemented with 0.1-1.0 mg/l NAA and 3.8 mg/l adenine. Hoque *et al.* (19) found that a combination of 1.5 mg/l BA and 0.1mg /l NAA was more suitable for adventitious multiple shoot formation in teastle grourd whereas in this experiment 1.0 mg/l BA was observed to be best for the production of multiple shoots in watermelon. There are some reports on callus culture and organogenesis of plant of several related species. Baskaran and Jayabalan (15) showed that higher shoot regeneration (89.5-1.18) was achieved in enriched L2 medium supplemented with 2 mM BA, 4 mM TDZ and 50 mg /l BVN in *Psoralea corylifolia* L.

The effect of BA and NAA on callus differentiation was examined using callus from leaf. Data presented in Table (3) show that BA was required to promote shoot organogenesis. Optimum concentration of different growth regulators for adventitious shoot regeneration was examined. The adventitious shoot regeneration frequency is 70 %dependence on the type and concentration of growth regulator. Adventitious shoot were observed by eye on the callus after about 12 weeks. The maximum frequency of adventitious shoot number was obtained when callus were cultured onto MS medium with 1.0 BA+0.2 NAA (4.7 shoots) Fig. (3). The number of shoots and average length shoots per culture were (4.7 and 2.4cm respectively). The results of the present investigation agree with Manye *et al.* (20) showed that in the process of callus differentiating adventitious buds, the kind, proportion and quantity of phytohormone and the type of callus made different result in *Momordica charantia* L. They obtained adventitious buds from yellowish green callus on MS medium with BAP and Kin. Sultana *et al.* (3) showed that MS medium supplemented with 1.0mg/l BA+0.2mg/l NAA gave the best result of callus differentiation in watermelon plants.

Establishment of Transformation System of Watermelon:

Sensitivity of Different Explants (Non Transformed) to Kanamycin: Kanamycin resistance is the most widely used selectable marker for plant transformation and the sensitivity of a particular species or explant to kanamycin is a key element in the establishment of any new transformation system in which a kanamycin resistance gene will be employed. From the presented results it could be observed that the type of explant affects on the survival number and percentage. The highest number of survival was recorded when using cotyledon as explant (20.8), while the lowest mean value was record with leaf and shoot tip (15.40 and 15,50 respectively).Mean while, the highest number of survival was recorded with control (50.00), while the lowest number of survival was recorded when using 100mg/l kanamycin. The interaction between explant type and kanamycin concentration was significant at 5% level. The highest mean value of survival was recorded at control (50.00) when using all Types of explant. No survived (100% mortality) explants were observed when 100 mg/I kanamycin was used in all types of explants Table (4). These results are in line with Raharjo *et al.* (21) showed that the kanamycin-resistant embryogenic calli were used to initiate suspension cultures (in liquid MS medium with 1.0 µM 2,4-D/BA, 50 mg/l kanamycin) for multiplication of embryogenic cell aggregates. Joung *et al.* (22) mentioned that kanamycin was used for the selection of putative transformants. The minimum concentration of kanamycin require to kill non-transformed leaf explant of *Campanula* was 50mg/l, and death of explant occurred after 3 months of culture.

***Agrobacterium* Infection and Co-cultivation:** Transformation experiments were performed to optimize the effective *Agrobacterium tumefaciens* co-cultivation period and the best explant type for transformation. In the present study, co-cultivation period was assessed for each hour from 12 to 72 hour. In this explants the best explant for transformation is the cotyledon (21.67) with *Agrobacterium*. A 48 and 72-hours co-cultivation were found optimal for Giza and led to the production of significantly higher rate of GUS expression frequency % as compared to other hours of co-cultivation Table (5) and Fig (4). The interaction between explant type and co-cultivation period it was clear that 48 and 72 hour with cotyledon explant produced the highest gene expression (48.33 and 38.33 respectively) than other treatments, and recorded the highest GUS percentage (76.66% and 96.66% respectively). Eissa (23) found that a method was developed for the organogenesis and regeneration from cotyledons as well as *Agrobacterium tumefaciens* mediated transformation of watermelon *in vitro*.

Selection of Transformants: After co-cultivation, explants with *Agobacterium* were transferred to MS medium containing BA (2.00mg/l), carbincillin (250mg/l) and Kanamycin (100 mg/l) for selection of transformed explant. Concerning to the effect of explant type, the highest number of explant resistant to

kanamycin is the explants of (cotyledon and callus) than other explants as shown in Table (6) and Fig (5). Mean while, co-cultivation period with *Agobacterium* effect of resistance explant to kanamycin, 48 and 72 hr produced highest resistance explants to kanamycin (33.75 and 43.33respectively) than other cocultivation periods with *Agobacterium*. Interaction between explants types and cocultivation period with *Agobacterium* at different times, it was clear that the highest resistance explant to kanamycin is cotyledon after 72 hour (50.00, 100%) than other treatments. This result agree with Vasudevan *et al.* (6) found that an efficient transformation protocol for cucumber poinsett 76 using *Agrobacterium* strain EHA 105. Five-day-old mature cotyledon explants was used for transformation study. The infected explants were co-cultivated for 2 days in MS medium containing BA (1.0 mg/l) in cucumber. Choi (4) showed that cotyledonary explants of *Citrullus lanatus* Thumb (cv. Daesan) were co-cultivated with *Agrobacterium* strains (LBA4404, GV3101, EHA101) containing pPTN289 carrying with bar gene and pPTN290 carrying with nptII gene, respectively. There was a significant difference in the transformation frequency between bacteria strains and selective markers. The EHA101/pPTN289 showed higher transformation frequency (1.16 %) than GV3101/pPTN289 (0.33 %) and LBA4404/pPTN289 or /pPTN290 (0 %) in watermelon plants.

PCR detection: The PCR is a sensitive technique allowing single-copy genes to be amplified and extracted out of a complex mixture of genomic sequence. Amplified DNA is visualized as distinct bands on agarose gels. PCR was utilized in this investigation for rapid screening of GUS gene explants. For screening, total genomic DNA was isolated from explants of sever transformants. Using primer specific to GUS gene (F&R) a PCR product with a size of about 200bp was amplified when the total nucleic acid extracts prepared from the transformants were used as templates. The obtained result confirmed the presence of GUS in the transformants. DNA of non-transformed explants was used as a negative control and DNA of pB1121 was used as a positive control as shown in Fig. (6).

Table 1: Effect of 2,4-D on callus formation from cotyledons of watermelon.

2,4 -D Con. (mg/l)	No. of initial explant	No. explant formed callus	% of explants induced callus	Weight (g)	Callus color	Degree of callus formation	Callus type
Control	50	2	4	1.55	Cr	+	friable
0.5 mg	50	50	100	10.54	Crw	++++	compact
1.0 mg	50	45	90	3.44	Cr	+++	compact
2.0 mg	50	45	90	3.80	Cr	+++	compact
2.5 mg	50	48	96	5.50	Cr	++	compact
3.0 mg	50	45	90	3.97	B	++	friable
3.5 mg	50	45	90	2.83	B	++	friable
4.0 mg	50	45	90	2.41	B	++	friable
			LSD at level 5%	1.314			

- = No callus formation; + = Low callus formation; ++ = Moderate callus formation; ++++ = High callus formation; + + + += Very high callus formation. Cr= Creamy; Crw= Creamy/white; B=Brown; LB = Light Brown

Table 2: Effect of 2,4-D on callus formation from leaf of watermelon.

2,4 D Con. (mg/l)	No. of initial explant	No. explant formed callus	% of explants induced callus	Weight (g)	Callus color	Degree of callus formation	Callus type
Control	50	0	0	0.00	-	-	-
0.5 mg	50	0	0	0.00	-	-	-
1.0 mg	50	50	100	0.59	Crw	+++	compact
2.0 mg	50	50	100	0.22	Crw	+++	compact
2.5 mg	50	49	98	0.10	Crw	+++	compact
3.0 mg	50	0	0	0.00	-	-	-
3.5 mg	50	0	0	0.00	-	-	-
4.0 mg	50	0	0	0.00	-	-	-
			LSD at level 5%	0.058			

- = No callus formation; ++ = Moderate callus formation; +++= High callus formation; Crw = Creamy/white

Table 3: Effect of BA and NAA at different concentration and combination on plant regeneration from the callus derived from cotyledon and leaf tissue of watermelon.

Cytokinin and Auxine type Conc. mg/l		Regeneration percentage		Shoot number		Leaves number		Shoot length (cm)	
BA (mg/l)	NAA (mg/l)	Callus of cotyledon	Callus of leaf	Callus of cotyledon	Callus of leaf	Callus of cotyledon	Callus of leaf	Callus of cotyledon	Callus of leaf
0.0	0.0	-	-	-	-	-	-	-	-
	0.1	-	-	-	-	-	-	-	-
	0.2	-	-	-	-	-	-	-	-
	0.3	-	-	-	-	-	-	-	-
0.5	0.0	75	-	6.4	-	15	-	2.5	-
	0.1	-	-	-	-	-	-	-	-
	0.2	-	-	-	-	-	-	-	-
	0.3	-	-	-	-	-	-	-	-
1.00	0.0	70	-	3.6	-	9.6	-	2.4	-
	0.1	-	-	-	-	-	-	-	-
	0.2	-	70	-	4.7	-	19.8	-	2.4
	0.3	-	-	-	-	-	-	-	-
2.00	0.0	-	-	-	-	-	-	-	-
	0.1	-	-	-	-	-	-	-	-
	0.2	-	-	-	-	-	-	-	-
	0.3	-	-	-	-	-	-	-	-
3.0	0.0	-	-	-	-	-	-	-	-
	0.1	-	-	-	-	-	-	-	-
	0.2	-	-	-	-	-	-	-	-
	0.3	-	-	-	-	-	-	-	-

Table 4: Effect of kanamycin concentrations on explants survival and percentage of non transformed of watermelon.

Kanamycin con. mg/l(B)	Explant type(A)									
	Survival explants number					Survival explants percentage (%)				
	Cotyledon	Leaf	Shoot tip	Callus	Mean	Cotyledon	Leaf	Shoot tip	Callus	Mean
Control	50.00	50.00	50.00	50.00	50.00	100.00	100.0	100.0	100.00	100.00
25 mg/l	40.33	35.33	32.33	42.33	37.58	80.66	70.66	64.66	75.16	72.79
50 mg/l	35.00	20.33	17.33	25.33	24.50	70.00	40.66	34.66	49.00	48.58
75 mg/l	20.33	2.43	9.33	13.33	11.34	40.66	4.86	18.66	22.68	21.72
100 mg/l	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
125 mg/l	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
150mg/l	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	20.8	15.4	15.5	18.7		41.62	30.88	31.14	35.26	
L.S.D at 5%	A	0.26								
	B	0.20								
	AxB	0.53								

Table 5: Gus expression from explants of watermelon after 12, 24, 48 and 72 hour from co-cultivated with *Agobacterium tumefaciens*.

Co-cultivation period(hour) (B)	Explant type(A)									
	Gus expression number					Gus expression percentage				
	Cotyledon	Leaf	Shoot-tip	Callus/ Petri dish	Mean	Cotyledon	Leaf	Shoot-tip	Callus/ Petri dish	Mean
12	0.00	0.00	0.00	0.00	0.00	00.00	00.00	00.00	00.00	00.00
24	0.00	0.00	0.00	0.00	0.00	00.00	00.00	00.00	00.00	00.00
48	38.33	0.00	5.00	10.00	15.00	76.66	13.33	10.00	20.00	30.00
72	48.33	6.66	10.00	28.33	24.33	96.66	23.33	20.00	56.66	49.16
Mean	21.67	4.33	10.67	9.58		43.33	9.17	7.5	19.17	
L.S.D at 5%	A	2.46								
	B	2.46								
	AxB	4.93								

Table 6: Selection of kanamycin resistant of different watermelon explants after co-cultivation with *Agobacterium tumefaciens*.

Co-cultivation period (hour) (B)	Explant type(A)									
	Resistance explants number					Resistance explants percentage				
	Cotyledon	Leaf	Shoot-tip	Callus/ Petri dish	Mean	Cotyledon	Leaf	Shoot-tip	Callus/ Petri dish	Mean
12	0.000	0.000	0.000	0.000	0.00	00.00	00.00	00.00	00.00	0.00
24	15.00	0.000	0.000	0.000	3.75	30.00	0.000	00.00	00.00	7.50
48	48.33	38.33	0.000	46.76	33.75	96.66	76.66	0.00	93.33	66.66
72	50.00	43.33	33.33	48.33	43.33	100.00	86.66	66.66	96.66	87.50
Mean	28.33	20.42	8.33	23.75		56.67	40.83	16.67	47.50	
L.S.Dat5%	A	2.26								
	B	2.26								
	AxB	4.52								

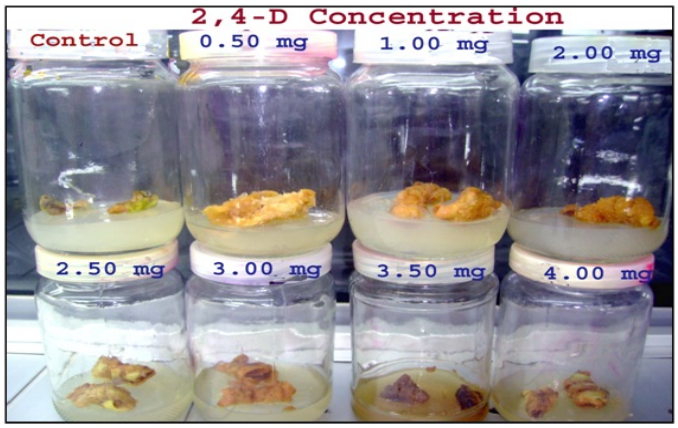

Fig. 1: Effect of 2,4-D on callus formation from cotyledons of watermelon.

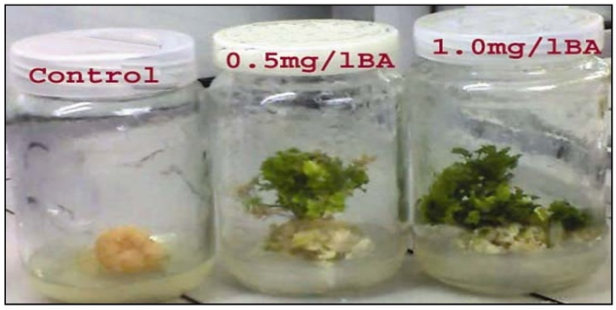

Fig. 2: Development of organogenesis from callus derived cotyledons of watermelon.

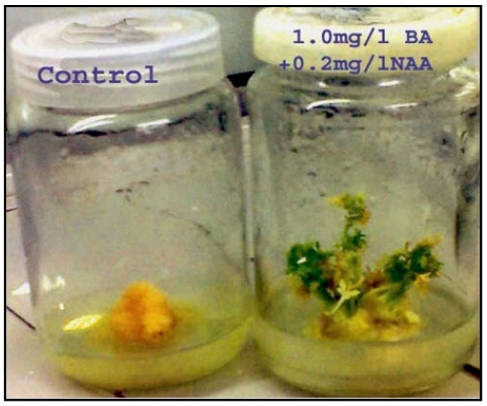

Fig. 3: Organogenesis formation from callus derived from watermelon leave.

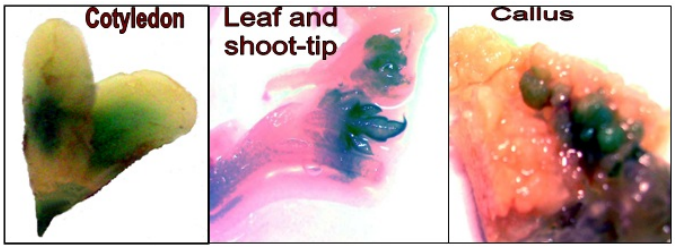

Fig. 4: Gus expression from cotyledon, leaf, shoot-tip and callus of watermelon after co-cultivation with *Agrobacterium tumefaciens.*

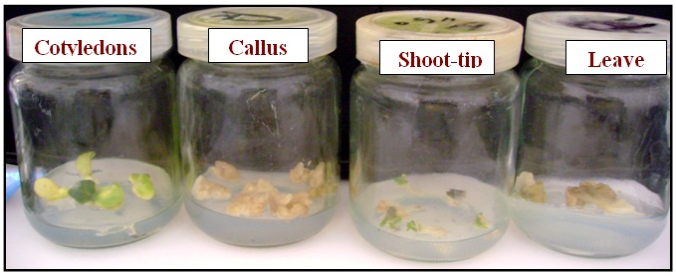

Fig. 5: Selection of kanamycin resistant after 72 hr. from co cultivated of watermelon from different explants with *Agobacterium tumefaciends.*

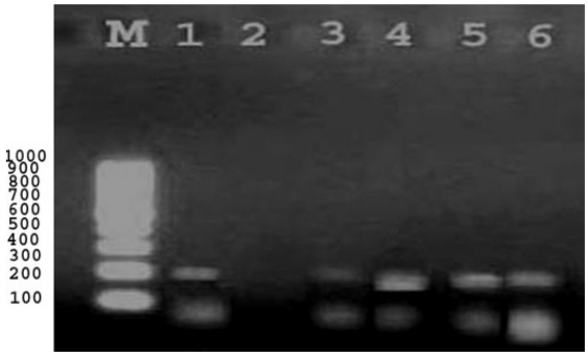

Fig. 6: PCR products (200bp) amplified from the total nucleic acids extracts prepared from some transformed (cotyledon, callus, leaf or shoot tip of watermelon).
M: Standard marker; 1: Positive control; 2: Negative control; 3: Cotyledon; 4: Leaf; 5: Shoot-tip; 6: Callus.

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