Effect of cytokinin and thidiazuron concentrations on proliferation and somaclonal variation of communis pear

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ABSTRACT

Uniform shoot tip explants of communis pear (Pyrus communis L) were cultured on Murashige and Skoog (MS)medium.Different concentrations of cytokinin [Kinetin (kin) and Benzyladenine(BA)] and thidiazuron(TDZ) were tested. Data indicated that higher kinetin concentrations (4.0 and 6.0 mg/l) enhanced shoot number, shoot length and proliferation percentage. However, BA at the rate of 2.0 mg/l enhanced proliferation, necrosis and greening. The best shoot number, shoot length and proliferation percentage were obtained when 1.0 mg/l TDZ was added to the medium. Somaclonal variability was studied in vitro on plantlets of pear by RAPD analysis. The explants were cultured on MS medium supplemented with different concentrations of different hormones; all treatments were screened using seven 10-mer primers. The analysis revealed that two out of the seven primers succeeded to amplify the DNAs and indicated that some polymorphic bands in DNA patterns of plantlets were obtained through the shoot apices, in case of TDZ treatment and BA but in case of Kin treatment the % of polymorphism was low or none. The work showed the potential for using RAPD analysis to study somaclonal variability in pear.

Key words: *Pear, tissue culture, somaclonal variation, RAPD, cytokinin, thidiazuron.*

INTRODUCTION

Tissue culture has become an important technique for propagation and breeding of woody plants. Communis pear is the most valuable and compatible rootstock for pear under Egyptian conditions. It is mainly propagated by seeds imported from abroad. Consequently, high costs for transportation, agricultural quarantine and relatively low seed viability are the main problems facing the use of communis pear seeds. Thus, micropropagation may be the alternative method to overcome such problems. In general, *in vitro* rejuvenation has been recognized based on changes in vegetative characteristics such as leaf shape and enhancement of shoot vigour and increase in rooting ability (Hackett, 1985 and Pierik,1990). The occurrence of somaclonal variation during culture has led to look for in the possibility of selecting disease- resistant plants through *in vitro* culture. (Reuveni *et al.*,1996).

In many species, micropropagated by tissue culture, some of the regenerated plants do not conform to the source plant material. The term somaclonal variation has been proposed to describe the variability produced by *in vitro* multiplication (Larkin and Scowcroft, 1981 and Meins 1983). Somaclonal variations have been shown

recently in many plant species to appear *in vitro* mainly in callus, cell suspension and isolated protoplast cultures. These somatic variations may result from cytological changes, point mutations and other less well-defined alterations to the nuclear and cytoplasmic (mitochondrial and chloroplast) genomes. Somaclonal variation applies to stable and transmissible modifications, i.e., changes which persist in the absence of the events that induced them and which are transmitted during mitosis. The possible origins of variations have been discussed in several reviews (Swartz, 1990; Karp, 1995). In many *in vitro* multiplication systems, it has been observed that variant rates increase with the number of multiplication cycles or with interval period of subculture (Gözükirmizi *et al.*, 1990; Morrish *et al.*, 1990; Müller *et al.*, 1990; Wang *et al.*, 1992; Gavidia *et al.*, 1996; Yang *et al.*, 1999). However, in several *in vitro* regeneration systems, it has also been observed that progenies of plants derived from the same initial source material and multiplied following a strictly identical culture protocol, including the same interval time of culture, exhibit variable percentages of off-types (Benzion and Phillips, 1998; Wang *et al.*,, 1992). Clonal micropropagation is usually performed by stimulation of axillary meristems using growth regulators. At the same time, the hormonal effects may cause callus formation and spontaneous regeneration of callus-derived plants which may vary somaclonally (Adams, 1972). The role of growth regulators, as agents of mutation, is under study by several investigators. Arnholdt-Schmitt (1993) observed that indole-3-acetic acid (IAA) and inositol in the growth medium induced DNA rearrangements and methylation changes in carrot (*Daucus Carota* L.) callus cultures. However, tissue culture medium cytokinin concentration did not influence cropping as vegetative performance of runner progeny of micropropagated strawberry plants (Beech *et al.*, 1998).DNA analysis itself seems to be a useful tool to differentiate stable somaclones. The RFLP analysis has been used for molecular characterization of tissue culture derived plants. However, RFLPs analysis isn't technically easy and it has high costs for a routin application in screening varied clones in comparison to RAPD analysis (Williams *et al.*, 1990), which also has the great advantage of being quick to be performing and to require small amounts of DNA. Recently, RAPDs was shown to be applicable to assay somaclonal variations, resulting from gene mutations, in *in-vitro* culure of various species, among them *Populus deltoides* and peach (Rani *et al.*, 1995). The objective of this study was to determine whether benzyladenine (BA), kinetin (Kin) and thidiazuron (TDZ) concentration in the culture medium during multiplication influenced genetic stability of pear (communis) plantlets as detected by RAPD molecular markers in plantlets *in vitro*.

MATERIALS AND METHODS

This study was carried out at the tissue culture laboratory of Plant Biotechnology Dept., National Research Center. Shoot tip of communis pear (*Pyrus communis* L) rootstocks was excised from terminal parts with 5mm long as explants. The explants were washed with running water for 20-30 min, then sterilized with 20% Clorox (commercial bleach) with 0.1% Tween- 20 for 15 min then, washed in sterilized distilled water 3 times for 5 min each. The prepared explant were cultured on MS medium (Murashige and Skoog, 1962) supplemented with 100mg/l myo-instol, 0.5 mg/l BA (benzyladenine), 0.1 mg/l IBA (Indole 3- butyric acid), 30g/l sucrose and 7g/l Difico- Bacto agar which was considered as basal medium. The pH of the medium was adjusted to 5.8 and autoclaved at

121°C and 1.5 Ib/inch^2 for 25 min. The cultured explants were incubated under 16 hr of artificial light (fluorescent light at 30UM/m^2/sec) and 8 hours of dark at 4 weeks intervals in all stages and experiments.After four subcultures shoots were transferred to MS medium solidified with 0.7% agar and supplemented with sucrose at 3%. Thus, the following experiments were carried out:

Effect of kinetin concentration

Different concentrations of kinetin i.e (0.0, 2.0, 4.0 and 6.0 mg/l) were evaluated to identify the most suitable concentration that induced the highest proliferation and lowest somaclonal variation.

Effect of benzyladenine (BA) concentration

Different BA concentrations i.e (0.0, 2.0, 4.0 and 6.0 mg/l) were investigated to decide the best concentration that induced the greatest proliferation and lowest somaclonal variation.

Effect of thidiazuron(TDZ) concentration

Different TDZ (Thidiazuron) concentrations i.e. (0.0, 1.0, 2.0, 3.0and 4.0mg/l) were supplemented to the culture medium to identify the best TDZ concentration maximizes proliferation and lowers somaclonal variation.

Statistical analysis

Treatments were arranged in a completely randomized design, each treatment was replicated three times; each replicate involved 5 jars, each contained a single explant or two shoots developed *in vitro*. Means were compared according to the method described by Snedecor and Cochran (1989).

Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR)

Isolation of plant DNA

Genomic DNA was isolated as mentioned by Murray and Thompson, (1980). Pear tissue culture derived plantlets of weights 0.5 gm were extracted by freezing in liquid nitrogen in eppendorf tubes and homogenized in 500 µl of extraction buffer containing 2% CTAB (1.4 M Nacl, 100mM EDTA (pH.8.0), 100 mM Tris-Hcl (pH 8.0) and 0.1 M β-Mercaptoethanol).

The extract was incubated at 60°C for 20 min. To this added 500 µl of phenol: chloroform: isoamylacohol (24:24:1) and mixed by vortexing for 30 sec., followed by centrifugation at 10000xg for 12 min. at room temperature. The aqueous phase was transferred to another tube. This was once again extracted with 500 µl of chloroform: isoamyle alcohol (24:1). To the aqueous phase added 0.6 volume of isopropanol and cooled for 20 min, followed by centrifugation at 10000xg for 10 min at 4°C to precipitate the genomic DNA and washed thrice with 70% ethanol, dried in vacuum and dissolved in TE buffer containing (10 mM Tris-Hcl pH 8.0 and 1 mM EDTA).

DNA amplification

The PCR reaction was performed in 25 µl mix containing 10 mM Tris-HCl pH 8.3, 50 mM KCl, 5 mM $MgCl_2$, 200 µ M each of dATP, dCTP, dGTP, dTTP, 0.5µg of the random primer, 0.5 unit of Taq DNA polymerase (Promega) and 20 ng of DNA. The mixture was incubated at 94°C/5min; DNA amplification was performed for 45 cycles. Each cycle was at 94°C/30 sec. (denaturation), 36°C/30 sec (annealing) and 72°C/2min (extension). Reactions were finally incubated at 72°C for 7 min. Seven random oligonucleotide primers of 10 bp (Operon Technologies Inc., Alameda, California) were designed for use in RAPD analysis. The primers are shown in Table (1).

Electrophoresis in agarose gel

The RAPD products were analyzed by electrophoresis in 1% agarose in 100 ml (1x)

TBE buffer containing (89 mM Tris-Base, 89 mM EDTA and 2.5 mM Boric acid for 10 x buffer), stained with 3 µl ethidium bromide and photographed under ultraviolet light.

Preparation of samples

Added 2µl from loading buffer (0.25 g bromophenol Blue, 0.25 g Xylene cyanol and 30% glycerol in 100 ml sterile distilled water) to 5-10 µl of the DNA sample and then loaded into the agarose gel. Runing was performed at 70 volt for 1 h. The DNA was detected under UV- transilluminator and photographed by Polaroid camera.

Band-Sharing analysis

Bands on agarose gels were scored as present (+) or absent (-) and a pair wise similarity matrix was constructed using the Dice similarity (DS) index (Sneath and Sokal, 1973). DS values were calculated as the doubled number of shared bands between two patterns divided by the sum of all bands in the same patterns. An UPGMA cluster based on DS values was generated using the NTSYS (Numerical Taxonomy System, Applied Biostatistics, Setauket, New York) Computer program.

Table (1): Random oligonucleotide primers used for RAPD analysis.

Code	Seq. (5'- 3')	Length (bp)
Operon K1	TGC CGA GCT G	10
Operon K2	GTG AGG CGT C	10
Operon K3	CCC TAC CGA C	10
Operon K4	TCG TTC CGC A	10
Operon K5	CAC CTT TCC C	10
Operon K6	GAG GGA AGA G	10
Operon K7	CCA CAG CAG T	10

RESULTS AND DISCUSSION

Effect of kinetin concentration

It is clear from Table (2) and Fig. (1) that addition of high concentrations (4.0 and 6.0 mg/l) of kinetin to the culture medium was valuable in significantly reducing necrosis and improving green colour in comparison with low concentrations (0.0 and 2.0 mg/l).

Meanwhile, supplementation of the culture medium with kinetin at 6.0 mg/l level maximized shoot number, shoot length and proliferation percentage in comparison to the other concentrations under study. On the other hand, no statistical differences were noticed among concentrations under study when percentage of shoots developed calli was evaluated.

Effect of benzyladenine (BA) concentration

Table (3) and Fig. (1) indicate that shoot length and proliferation percentage are greatly adversely affected by increasing of BA concentrations; the low level (2.0 mg/l) is the best. However, the reverse was true in case of necrosis and greening parameters. Moreover, number of shoots and shoots developed calli percentage showed a positive response with increasing BA concentrations up to 6.0 mg/l.

Table (2): Effect of different kinetin concentrations on shoot proliferation of Communis pear after four subcultures.

Kinetin Concentration (mg/l)	Necrosis (Score)	Shoot number	Shoot length (cm)	% Proliferation	% Shoots developed calli	Greening
0.0	2.0A	1.0C	1.6D	0.0B	0.0A	3.0B
2.0	2.0A	1.0C	3.2C	0.0B	0.0A	3.0B
4.0	1.0B	3.0B	4.3B	0.0B	0.0A	4.0A
6.0	1.0B	7.3A	6.5A	40.0A	0.0A	4.0A

Table (3): Effect of benzyladenine (BA) concentrations on shoot proliferation of communis pear after four subcultures.

BA Concentration (mg/l)	Necrosis	Shoot number	Shoot length (cm)	% Proliferation	% Shoots developed callus	Greening
0.0	1.0C	1.0D	1.6D	0.0D	0.0B	4.0A
2.0	1.0C	16.6B	6.0A	100.0A	0.0B	4.0A
4.0	2.0B	12.0C	4.6B	40.0C	0.0B	3.0B
6.0	2.3A	30.0A	4.3C	60.0B	30.0A	2.6C

Table (4): Effect of thidiazuron concentration (TDZ) on shoot proliferation of communis pear after four subcultures.

TDZ Concentration (mg/l)	Necrosis	Shoot number	Shoot length (cm)	% Proliferation	% Shoots developed calli	Greening
0.0	2.0A	1.0E	1.6E	0.0E	0.0C	3.0B
1.0	1.0B	30.0C	2.0D	30D	0.0C	5.0A
2.0	1.0B	35.0B	2.3C	80C	20.0A	3.0B
3.0	2.0A	25.0D	3.0B	90B	10.0B	3.0B
4.0	2.0A	60.0A	3.6A	100A	0.0 C	3.0B

Table (5): Amplified fragments obtained from the DNAs of in vitro derived plantlets (kinetin treatments) of communis pear.

OPK6				OPK5			
K_0	K_2	K_4	K_6	K_0	K_2	K_4	K_6
0	0	0	0	1	1	1	1
0	0	0	0	0	0	0	0
0	0	0	0	1	1	1	1
0	0	0	0	1	1	1	1
0	0	0	0	1	0	1	1
0	0	0	0	1	1	1	1
0	0	0	0	1	1	1	1
0	0	0	0	1	1	1	1
0	0	0	0	0	0	0	0
0	0	1	0	1	1	1	1
0	0	0	0	0	0	0	0
1	1	1	1	1	1	1	1
1	1	0	1	0	0	0	0
0	0	0	0	1	0	1	1
1	1	1	1	0	1	0	0
0	0	0	0	1	0	1	1
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
3	3	3	3	11	9	11	11

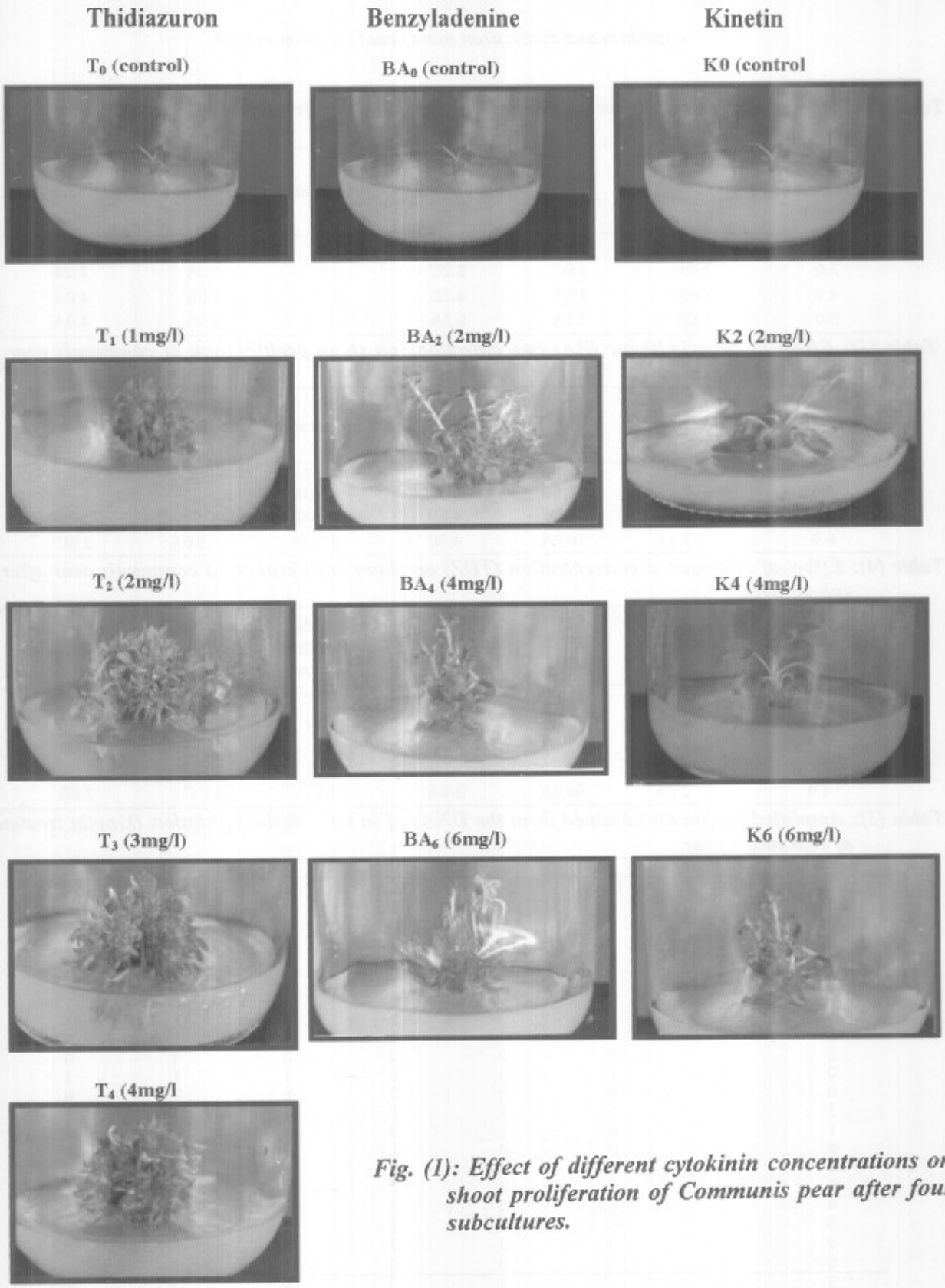

Fig. (1): Effect of different cytokinin concentrations on shoot proliferation of Communis pear after four subcultures.

Effect of thidiazuron (TDZ) concentration

Table (4) and Fig. (1) indicated that supplementation of the culture medium with 4.0 mg/l TDZ caused a significant increase in the number of shoots, proliferation percentage and shoot length, while significantly decreased

shoots developed calli percentage and greening parameters. On the other hand, either 1.0 or 2.0 mg/l TDZ induced significant decrease in necrosis compared with other used concentrations. However, addition of 1.0 mg/l to the culture medium caused a significant increase in greening.

In general, summarizing the above results indicated that both BA and TDZ enhanced the best proliferation. Also, kinetin surpassed others in improving growth and greening while TDZ maximized proliferation. These results are in harmony with the findings of Caboni *et al.*, (1999) and Dantas *et al.* (2002) who pointed out that the highest proliferation of pear cultivars was noticed when cultured on ¾ strength MS medium with 1.6 µM/l BA. In addition, Camargo *et al.* (1998) and Aziza (2004) recommended for apple rootstocks 2mg/l kinetin for enhancing growth and greening, while 2.0 mg/l of BA was suitable for proliferation. Also, Zaied (1997) indicated that the highest multiplication occurred when 2.0 mg/l TDZ for apricot, peach and almond plants.

Molecular analysis

Out of seven primers (10-mer), two turned out to give satisfactory banding patterns in the gel with all tested pear genomic DNAs with the different treatments. Under our PCR conditions, 10-mer primers can effectively substitute for the shorter primers as suggested by Caetano-Anolles and Gesshoff (1992) for DNA fingerprinting. Two 10-mer oligonucleotide primers succeeded to amplify the DNAs of pear plantlets derived tissue culture. First one is OPK5 (CAC CTT TCC C), it produced 42, 36 and 30 bands in case of plantlets exposed to kin, BA and TDZ, respectively. However, OPK6 (GAG GGA AGA G) produced 12, 16 and 39 bands in case of plantlets exposed to Kin, BA and TDZ, respectively, as shown in Tables (5, 6 and 7), as well as in Table (8) and Fig (2). OPK5 produced 20, 22 and 22 bands in all DNAs of pear exposed to different concentrations of Kin (2,4 and 6 mg/l), ranging from 200 bp to 1200 bp and no polymorphism bands were appeared, except in case of 2 mg/l. Furthermore, no variation was found in the patterns obtained with the DNA from plantlets exposed to 4 and 6 mg/L of kinetin. Otherwise, OPK6 produced 6 bands in all DNAs of plantlets exposed to different concentrations of kin (2,4 and 6 mg/l) ranging from 300 to 550 bp and no polymorphism bands in the gel were appeared except 2 bands that appeared in case of 4mg/l of kinetin. Their sizes are 400 and 550bp. It does mean that % of polymorphism was 0.0, 33.3 and 0.0% in kinetin of 2..., 4 and 6 mg/l and could be concluded that kinetin concentration ranging from 2 to 6 mg/l induced a little polymorphism % in the DNA pattern of pear *in vitro*.

On the other hand, OPK5 produced 18, 19 and 21 bands in all DNAs of plantlets exposed to different concentrations of BA (2, 4, 6 mg/l) ranging from 200 to 1200 bp and five polymorphism bands appeared in case of 6 mg/L of BA but 6 and 7 polymorphic bands appeared in case of 2 and 4 mg/l concentrations. OPK6 produced 8, 7 and 7 bands in all DNAs of plantlets exposed to 2, 4 and 6 mg/l of BA and produced 2, 1 and 1 polymorphic bands ranging from 650-800bp. This does mean that polymorphism was 25, 14.3 and 14.3% in case of 2, 4 and 6 mg/l of BA, respectively.

In case of thidiazurone, OPK5 produced 14,15,14 and 20 bands in the gel in all DNAs of plantlets exposed to 1,2,3 and 4 mg/L of TDZ ,respectively, ranging from 200 to 1200 bp and produced 8,7,8 and 6 polymorphic bands under the same conditions ranging from 200 to 1200 bp. But in case of OPK6, it produced 12 bands in all concentrations of TDZ ranging form 300 to 1200 bp with 6

polymorphic bands, i.e 50% polymorphism.

The greatest disadvantage of some *in vitro* methods of vegetative propagation is the chance that genetic variation and mutations may occur. The term variant is used when the nature of the heritable change(s) in not known (Meins, 1983). When genetic variation is observed in plants which have been regenerated *in vitro*, then the term somaclonal variation is used (Scowcroft, 1985).

If cytokinin is added, as is usually the case *in vitro*, then the chances of mutation are increased (Scowcroft, 1985).

Moreover, somaclonal epigenetic variation can also take place, which is reversible and not hereditary. For example, different pheno-types can occur as a result of the use of cytokinin or auxin, which although are not mutations, but they have the appearance of being so.

The use of some growth regulators such as 2,4-D, NAA and synthetic cytokinins lead to a high increase in the number of mutations. Kinetin appears to induce selectively cell division in cells which are naturally (*in vivo*) polypolid (D'Amato, 1977).

Genetic instability may be occured by using auxin and cytokinin and polyploid cells can be selectively induced to divide, as has been demonstrated for pear. On the other hand, it appears that synthetic auxins like NAA and 2,4-D which mainly result in non-organized growth of cells can give rise to abnormalities during cell division. Also, when aneuploid cell arise (e.g. with *Nicotiana tabacum* and sugar cane hybrids) it is assumed that these are a result of *in vitro* culture, since very few aneuploids arise *in vivo*. The auxins NAA and 2,4-D are often assumed to be responsible for occurrence of aneuploids *in vitro* although this has yet to be authenticated .

Table (6): Amplified fragments obtained from the DNAs of in-vitro derived plantlets (benzyladenine treatments) of communis pear.

OPK6				OPK5			
B_0	B_2	B_4	B_6	B_0	B_2	B_4	B_6
0	0	0	0	1	1	0	0
0	0	0	0	0	0	0	0
0	0	0	0	1	1	0	0
0	0	0	0	1	0	0	0
0	1	1	1	1	1	1	1
0	0	0	0	1	0	0	1
0	0	0	0	1	1	1	1
0	1	0	0	1	1	1	1
0	0	0	0	0	0	0	0
0	0	0	0	1	1	1	1
0	0	0	0	0	0	0	0
1	1	1	1	1	0	1	1
1	1	1	1	0	1	1	0
0	0	0	0	1	0	0	1
1	1	1	1	0	0	0	1
0	0	0	0	1	0	1	1
0	0	0	0	0	0	1	1
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
3	5	4	4	11	7	8	10

Table (7): Amplified fragments obtained from the DNAs of in vitro derived plantlets (Thidiazuron treatments) of communis pear.

OPK6					OPK5				
T_0	T_1	T_2	T_3	T_4	T_0	T_1	T_2	T_3	T_4
0	1	1	1	1	1	0	0	0	1
0	1	1	1	1	0	0	0	0	0
0	1	1	1	1	1	0	0	0	1
0	1	1	1	1	1	0	0	0	1
0	1	1	1	1	1	0	1	1	0
0	0	0	0	0	1	0	0	0	1
0	0	0	0	0	1	0	0	0	1
0	0	0	0	0	1	1	1	1	1
0	1	1	1	1	0	0	0	0	1
0	0	0	0	0	1	1	0	0	0
0	0	0	0	0	0	0	0	0	0
1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	0	0	0	0	0
0	0	0	0	0	1	0	1	0	0
1	1	1	1	1	0	0	0	0	1
0	0	0	0	0	1	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
3	9	9	9	9	11	3	4	3	9

Table (8): Primers with arbitrary sequence tested for their effectiveness in the RAPD-PCR analysis that produced polymorphic bands of pear.

Treatment	Primers	No of Bands in the gel		% of polymorphism
		total	polymorphic	
Kinetin				
2mg/L	OPK_5	20	4	20.0
	OPK_6	6	0.0	0.0
4mg/L	OPK_5	22	0.0	0.0
	OPK_6	6	2.0	33.3
6mg/L	OPK_5	22	0.0	0.0
	OPK_6	6	0.0	0.0
Benzyladenine				
2mg/L	OPK_5	18	6	33.3
	OPK_6	8	2	25.0
4mg/L	OPK_5	19	7	36.8
	OPK_6	7	1	14.3
6mg/L	OPK_5	21	5	26.3
	OPK_6	7	1	14.3
Thidiazuron				
1 mg/L	OPK_5	14	8	57.1
	OPK_6	12	6	50.0
2mg/L	OPK_5	15	7	46.7
	OPK_6	12	6	50.0
3mg/L	OPK_5	14	8	57.1
	OPK_6	12	6	50.0
4mg/L	OPK_5	20	6	30.0
	OPK_6	12	6	50.0

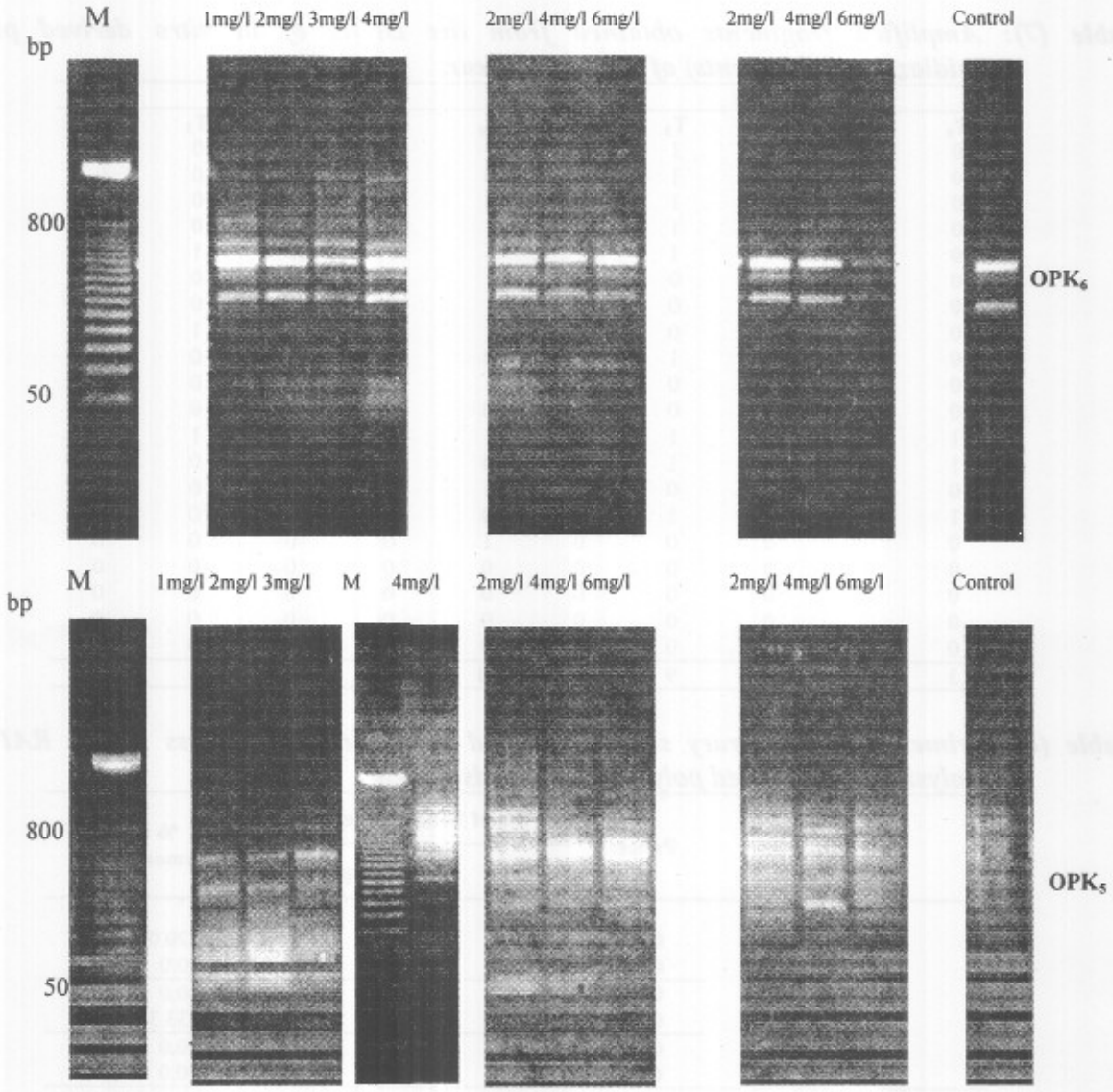

Fig. (2): Gel electrophoresis of RAPD fragments obtained with random primers (OPK5 and OPK6) by PCR amplifications of the DNAs prepared from in vitro derived pear plantlets exposed to different treatments of kinetin(2, 4 ,6 mg/l), Benzyladenine(2, 4 and 6 mg/l) and thiadiazuron (1, 2, 3 and 4 mg/l). M → 1 Kb DNA ladder 50 bp (promega).

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الملخص العربي

تأثير تركيزات مختلفة من السيتوكينينات والثايدزورن على الزيادة العددية و حدوث الطفرات فى الكمثرى

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أجرى هذا البحث بمعمل زراعة الأنسجة بالمركز القومى للبحوث وذلك بهدف دراسة تأثيراضافة السيتوكينينات والثايدزورن بتركيزاتها المختلفة فى بيئة الإكثار الدقيق للكمثرى على التكشف وحدوث التباين الوراثى فى النباتات الناتجة. وتم فصل القمة النامية من النموات الصغيرة للكمثرى حيث زرعت على بيئة موراشيج وسكوج الصلبة وبعد النقلة الرابعة على بيئة التكشف تم دراسة تأثير الكينيتين والبنزايل امينوبيورين والثايدزورن وتم عمل بصمة وراثية باستخدام تكنيك الـ RAPD-PCR . ووجد أن الكينيتين بتركيزاته المرتفعة 4و6 ملليجرام/ لتر ساعد على الزيادة العددية للسيقان وشجع استخدام 2 ملليجرام/ لتر من البنزايل ادنين الزيادة العددية وساعد استخدام الثايدزورن بتركيز 6 ملليجرام / لتر على تحسين جميع قياسات النمو. وقد أشارت دراسة البصمة الوراثية باستخدام تكنيك الـ RAPD-PCR إلى وجود بعض الاختلافات البسيطة فى عدد وحجم الحزم الموجودة فى الحمض النووى من النباتات المعملية المعرضة لأنواع مختلفة من الهرمونات بتركيزات مختلفة، وهذه الاختلافات تختلف بتركيز الهرمون ونوعه، وكانت هذه الاختلافات واضحة جداً عندما استخدمت مادة الثايدزورن.