In vitro culture of *Gypsophila paniculata* L. and random amplified polymorphic DNA analysis of the propagated plants

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ABSTRACT

A protocol is established for regeneration of the economically important cut flower plant, Gypsophila paniculata L. using shoot tips explants. Multiple shoots were obtained on MS-medium fortified with 0.5 mg/l each of NAA and BA. Addition of 10 g/l agar promoted shoot proliferation and reduced the degree of shoot vitrification. Transfer to 3 mg/l IBA containing medium produced optimum root initiation and development. The produced plants as well as intact plants were subjected to the random amplified polymorphic DNA (RAPD) analysis. Using 9 primers, the total number of amplification products generated by PCR amplification was 142 bands (15.7 bands per primer), of which 7.74% showed polymorphism. The analysis of bands recorded, showed 92.25% similarity. The results indicated that variation at the DNA level has occurred during in vitro culture of Gypsophila but in a very low level.

Key words: *Gypsophila paniculata, micropropagation, RAPD analysis.*

INTRODUCTION

Gypsophila (*Gypsophila paniculata* L.), also known as baby's breath, belongs to *Caryophyllaceae* family. It is a perennial plant, but is often grown commercially as an annual crop. Gypsophila is now the main flowering shoot used fresh and dried as a filler in flower arrangements in both Europe and USA. The flowers of commercial *Gypsophila paniculata* plants are sterile and do not produce seeds; therefore, breeding programs are severely restricted (Shillo, 1985). Also, the low rooting frequency of vegetatively propagated cuttings hinders propagation so, the application of tissue culture techniques forgypsophila is very vital to overcome problems for breeding and propagation.

Now, commercial propagation of ornamental plants by tissue culture is being a new promising industry in horticultural markets (Debergh and Maene, 1981). Most of the previously published reports on *Gypsophila paniculata* have concentrated on developing adventitious shoot regeneration from inter nodes or leaf segment (Ahroni, *et*

al., 1997; Zuker *et al*., 1997 and Lee and Bae, 1999a). Recently, callus and cell suspension cultures were established from *G. paniculata* leaf segments (Salman, 2002). In addition, earlier publications on *Gypsophila* have included micropropagation from shoot-tips explants (Han *et al*., 1991a; Zamorano-Mendoza and Mejia-Munoz, 1994; Song *et al*., 1996; Lee and Bae, 1999b).

The introduction of molecular marker technology in micropropagation programs might increase our knowledge about effect of *in vitro* somaclonal variation on micropropagated plants. Therefore, introduction of valuable variation through tissue culture of *Gypsophila* plant may help in programmes designed to improve characteristics of the plant. Previous workers have reported multiplication of shoot cultures of *Gypsophila*, but no report is available for random amplified polymorphic DNA (RAPD) analysis of the produced plants. RAPD assays utilize arbitrary 10-mer oligonucleotide sequences as primers (Williams *et al*., 1990). Primers hybridise to two nearby sites in the template DNA that are complementary to the primer sequence. Deletions or insertions in the amplified regions or base changes altering primer binding sites will result in polymorphisms. The PCR-based RAPD technique was applied and proved effective in a number of reports. RAPDs have proved to be very useful for the analysis of large number of genotypes (Rafalski and Tingey, 1993). Detection of somaclonal variation using RAPD in garlic, *Allium sativum* L. was studied (Al-Zahim *et al*., 1999). RAPD analysis of *in vitro* produced turmeric plants was reported (Salvi *et al*., 2001). RAPD is referred as an appropriate tool for certification of genetic fidelity of *in vitro* propagated plants (Gupta and Rao, 2002). Recently, many authors used RAPD fingerprints to study genetic stability of *in vitro* propagated chestnut hybrids (Carvalho *et al*., 2004) and *Curcuma amada* Roxb. plantlets (Prakash *et al*., 2004).

The aim of this study was to improve the different stages of *in vitro* propagation of *Gypsophila* to allow commercial production of this plant in Egypt, through modification of culture media for initiation of shoots, shoot proliferation, and rooting of shoots. RAPD analysis as a simple molecular marker method for the genetic identification of tissue culture produced *Gypsophila* plants was employed.

MATERIALS AND METHODS

Plant material and tissue-culture conditions

Shoot tips excised from *Gypsophila paniculata* L. cuttings were surface sterilized with 70% ethanol for 1 min, followed by 20% commercial clorox (contained 5.25% sodium hypochlorite) for 20 min. After three successive rinses in sterile distilled water the explants (about 0.25 cm in length) were placed in glass tubes containing 20 ml of MS (Murashige and Skoog, 1962) basal medium supplemented with 30 g/l sucrose, 100 mg / l myo-inositol and solidified with 7 g/l agar. Prior to autoclaving (121°C and a pressure of 1.2 kg cm^{-2} for 20 min.) growth hormones were added to the media and the pH was adjusted to 5.8 (using 1 M NaOH or HCl). Four concentrations of the cytokinin BA (6-benzyladenine) (0.25, 0.5, 1, 2 mg/l), in combination with 0.5 mg/l NAA (naphthaleneacetic acid) as auxin. After 30 days of culture, shoots were transferred to MS medium supplemented with different concentrations of 3-indolebutyric acid (IBA) (0.0, 1, 2 and 3 mg/l). Well developed plantlets were subsequently grown in pots containing a mixture of peat-moss and perlite in 1: 1 ratio, and incubated in the growth chamber for 1 month and were used for RAPD analysis. All cultures were maintained in a growth room at 25 ± 2°C under a 16-h photoperiod (irradiance

of about 40 μmol m^{-2} s^{-1} provided by cool white fluorescent lamps).

Experimental design, statistical analysis

Experiments were set up in a completely randomized design and repeated two times. Each treatment has five replications. Shoot number (longer than 1 cm), shoot length (cm), roots length (cm), rooting and vitrification percentage (evaluated by visual observations) were recorded after one month of cultivation. Data obtained were subjected to statistical analysis as described by Snedecor and Cochran, (1967).

Genomic DNA extraction and RAPD analysis

Total genomic DNA was extracted from leaf material (100-200 mg) of *in vivo* plants (propagated by conventional method through cuttings) and *in vitro* propagated plants (regenerants) using the CTAB method of Doyle and Doyle (1990). PCR amplification was performed in 20 μl reaction mix containing 40 ng genomic DNA, 0.5 unit Taq polymerase (Appliegene, Germany), 200 μM each of (dNTPs) dATP, dCTP, dGTP, dTTP, 10 p mole random primers and appropriate amplification buffer.

Following an initial denaturation step at 92°C for 2 min, the amplification programme was 44 cycles of 30 sec at 92°C (denaturing step), 30 sec. at 36°C (annealing step), and 2 min. at 72°C (extension step) in Perkin Elmer thermocycler (USA). Reactions were finally incubated at 72°C for 10 min. All primers used were 10-mer random oligonucleotide sequences obtained from Operon Technologies Inc. (Alameda, CA, USA). The amplification products were separated on 2% agarose gel in TAE buffer (pH 8.0), stained with 0.2 μg/ml ethidium bromide and photographed under UV light using red filter.

RESULTS AND DISCUSSION

Effect of NAA and BA on shoot proliferation

In a preliminary study the proliferate capacity of cultured shoot-tips depended on the concentration of the BA used. The effect of different levels of BA with a constant level of NAA on adventitious shoot proliferation by shoot tip culture of *G. paniculata* was shown in Table (1). It could be observed that the inclusion of 0.5 mg/l each of NAA and BA in the culture medium led to the highest number of shoot per explant and shoot length (4.2 and 3.3, respectively) as compared to that recorded with other treatments (Fig. 1-A). At low BA concentration (0.25 mg/l) fewer shoot and shoot length were obtained (3.1 and 1.9, respectively). However, no callus formation in all treatments was observed.

In general, high or low concentration of BA with NAA resulted in lower values for number of shoot and shoot length. The synergistic effect of NAA in combination with BA on promotion of *G. paniculata* shoot cultures is in agreement with observations of Song *et al*., (1996) who obtained regenerated plants from shoot tips of *Gypsophila paniculata.* They reported that the best culture medium was MS medium with 0.5 mg/l BA + 0.5 mg/l NAA + 1.0 mg/l Kinetin which resulted a high multiplication rate (10.4). Lee and Bae, (1999a) found that the best levels of BA and NAA in the culture medium for *in vitro* shoot tip proliferation of *Gypsophila paniculata* L. was 0.2 mg/l BA + 0.1 or 0.2 mg/l NAA. However, Han *et al*., (1991a) found that BA at 0.5 - 2 mg/l was the most effective cytokinin for shoot proliferation of *Gypsophila paniculata*. They reported that a combination of BA and IAA each at 0.1 - 0.3 mg/l gave better shoot proliferation and growth than BA alone.

Table (1): Means of shoots /explant, shoot length and vitrification percentage of G. paniculata as affected by NAA and BA.

Growth regulator (mg/l)		* Number of shoots per explant	* Shoot length (cm)	Vitrification (%)
NAA	BA			
0.5	0.25	3.1 ± 0.90 a	1.9 ± 0.56 a	15
0.5	0.50	4.2 ± 1.00 a	3.3 ± 0.98 a	18
0.5	1.00	3.4 ± 0.63 a	2.8 ± 0.67 a	19
0.5	2.00	3.0 ± 0.88 a	3.1 ± 1.00 a	23

*Means of number of shoots and shoot length are followed by ± standard error (SE).
Data (mean ± SE) sharing the same letter in the same column are not significantly different ($P < 0.05$).

On the other hand, in cultures of shoot tips of *G. paniculata* different growth regulators were used for proliferation of shoots. For example, Zamorano-Mendoza and Mejia-Munoz, (1994) concluded that the presence of 0.5 mg/l IAA + 3.0 mg/l BA in the medium gave the highest number of shoots (8/shoot tip explant, within 5 weeks) from cultured shoot tips of G. *paniculata* cv. Perfecta. Also, Ahroni, *et al*., (1997) found that thidiazuron (TDZ) was the most effective cytokinin, with up to 100% of the explants (internodes of the stem of *G. paniculata*) forming shoots, at an average of up to 19 shoots per explant have regenerated. Similarly, Lee and Bae, (1999 b) found that thidiazuron was remarkably effective for the regeneration of leaf segment in *G. paniculata* as compared with BA and kinetin.

Results presented in Table (1) show also vitrification percentage of proliferated *G. paniculata* shoots. Degree of vitrification was highest (23%) in shoots grown on medium contained high levels of BA (2 mg/l). Less vitrified shoots were observed when low level of BA (0.25 mg/l) was added in the culture medium. Generally, it could be observed that increasing levels of BA in the medium caused an increase in vitrified shoots. In this respect, Han *et al*., (1991b) and Lee and Bae, (1999 b) also reported that addition of BA to the culture medium in *in vitro* cultures of *G. paniculata* increased the percentage of vitrified plants.

Effect of agar levels on shoot proliferation and vitrification

This part of study was conducted in order to minimize the percentage of vitrification in shoot cultures of gypsophila. Hyperhydricity (vitrification) is a well-known phenomenon in tissue culture of gypsophila. This serious problem limits the success of micropropagation due to the poor rate of survived tissue culture derived plants. However, many workers studied this phenomenon in *Gypsophila* tissue culture (Dillen and Buysens, 1989; Han *et al*., 1991a,b and Lee and Bae, 1999a,b) and found its relation with agar levels in the culture medium.

Table (2): Mean number of shoots, shoot length and vitrification percentage of G. paniculata as affected by agar concentrations.

Agar concentration (%)	Number of shoots per	Shoot length (cm)	* Vitrification (%)

	explant		
0.6	4.5 ± 0.87 ab	4.1 ± 1.00 a	17
0.8	5.6 ± 0.98 a	5.5 ± 0.94 a	10
1.0	8.1 ± 0.55 ac	5.0 ± 1.10 a	5
1.2	6.2 ± 0.54 a	6.1 ± 1.23 a	3

Values are means ± standard error (SE).

* Number of vitrified shoots / Total number of shoots × 100

Data (mean ± SE) sharing the same letter in the same column are not significantly different ($P < 0.05$).

As shown in Table (2) different levels of agar was added to the culture MS-medium supplemented with 0.5 mg/l each of NAA and BA. The maximum number of shoots (8.1) was obtained from explants initially cultured in medium containing 1.0% agar. It could be observed that the addition of 1.2% agar to the medium enhanced the elongation of shoots (6.1 cm). At low agar level (0.6 %) fewer shoots (4.5) and shoot length (4.1 cm) were obtained.

Visual observation for proliferated shoots demonstrated that high percentage of vitrification (17) was observed in shoots cultured on medium containing 0.6% agar. The lowest degree of vitrification (5 and 3%) was recorded when 1.0 and 1.2% agar, respectively were added to the culture medium (Fig. 1-B).

In general, the low agar level in the medium reduces the number of shoots produced and increases degree of vitrification, whereas increasing agar level promoted shoot proliferation and length and reduced percentage of vitrification. In this respect, It could be mentioned that high percentage of vitrification may be due to the presence of cytokinins in the medium as they may stimulate stress ethylene production which is regarded as a possible trigger of vitrification (Kevers *et al*., 1984). Some workers observed that vitreous shoots did not root vigorously. This may partly be due to the poor development of wax layer which controls excessive evaporation through the cuticle (John and Webb, 1987). Results of this study agree with other authors who reported that in *Gypsophila* cultures, increasing agar levels or nitrate:ammonium ratio of the medium and incubation of cultures under low temperature (Han *et al*., 1991a,b) or using special vessels in culture (Lee and Bae, 1999b) could reduce vitrification of produced shoots.

Effect of IBA levels on rooting

The effect of IBA concentration on the percentage of shoots forming roots and root length of *G. paniculata* shoots is illustrated in Fig. (2). In the present study, It should be noticed that the first root emergence was observed after 20 days of cultivation on medium containing 3 mg/l IBA (Fig. 1-C), whereas roots were appeared after 22 days of cultivation on medium containing 2 mg/l IBA. However, medium avoided with IBA or with 1.0 mg/l IBA did not induce roots on *Gypsophila* shoots. Twenty five percent of shoots developed roots when cultured on MS-medium with 2.0 mg/l IBA while ninety percent of shoots developed roots when cultured on MS-medium with 3.0 mg/l IBA. The longest root length (4.3 cm) was recorded when shoots were grown on medium contained 3.0 mg/l IBA, whereas root length of shoots grown in medium with 2.0 mg/l IBA was 3.5 cm. Previous investigations on root initiation of *G. paniculata* using various growth regulators showed that 0.75 mg/l IBA gave the longest root length Zamorano-Mendoza and Mejia-Munoz, (1994). In cultures of shoot tips of *G. paniculata* Han *et al*. (1991a) found that addition of 1 mg/l IBA induced root formation. Song *et al*. (1996) reported that *G. paniculata* plantlets rooted on MS medium supplemented

with 5.0 mg/l IAA + 0.1 mg/l NAA + 0.2 mg/l IBA (95% rooting after 14 days). Half-strength MS basal medium containing 0.1 mg/l GA_3 and 0.1 mg/l NAA induced root formation in *G. paniculata* adventitious shoot, Zuker *et al*. (1997). Recently, Qian *et al*. (2000) reported that MS-medium supplemented with 0.01 mg/l NAA and 0.10 mg/l PP333 (Paclobutrazol) induced rooting in cultured *G. paniculata* buds.

Plantlets with well-developed roots were potted in a mixture of equal parts of peat-moss and perlite (Fig. 1-D). The plantlets were acclimatized to growth chamber conditions with gradual exposure to reduced relative humidity by removing a plastic cover over a period of 2 weeks. Once the acclimatization was accomplished, the plants were subjected to RAPD analysis.

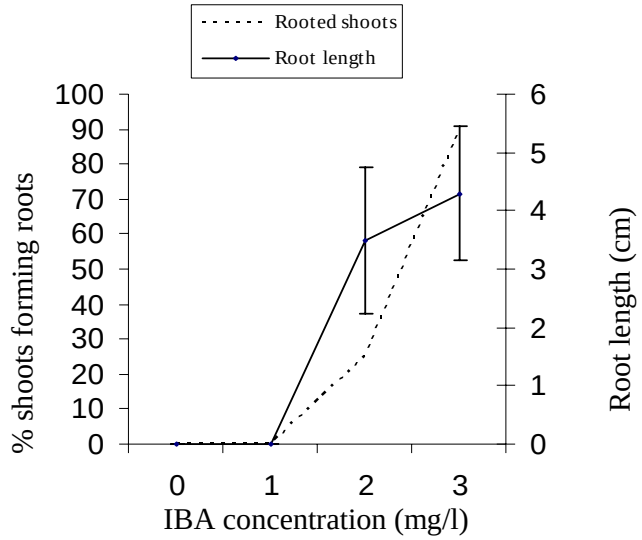


Fig. (2): Effect of IBA concentrations on rooting of G. paniculata shoots.

RAPD analysis of *in vivo* and *in vitro* gypsophila plants

DNA of *in vivo* and *in vitro* regenerated plants was prepared from leaves and amplified by PCR using random oligonucleotide primers. Amplification products were separated by agarose-gel electrophoresis to reveal band polymorphism. Data in Table (3) demonstrated the results of RAPD analysis of *in vivo* and regenerated gypsophila plants. Out of the 20 random primers screened, only nine primers produced clear reproducible bands (sequences presented in Table 3). The 9 primers yielded 142 scorable bands (with an average of 15.7 bands per primer), including eleven polymorphic bands. The number of bands from each primer varied from 12 to 27. The primer OPM16 in particular, produced a large number of strongly amplified individual fragments (27), whereas, primers OPK4, OPK5 and OPM13 produced the lowest number (12) of amplicons. On the other hand, primer OPK10 gave the highest percentage of polymorphism (21.42) while the lowest percentage (zero) was

obtained by using the primers OPK5, OPM13 and OPK9.

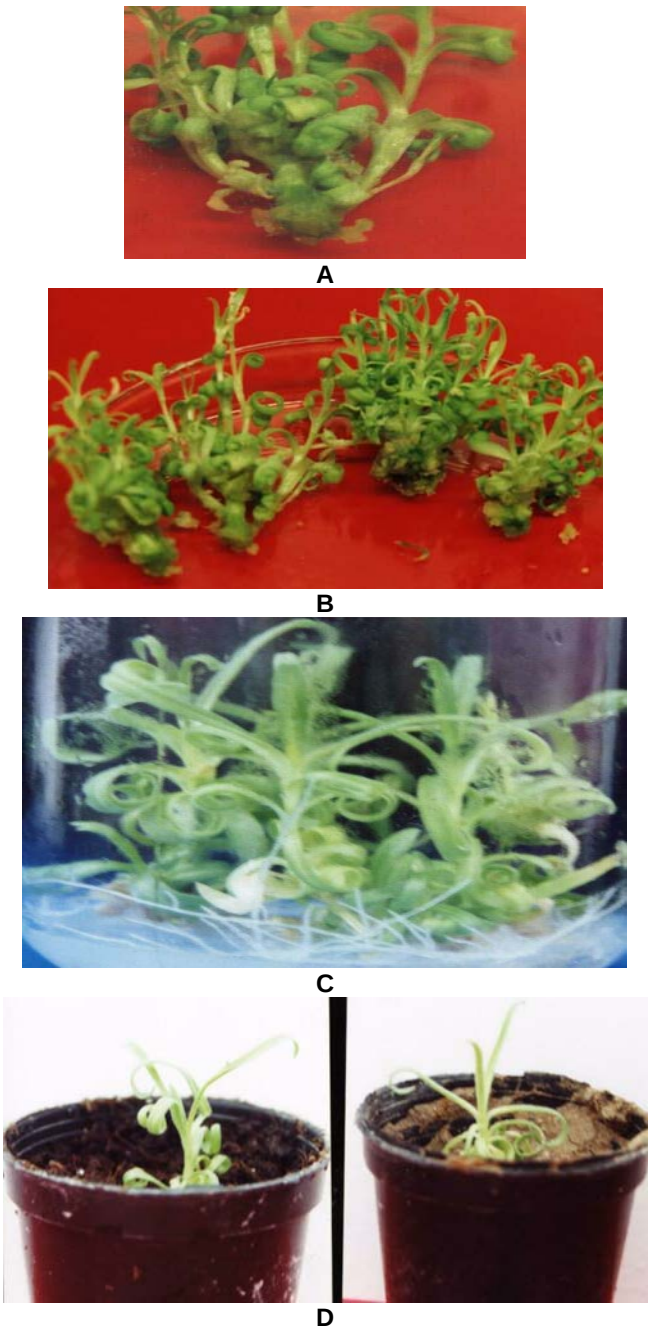
Table (3): RAPD-PCR amplification products of DNA extracted from in vivo and in vitro produced gypsophila plants using nine random primers.

Primer	Sequence 5′----------3′	Total number of scorable bands	Number of Polymorphic bands	% of polymorphism
OPK1	TGCCGAGCTG	14	1	7.14
OPK4	TCGTTCCGCA	12	2	16.66
OPK10	GTGCAACGTG	14	3	21.42
OPK5	CACCTTTCCC	12	---	---
OPM13	GGTGGTCAAG	12	---	---
OPM16	GTAACCAGCC	27	2	7.40
OPN1	CTCACGTTGG	18	2	11.11
OPK6	GAGGGAAGAG	18	1	5.55
OPK9	CCCTACCGAC	15	---	---
Overall totals		142	11	7.74

% of polymorphism = (No. of polymorphic bands ÷ Total No. of scorable bands) × 100

Of the 9 primers tested, 3 (OPK5, OPM13 and OPK9) produced amplification products that were monomorphic across all the *in vivo* and regenerated gypsophila plants (Fig. 3-B) and (Fig. 4-A and B). The number of amplification products generated by the 3 primers was 39 bands ranged from 12 in OPK5 and OPM13 to 15 in OPK9 (Table 3). The size of the 39 monomorphic bands produced by these primers ranged from 250 bp in OPK5 (Fig. 3-A) to 1100 bp in OPM13 (Fig. 4-A). The other 6 primers (OPK1, OPK4, OPK10, OPM16, OPN1, and OPK6) revealed scorable polymorphisms. The number of amplification products generated by the 6 primers was 103 bands ranged from 12 in OPK4 to 27 in OPM16 (Table 3), with a size range of 250 bp in OPK1 (Fig. 3-A) to 1300 in OPM16 (Fig. 4-A).

Using the primer OPK1, one polymorphic band with a molecular weight 250 bp in regenerant plant (2) was absent (Fig. 3-A and Table 4), with the primer OPK4, two polymorphic bands with molecular weights 250 and 270 bp were absent in regenerant plant (2). Also, using the primer OPK10, three polymorphic bands with molecular weights 350, 250 and 240 bp in regenerant plant (2) were absent (Fig. 3-B and Table 4).



Fi g. (1): Micropropagation of Gypsophila paniculata L.

- ***A) Adventitious shoots from shoot-tip explant after 4 weeks of culture.***
- ***B) Multiple shoot cultured on media contained different levels of agar.***
- ***C) Rooting of shoots on MS-medium contained 3 mg/l IBA.***
- D) ***Propagated plants established on pots contained peat-moss and perlite (ratio 1 : 1).***

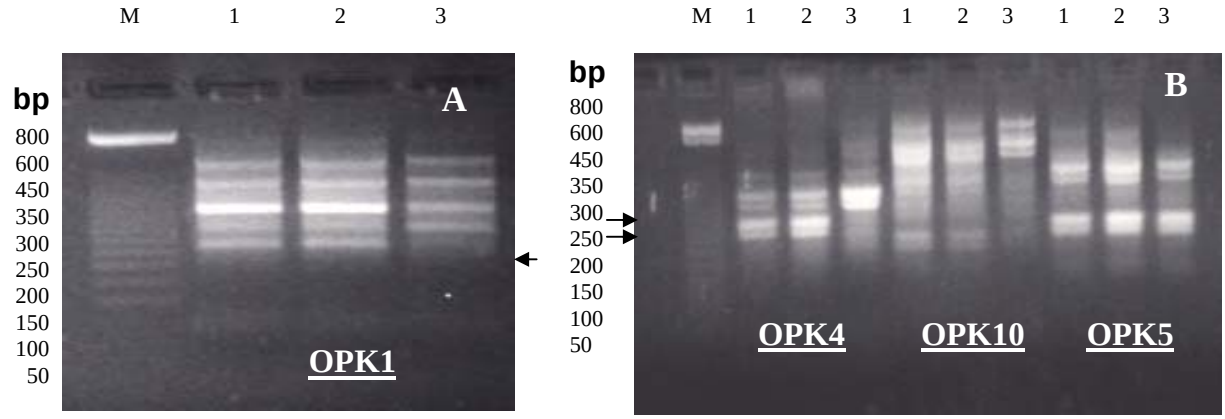


Fig. (3): Gel electrophoresis of RAPD fragments generated by primers OPK1 (A); OPK4, OPK10 and OPK5 (B). Lane (1) represents intact plant; lanes (2) and (3) represent in vitro produced plants; lane (M) indicates molecular weight DNA marker by bp.
Primers used are designated below the gels.
Polymorphic DNA fragments are identified by an arrowhead.

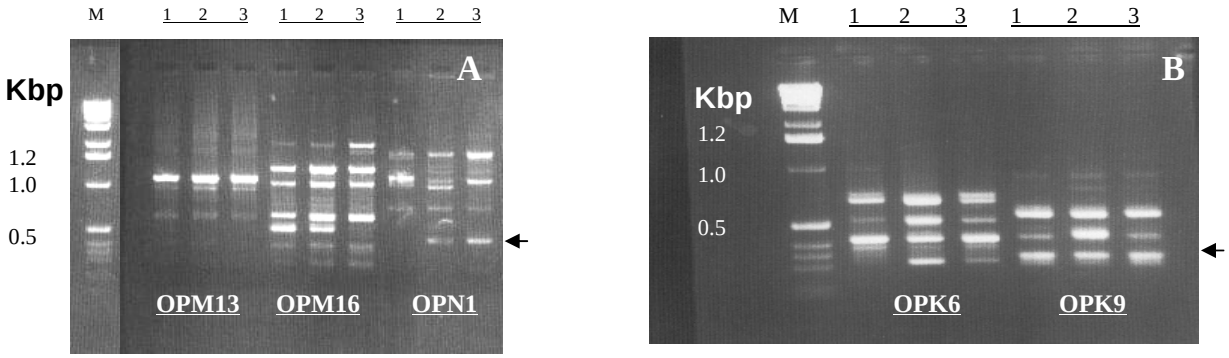


Fig. (4): Gel electrophoresis of RAPD fragments generated by primers OPM13, OPM16 and OPN1 (A); OPK6 and OPK9 (B). Lane (1) represents intact plant; lanes (2) and (3) represent in vitro produced plants; lane (M) indicates molecular weight DNA marker by K bp.
Primers used are designated below the gels.
Polymorphic DNA fragments are identified by an arrowhead.

With the primer OPM16, two polymorphic bands with molecular weights 1200 bp in regenerant plant (2), (lane 3) and 500 bp in *in vivo* and regenerated plants (1), (lane 1 and 2) were detected (Fig. 3-A-black arrow). However, for the primer OPN1 one polymorphic band with a molecular weight 1000 bp in regenerant plants (2) (lane 3) was absent and one polymorphic band with a molecular weight 400 bp in regenerants plants (lane 2 and 3) was present (Fig. 3-A-black arrow). From the profiles obtained with the primer OPK6 one polymorphic band with a molecular weight 250 bp in *in vivo* plant (lane

1) was absent (Fig. 4-B). RAPD variation has also been reported in many studies. For example, reports have indicated the occurrence of somaclonal variation in micropropagated banana plants raised from meristem culture (Schoofs, 1992). *In vitro* culture environment may be mutagenic as reported by Larkin and Scowcroft (1981). Thirty five of garlic (*Allium sativum* L.) plants regenerated by somatic embryogenesis were subjected to RAPD analysis (Al-Zahim *et al*., 1999) who found that the frequency of variation was found to be cultivar dependent. Certain band changes were found in regenerants of different cultivars, suggesting the existence of a mutation-sensitive part of the garlic genome. Similarly, Salvi *et al*., (2001) reported that RAPD analysis of eight regenerated turmeric plants using 14 primers showed 38 novel bands. About 51 bands present in the control were absent in the regenerants. Their results indicate that variation at DNA level has occurred during *in vitro* culture.

Table (4): Distribution and size of polymorphic bands from in vivo and in vitro produced Gypsophila plants using nine random primers.

Primer	Size of polymorphic band (bp)	Distribution of polymorphic bands		
		In vivo plant	*Regenerant plant (1)*	*Regenerant plant (2)*
OPK1	250	+	+	---
OPK4	250	+	+	---
	270	+	+	---
	350	+	+	---
OPK10	250	+	+	---
	240	+	+	---
OPK5	---	---	---	---
OPM13	---	---	---	---
OPM16	1200	---	---	+
	500	+	+	---
OPN1	1000	+	+	---
	400	---	+	+
OPK6	250	---	+	+
OPK9	---	---	---	---

+, refers to the presence and ---, absence of a RAPD marker (polymorphic band).

Table (4) shows distribution and size of polymorphic bands from *in vivo* and *in vitro Gypsophila* plants using nine primers. It could be observed that using the primers OPK1, OPK4 and OPK10, 6 polymorphic bands were absent in regenerant plant (2) but were present in *in vivo* and regenerant (1) plant. Failure of amplification for regenerant plant (2) may be due to a single base change or two completely different sequences (Williams *et al*., 1990 and Vierling and Nguyen 1992). However, using the primers OPM16, OPN1 and OPK6, 3 bands were recorded in regenerants (1) and (2) plants but were absent in the *in vivo* plant. On the other hand, with 6 out of 9 primers tested RAPD profiles of regenerant plant (1) exhibited similar banding patterns (monomorphic RAPD profiles) to that obtained with *in vivo* plant which suggests homology among the two plants. The absence of polymorphic fragments presented here is in accordance with RAPD comparisons of *Pyrus* propagated *in vitro* with *in vivo* donor plants (Oliveira *et al*., 1999). This is also in

agreement with the observation of Rout *et al*., (1998) who found that micropropagated ginger plants which obtained from axillary bud proliferation showed monomorphic RAPD profiles in comparison with control plants. Moreover, Carvalho *et al*., (2004) used RAPD fingerprints to study genetic stability of *in vitro* propagated chestnut hybrids and found that no polymorphism was detected between *in vitro* plants and the donor plants they originated from. RAPD analysis of the regenerated plantlets of *Curcuma amada* Roxb were also similar to the mother plants (Prakash *et al*., 2004).

In general, in the present study using 9 primers resulted in a total of 142 bands including eleven polymorphic bands, of which 7.74% showed polymorphism. Earlier reports indicate higher polymorphism, in garlic, (Al-Zahim *et al*., 1999) using five varieties, 50 polymorphic bands were obtained from a total of 7903 bands (0.63% showed polymorphism). Yang *et al*. (1999) obtained more than 50 polymorphic fragments using four primers from regenerated rice plants derived from callus cultures. In turmeric plant, Salvi *et al*. (2001) using 14 primers led to a total of 231 bands of which 16.5% showed polymorphism. Also, with *Dioscorea floribunda* plants derived from cryopreserved shoot tips, 10 primers produced 64 clear reproducible bands. A total of 5120 bands obtained from this study exhibited no aberration in RAPD banding except 1 being polymorphic (Ahuja *et al*., 2002). Recently, Prakash *et al*. (2004) reported that RAPD analysis of *Curcuma amada* Roxb. regenerated plantlets revealed 103 scorable bands from 10 primers, including nine polymorphic bands, (of which 8.7% showed polymorphism) which were absent in control.

In conclusion, the present investigation reveals the varied response of gypsophila shoot tips to BA levels in the culture medium. Vitrification were avoided by the addition of a high level of agar in the culture medium. The present study provides the first information on the molecular basis of polymorphism detected as RAPD markers in gypsophila micropropagated plants. Furthermore, in this study RAPD analysis demonstrated that the polymorphism observed (7.74%) is very low. The similarity obtained from the analysis of all the bands recorded showed 92.25% similarity (11 polymorphic fragments out of a total of 142). It might be stated that the reliability of RAPD as a marker system to certify genetic stability of *in vitro* produced *Gypsophila* plants needs to be confirmed by adult phenotypic characteristics which will be investigated in a further study.

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الملخص العربي

الإكثار المعملي والبصمة الوراثية لمزارع أنسجة نبات الجبسوفيلا

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فى هذا البحث تم تشييد بروتوكول لإكثار نبات الجبسوفيلا معمليا بأستخدام القمم الخضرية لنباتات الحقل وقد كانت بيئة (MS) موراشيج وسكوج (١٩٦٢) المحتوية على بنزيل الأدنين BA ونفثالين حمض الخليك NAA بتركيز ٠,٥ ملليجرام/لتر لكل منهما هي أفضل البيئات لأنتاج أعلى المعدلات من النموات الخضرية. كما أتضح من النتائج أيضا بأن إضافة الأجار إلى بيئة الزراعة بتركيز ١٠ جرام/لتر أدى إلى تحسين معدل النموات الخضرية الناتجة وتقليل درجة التزجيج Vitrification لها . أوضحت النتائج أيضا أن إضافة ٣ ملليجرام/لتر أندول حمض البيوتريك IBA إلى البيئة أدى إلى تكوين الجذور على النموات الخضرية الناتجة. وبأستخدام تقنية التكبير العشوائي لأختلافات الحمض النووي (RAPD) للنباتات الناتجة معمليا والنباتات الحقلية بأستعمال تسعة بوادئ كان العدد الإجمالي للشرائط المتضاعفة بواسطة تفاعل البلمرة المتسلسل (PCR) هو ١٤٢ شريطا (حوالي ١٥,٧ شريط لكل بادئ) حيث أظهرت ٧,٧٤% من الشرائط الناتجة أختلافات وبالتالي كانت نسبة الشرائط المتشابهة ٩٢,٢٥% من إجمالي الشرائط. وعموما فأن النتائج تشير إلى وجود أختلافات على مستوى الحمض النووي لنباتات الجبسوفيلا الناتجة معمليا والنباتات الأم ولكن هذه الأختلافات كانت على مستوى ضعيف نسبيا.