BIOLOGIA PLANTARUM 50 (4): 507-513, 2006

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

M.R. RADY

Plant Biotechnology Department, Genetic Engineering and Biotechnology Division, National Research Centre, El-Tahrir St., P.O. Box 12311, Dokki Giza, Cairo, Egypt

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 Murashige and Skoog medium fortified with 0.5 mg dm^{-3} each of α-naphthaleneacetic acid and 6-benzyladenine. Addition of 10 g dm^{-3} agar promoted shoot proliferation and reduced the degree of shoot vitrification. Transfer to 3 mg dm^{-3} indole-3-butyric acid 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 polymerase chain reaction 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 very low variation at the DNA level occurred during *in vitro* culture of *Gypsophila*.

Additional key words: auxins, cut flower plant, cytokinins, micropropagation, molecular marker, polymerase chain reaction.

Introduction

Gypsophila paniculata L. belongs to *Caryophyllaceae* family. It is a perennial plant often grown commercially as an annual crop. The flowers of commercial *G. 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 for *Gypsophila* is very vital to overcome these problems.

Most of the previously published reports on *G. paniculata* have concentrated on developing adventitious shoot regeneration from internodes or leaf segment (Ahroni *et al*. 1997, Zuker *et al*. 1997, Lee and Bae 1999a). Recently, callus and cell suspension cultures were established from 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. 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 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) and for detecting intra-clonal genetic variability in vegetatively propagated tea (Singh *et al*. 2004). However, RAPD analysis was used in detecting mutations in embryogenic cultures of soybean (Hofmann *et al*. 2004). Recently, many authors used RAPD fingerprints to study genetic

Received 1 November 2004, *accepted* 21 September 2005.

Abbreviations: BA - 6-benzyladenine; CTAB - cetyltrimethylammonium bromide; IBA - indole-3-butyric acid; NAA - α-naphthaleneacetic acid; PCR - polymerase chain reaction; RAPD - random amplified polymorphic DNA; TBE - Tris borate EDTA.

Fax: (+20) 23370931, e-mail: drrady1@yahoo.com

stability of *in vitro* propagated chestnut hybrids (Carvalho *et al*. 2004) and *Curcuma amada* plantlets (Prakash *et al*. 2004).

The aim of this study was to improve the different stages of *in vitro* propagation of *Gypsophila* through modification of the culture media for initiation of shoots, shoot proliferation, and rooting of shoots. RAPD analysis 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 cm^{-3} of Murashige and Skoog (1962; MS) basal medium supplemented with 30 g dm^{-3} sucrose, 100 mg dm^{-3} myo-inositol and solidified with 7 g dm^{-3} agar. Four concentrations of the cytokinin 6-benzyladenine (BA; (0.25, 0.5, 1.0, 2.0 mg dm^{-3}), in combination with 0.5 mg dm^{-3} α-naphthaleneacetic acid (NAA) as auxin were added to the media prior to autoclaving (121 °C and a pressure of 1.2 kg cm^{-2} for 20 min) and the pH was adjusted to 5.8 (using 1 M NaOH or HCl). After 4 weeks shoot-tips were excised from proliferating shoot cultures and placed on MS-medium supplemented with 0.5 mg dm^{-3} each of NAA and BA (as the best medium in starting stage) and containing various concentrations of agar (0.6, 0.8, 1.0 and 1.2 %). For rooting shoots were transferred (after 30 d of cultivation) to MS medium supplemented with different concentrations of indole-3-butyric acid (IBA; 0, 1, 2 and 3 mg dm^{-3}). Well developed plantlets were sub-sequently 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 and 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 and root length, 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).

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 0.02 cm^3 reaction mix containing 40 ng genomic DNA, 0.5 unit Taq polymerase (*Appliegene*, Heidelberg, Germany), 200 μM each of dNTPs, dATP, dCTP, dGTP, dTTP, 10 pmol 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 s at 92 °C (denaturing step), 30 s at 36 °C (annealing step), and 2 min at 72 °C (extension step) in *Perkin Elmer* thermocycler (Norton, USA). Reactions were finally incubated at 72 °C for 10 min. All primers used were 10-mer random oligonucleotide sequences obtained from *Operon Technologies* (Alameda, CA, USA). Amplification was performed using 9 primers and reactions repeated on different days with different DNA template preparations and reproducibility of bands verified. The amplification products were separated on 2 % agarose gel in TBE buffer (pH 8.0), stained with 0.2 mg dm^{-3} ethidium bromide and photographed under UV light using red filter.

Results and discussion

Effect of phytohormones 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 concentrations of BA with a constant concentration of NAA on adventitious shoot proliferation by shoot tip culture of *G. paniculata* was shown (Table 1). The inclusion of 0.5 mg dm^{-3} 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 dm^{-3}) 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 *G. paniculata*. They reported that the best culture medium was MS medium with 0.5 mg dm^{-3} BA + 0.5 mg dm^{-3} NAA + 1.0 mg dm^{-3} 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 *G. paniculata* L. was 0.2 mg dm^{-3} BA + 0.1 or 0.2 mg dm^{-3} NAA. However, Han *et al.* (1991a) found that BA at 0.5 - 2 mg dm^{-3} was the most effective cytokinin for shoot proliferation of *G. paniculata*. They reported that a combination of BA and IAA each at 0.1 - 0.3 mg dm^{-3} gave better shoot proliferation and growth than BA alone.

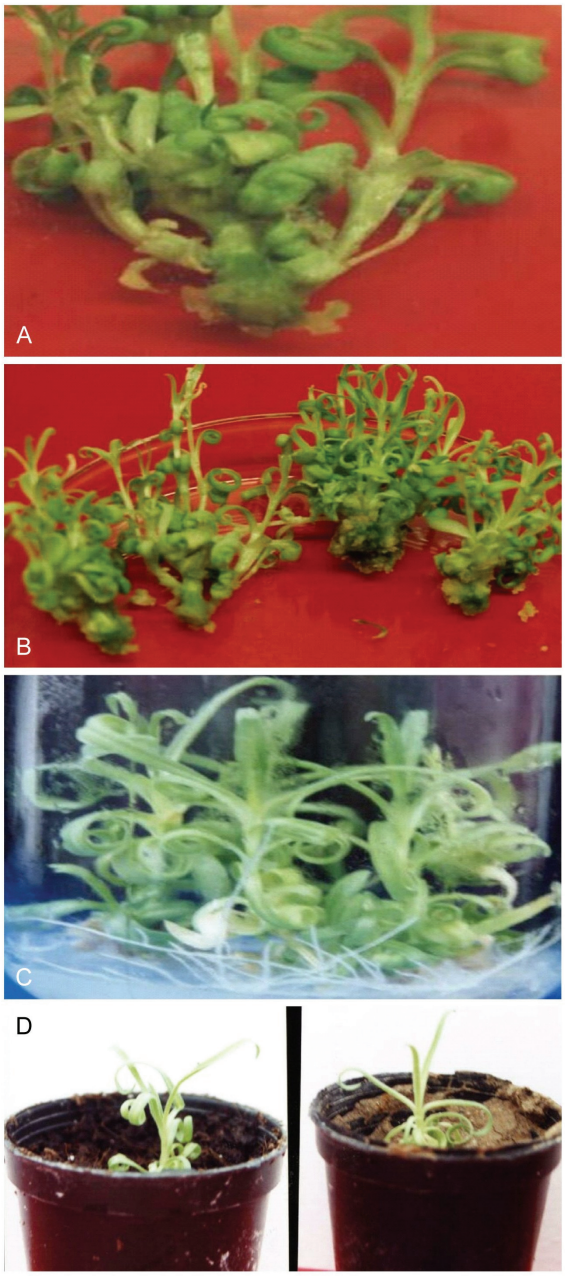

Fig. 1. Micropropagation of *Gypsophila paniculata*. *A* - Adventitious shoots from shoot-tip explant after 4 weeks of culture; *B* - multiple shoots cultured on media contained 1 % agar; *C* - rooting of shoots on MS medium contained 3 mg dm^{-3} IBA; *D* - micropropagated plants established in pots contained peat-moss and *Perlite* (1:1).

Table 1. Means number of shoot per explant, shoot length and vitrification percentage of *G. paniculata* as affected by NAA and BA concentrations. Means ± SE, n = 5. Means sharing the same letter in the same column is not significantly different ($P < 0.05$).

NAA [mg dm^{-3}]	BA [mg dm^{-3}]	Number of shoots [explant^{-1}]	Shoot length [cm]	Vitrification [%]
0.5	0.25	3.1 ± 0.90a	1.9 ± 0.56a	15
0.5	0.50	4.2 ± 1.00a	3.3 ± 0.98a	18
0.5	1.00	3.4 ± 0.63a	2.8 ± 0.67a	19
0.5	2.00	3.0 ± 0.88a	3.1 ± 1.00a	23

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 dm^{-3} IAA + 3.0 mg dm^{-3} BA in the medium gave the highest number of shoots (8 per 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.

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. Degree of vitrification was highest (23 %) in shoots grown on medium contained high concentrations of BA (2 mg dm^{-3}). Less vitrified shoots were observed when low concentration of BA (0.25 mg dm^{-3}) was in the culture medium. In this respect, Han *et al.* (1991b) and Lee and Bae (1999b) 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 concentration on shoot proliferation and vitrification: The maximum number of shoots (8.1) was obtained from explants cultured in medium containing 1.0 % agar. The addition of 1.2 % agar to the medium enhanced the elongation of shoots (6.1 cm) (Table 2). Visual observation for proliferated shoots demonstrated that high 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*). Many workers studied vitrification in *Gypsophila* tissue culture (Dillen and Buysens 1989, Han *et al*. 1991a,b, Lee and Bae 1999a,b) and found its relation with agar concentration in the culture medium.

Table 2. Means number of shoots, shoot length and vitrification percentage of *G. paniculata* as affected by agar concentrations. Means ± SE, $n = 5$. Means sharing the same letter in the same column is not significantly different ($P < 0.05$).

Agar [%]	Number of shoots [explant^{-1}]	Shoot length [cm]	Vitrification [%]
0.6	4.5 ± 0.87ab	4.1 ± 1.00a	17
0.8	5.6 ± 0.98a	5.5 ± 0.94a	10
1.0	8.1 ± 0.55ac	5.0 ± 1.10a	5
1.2	6.2 ± 0.54a	6.1 ± 1.23a	3

In general, the low agar concentration in the medium reduces the number of shoots produced and increases degree of vitrification, whereas increasing agar concentration 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 that increasing agar content could reduce vitrification of produced shoots (Han *et al*. 1991a,b).

Effect of IBA concentration on rooting: The first root emergence was observed after 20 d of cultivation on medium containing 3 mg dm^{-3} IBA (Table 3, Fig. 1*C*), whereas roots were appeared after 22 d of cultivation on medium containing 2 mg dm^{-3} IBA. However, medium avoided of IBA or with 1.0 mg dm^{-3} IBA did not induce root development. 25 % of shoots developed roots when cultured on MS-medium with 2.0 mg dm^{-3} IBA while 90 % of shoots developed roots when cultured on MS-medium with 3.0 mg dm^{-3} IBA. The longest roots (4.3 cm) were recorded when shoots were grown on medium contained 3.0 mg dm^{-3} IBA, whereas mean root length of shoots grown in medium with 2.0 mg dm^{-3} IBA was 3.5 cm. Previous investigations on root initiation of *G. paniculata* using various growth regulators showed that 0.75 mg dm^{-3} IBA gave the longest root length (Zamorano-Mendoza and Mejia-Munoz 1994). In cultures of *G. paniculata* Han *et al.* (1991a) found that addition of 1 mg dm^{-3} IBA induced root formation. Song *et al*. (1996) reported that *G. paniculata* plantlets rooted on MS medium supplemented with 5.0 mg dm^{-3} IAA + 0.1 mg dm^{-3} NAA + 0.2 mg dm^{-3} IBA (95 % rooting after 14 d). Half-strength MS basal medium containing 0.1 mg dm^{-3} GA_3 and 0.1 mg dm^{-3} 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 dm^{-3} NAA and 0.10 mg dm^{-3} 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.

Table 3. Effect of IBA concentration on rooting of *G. paniculata* shoots. Means ± SE, $n = 5$. Means sharing the same letter in the same column is not significantly different ($P < 0.05$).

IBA [mg dm^{-3}]	Rooting time [d]	Shoot forming roots [%]	Root length [cm]
2.0	22	25 ± 0.86b	3.5 ± 1.25b
3.0	20	90 ± 0.80c	4.3 ± 1.16b

RAPD analysis of *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. Out of the 20 random primers screened, only nine primers produced clear reproducible bands (sequences presented in Table 4). The 9 primers yielded 142 scorable bands

Table 4. RAPD-PCR amplification products of DNA extracted from *in vivo* and *in vitro* produced *G. paniculata* plants using nine random primers.

Primer	Sequence 5′----- 3′	Total number of bands	Number of polymorphic bands	Poly-morphism [%]
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	-	-

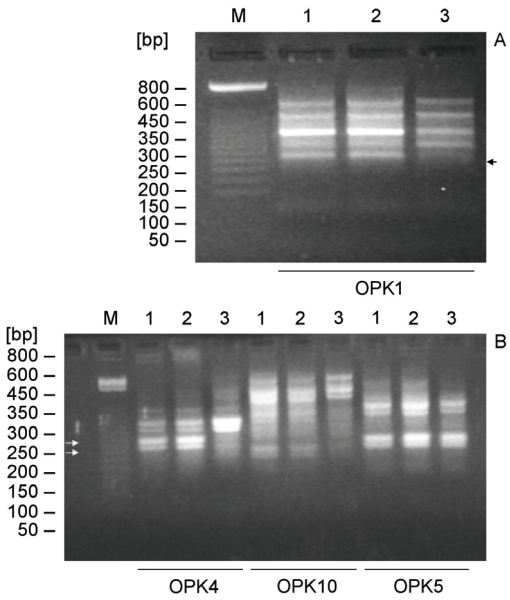

Fig. 2. Gel electrophoresis of RAPD fragments generated by primers OPK1 (*A*), and OPK4, OPK10 and OPK5 (*B*). *Lane 1* represent intact plant, *lanes 2 and 3* represent *in vitro* produced plants, *lane M* indicated molecular mass DNA marker. Polymorphic DNA fragments are identified by an *arrow*.

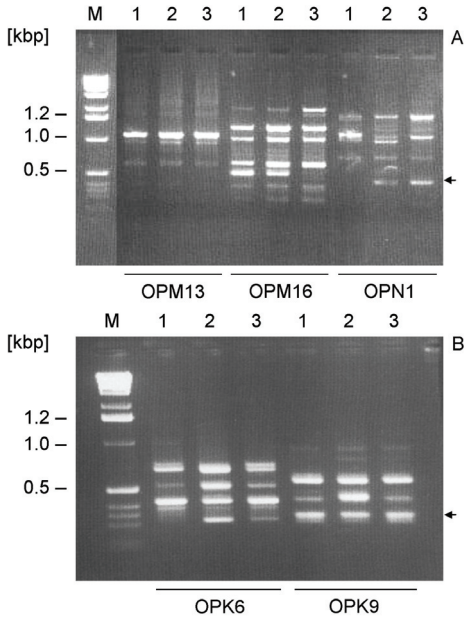

Fig. 3. Gel electrophoresis of RAPD fragments generated by primers PM13, OPM16, and OPN1 (*A*), and OPK6 and OPK9 (*B*). *Lane 1* represent intact plant, *lanes 2 and 3* represent *in vitro* produced plants, *lane M* indicated molecular mass DNA marker. Polymorphic DNA fragments are identified by an *arrow*.

(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.

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 (Figs. 2*B*, 3*A*,*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 4). The size of the 39 monomorphic bands produced by these primers ranged from 250 bp in OPK5 (Fig. 2*B*) to 1100 bp in OPM13 (Fig. 3*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 4), with a size range of 250 bp in OPK1 (Fig. 2*A*) to 1300 in OPM16 (Fig. 3*A*).

Using the primer OPK1, one polymorphic band with a molecular mass 250 bp in regenerant 2 was absent (Fig. 2*A*, Table 5), with the primer OPK4, two polymorphic bands with molecular mass 250 and 270 bp were absent in regenerant 2. Also, using the primer OPK10, three polymorphic bands with molecular mass 350, 250 and 240 bp in this regenerant were absent (Fig. 2*B*, Table 5). With the primer OPM16, two polymorphic bands with molecular mass 1200 bp in regenerant 2, and 500 bp in *in vivo* and regenerant 1 were detected (Fig. 3*A*). However, for the primer OPN1 one polymorphic band with a molecular mass 1000 bp in regenerant 2 was absent and one polymorphic band with a molecular mass 400 bp was present (Fig. 3A). From the profiles obtained with the primer OPK6 one polymorphic band with a molecular mass 250 bp in *in vivo* plant was absent (Fig. 3*B*).

RAPD variation has been reported in many studies. For example, reports have indicated the occurrence of

Table 5. Distribution and size of polymorphic bands from *in vivo* and *in vitro* produced *Gypsophila* plants (regenerants 1 and 2) using 6 primers producing polymorphic bands.

Primer	Size of polymorphic band [bp]	Distribution of polymorphic bands *in vivo* plant	reg. 1	reg. 2
OPK1	250	+	+	-
OPK4	250	+	+	-
	270	+	+	-
OPK10	350	+	+	-
	250	+	+	-
	240	+	+	-
OPM16	1200	-	-	+
	500	+	+	-
OPN1	1000	+	+	-
	400	-	+	+
OPK6	250	-	+	+

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 cultivars of garlic plants regenerated by somatic embryogenesis were subjected to RAPD analysis (Al-Zahim *et al*. 1999). They found that the frequency of variation was found to be cultivar dependent. 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.

Using the primers OPK1, OPK4 and OPK10, 6 polymorphic bands were absent in regenerant 2 but were present in *in vivo* and regenerant 1. Failure of amplification in regenerant 2 may be due to a single base change or two completely different sequences (Williams *et al*. 1990, Vierling and Nguyen 1992). However, using the primers OPM16, OPN1 and OPK6, 3 bands were recorded in both regenerants but were absent in the *in vivo* plant. On the other hand, with 6 out of 9 primers tested RAPD profiles of regenerant 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 cultivars, 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 tea plant, Singh *et al*. (2004) reported that 21 primers exhibiting amplified products gave monomorphic banding patterns. Only one primer gave a unique extra band of similar size in four plants.

In conclusion, the present investigation reveals the varied response of *Gypsophila* shoot tips to BA concentration in the culture medium. Vitrification was avoided by the high concentration of agar in the culture medium. 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.

References

Ahroni, A., Zuker, A., Rozen, Y., Shejtman, H., Vainstein, A.: An efficient method for adventitious shoot regeneration from stem segment explants of *Gypsophila*. - Plant Cell Tissue Organ Cult. **49**: 101-106, 1997.

Ahuja, S., Mandal, B.B., Dixit, S., Srivastava, P.S.: Molecular, phenotypic and biosynthetic stability in *Dioscorea floribunda* plants derived from cryopreserved shoot tips. - Plant Sci. **163**: 971-977, 2002.

Al-Zahim, M.A., Ford-Lloyd, B.V., Newbury, H.J.: Detection of somaclonal variation in garlic (*Allium sativum* L.) using RAPD and cytological analysis. - Plant Cell Rep. **18**: 473-477, 1999.

Carvalho, L.C., Goulao, L., Oliveira, C., Goncalves, J.C., Amancio, S.: RAPD assessment for identification of clonal identity and genetic stability of *in vitro* propagated chestnut hybrids. - Plant Cell Tissue Organ Cult. **77**: 23-27, 2004.

Dillen, W., Buysens, S.: A simple technique to overcome vitrification in *Gypsophila paniculata* L. - Plant Cell Tissue Organ Cult. **19**: 181-188, 1989.

Doyle, J.J., Doyle, J.L.: Isolation of DNA from fresh tissue. - Focus **12**: 13-15, 1990.

Gupta, P.K., Rao, J.K.: Molecular markers in crop improvement: Present status and future needs in India. - Plant Cell Tissue Organ Cult. **70**: 229-234, 2002.

Han, B.H., Paek, K.Y., Choi, J.K.: Micropropagation of *Gypsophila paniculata* using shoot tip culture *in vitro*. - J. korean Soc. hort. Sci. **32**: 394-400, 1991a.

Han, B.H., Paek, K.Y., Choi, J.K.: Prevention of vitrification of *Gypsophila paniculata* regenerated *in vitro*. - J. korean Soc. hort. Sci. **32**: 518-524, 1991b.

Hofmann, N.E., Raja, R., Nelson, R.L., Korban, S.S.: Mutagenesis of embryogenic cultures of soybean and detecting polymorphisms using RAPD markers. - Biol. Plant. **48**: 173-177, 2004

John, A., Webb, K.J.: Sitka spruce. - In: Bonga, J.M., Durzan, D.J. (ed.): Cell and Tissue Culture in Forestry. Pp. 30-41. Martinus Nijhoff, Dordrecht 1987.

Kevers, C., Coumans, M., Coumans-Gilles, M.F., Gaspar, T.: Physiological and biochemical events leading to vitrification of plants cultured *in vitro*. - Physiol. Plant. **61**: 69-74, 1984.

Larkin, P.J., Scowcroft, W.R.: Somaclonal variation - a novel source of variability from cell culture for plant improvement. - Theor. appl. Genet. **60**: 197-214, 1981.

Lee, S.W., Bae, J.J.: *In vitro* regeneration using leaf segment in *Gypsophila paniculata* L. Bristol Fairy. - Korean J. hort. Sci. Technol. **17**: 765-767, 1999a.

Lee, S.W., Bae, J.J.: Improvement of efficiency for multiplication of vigorous seedling by shoot cultured *in vitro* in *Gypsophila paniculata* L. - Korean J. hort. Sci. Technol. **17**: 768-769, 1999b.

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

Oliveira, C.M., Mota, M., Monte-Corvo, L., Goulao, L., Silva, D.M.: Molecular typing of *Pyrus* based on RAPD markers. - Sci. Hort. **79**: 163-174, 1999.

Prakash, S., Elangomathavan, R., Seshadri, S., Kathiravan, K., Ignacimuthu, S.: Efficient regeneration of *Curcuma amada* Roxb. plantlets from rhizome and leaf sheath explants. - Plant Cell Tissue Organ Cult. **78**: 159-165, 2004.

Qian, L.H., Zhang, Y.H., Chen, W.Y.: Study on the rooting cultivation of *Gypsophila paniculata* L.- Acta hort. sin. **27**: 226-227, 2000.

Rafalski, J., Tingey, S.: Genetic diagnostics in plant breeding: RAPDs microsatellites and machines. - Trends Genet. **9**: 275, 1993.

Rout, G.R., Das, P., Goel, S., Raina, S.N.: Determination of genetic stability of micropropagated plants of ginger showing random amplified polymorphic DNA (RAPD) markers. - Bot. Bull. Acad. sin. **39**: 23-27, 1998.

Salman, M.N.: Establishment of callus and cell suspension cultures from *Gypsophila paniculata* leaf segments and study of the attachment of host cells by *Erwinia herbicola* pv. *gypsophilae*. - Plant Cell Tissue Organ Cult. **69**: 189-196, 2002.

Salvi, N.D., George, L., Eapen, S.: Plant regeneration from leaf base callus of turmeric and random amplified polymorphic DNA analysis of regenerated plants. - Plant Cell Tissue Organ Cult. **66**: 113-119, 2001.

Schoofs, J.: Rapid propagation of fast growing woody species. - In: Baker, F.W.G. (ed.): Casava. Pp. 29-40. CAB Int., Wallingford 1992.

Shillo, R.: *Gypsophila paniculata.* - In: Halevy, A.H. (ed.): Handbook of Flowering. Vol. 3. Pp. 83-87. CRC Press, Boca Raton 1985.

Singh, M., Saroop, J., Dhiman, B.: Detection of intra-clonal genetic variability in vegetatively propagated tea using RAPD markers. - Biol. Plant. **48**: 113-115, 2004.

Snedecor, G.W., Cochran, W.G.: Statistical Methods. - Iowa State Univ. Press, Iowa 1967.

Song, R.H., Lu, J.P., Zhou, X.G., Zhu, Z.Y.: Rapid propagation of *Gypsophila paniculata* L. by tissue culture. - Acta Agr. Shanghai **12**: 71-73, 1996.

Vierling, R.A., Nguyen, H.T.: Use of RAPD markers to determine the genetic diversity of diploid wheat genotypes. - Theor. appl. Genet. **84**: 835-838, 1992.

Williams, J.G.K., Kubelik, A.R., Livak, R.J., Tingey, S.V.: DNA polymorphism amplified by arbitrary primers are useful as genetic markers. - Nucl. Acids Res. **18**: 6531-6535, 1990.

Yang, H., Tabei, Y., Kamada, H., Kayano, T., Takaiwa, F.: Detection of somaclonal variation in cultured rice cells using digoxogenin-based random amplified polymorphic DNA. - Plant Cell Rep. **18**: 520–526, 1999.

Zamorano-Mendoza, J.J., Mejia-Munoz, J.M.: *In vitro* propagation of gypsophila (*Gypsophila paniculata* L.) cv. Perfecta. - Revista-Chapingo. Ser. Hort. **1**: 67-71, 1994.

Zuker, A., Ahroni, A., Shejtman, H., Vainstein, A.: Adventitious shoot regeneration from leaf explants of *Gypsophila paniculata* L. - Plant Cell Rep. **16**: 775-778, 1997.