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In vitro mutagenesis, plant regeneration and characterization of mutants via RAPD analysis in Baby's breath *Gypsophila paniculata* L.

M. N. Barakat[*1] and Heba El-Sammak[2]

[1]Plant Genetic Manipulation and Genomic Breeding Group, Center of Excellence in Biotechnology Research, King Saud University, Riyadh, Saudi Arabia
[2]Department of Floriculture, Ornamental, Horticulture and Garden design, Faculty of Agriculture, Alexandria University, Alexandria, Egypt

***Corresponding author: mnrbarakat@yahoo.com** (On leave from Biotechnology Lab., Crop Science Department, Faculty of Agriculture, Alexandria University, Egypt)

Abstract

The objective of this study was to induce mutation in *Gypsophila paniculata* through *in vitro* mutagenesis by treating the shoot tips and lateral buds with four doses of gamma irradiation (0.25, 0.5, 0.75 and 1 Gy) and to apply RAPD analysis for the detection of genetic polymorphism among *Gypsophila* mutants and their parent. The results of analysis of variance revealed that callus induction (%), number of shoots per explant and shoot length (cm) were affected by gamma ray doses and gave highly significant differences influenced by radiation level, whereas shoot formation (%) were statistically insignificant. Radiation level × explant interaction significantly affected all studied characters except shoot formation (%) and shoot length (cm). The lateral bud explants gave significantly higher number of shoots (19.28) compared to shoot tip explants (14.68). Analysis of RAPD recognized 105 different amplification products. The genetic similarity among the ten genotypes ranged from 0.59 to 0.97. In conclusion, gamma rays irradiation can induct mutations which can be carefully acclimatized and commercially propagated under suitable condition. RAPD technique could be successfully applied to the newly *Gypsophila* variants and can differentiate mutants from their parents.

Keywords: *Gypsophila paniculata* L**,** *in vitro* mutagenesis, plant regeneration, RAPD analysis.
Abbreviations: BAP-6-benzylaminopurine; IBA-3-indole butyric acid; MS salts-Murashige and Skoog salts; NAA-1-Naphthyl acetic acid.

Introduction

Baby's breath *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* 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. Thus, there is a need to explore other more effective methods of propagation. Hence, the *in vitro* culture techniques can be the alternative for the continuous provision of plantlet stocks for large scale field cultivation. More and more medicinal plant species are now propagated via *in vitro* culture techniques (Tiwari et al., 2000; Santarém and Astarita, 2003; Malik et al., 2005; Mei, 2005). Plant tissue culture techniques have recently offered opportunities to be used in traditional medicine. Moreover, plant regeneration is essential and prerequisite for crop improvement. Recently, *In vitro* culture and plant regeneration from shoot tip and lateral bud explants of *Gypsophila paniculata* L have been reported (Barakat and El-Sammak, 2010). The application of *in vitro* mutagenesis has vast potential for increasing the available genetic variants in the years to come. Radiation has been successfully utilised for the development of new flower colour/shape mutants in Dendranthema. Therefore, induced mutagenesis through irradiation or chemical treatment has become a very important method for plant breeding, including flower breeding. By the year 2000, over 2200 mutant varieties of plants (mostly ornamental) had been released worldwide (IAEA, 2005), including 175 plant species with induced mutant varieties (Maluszynski et al., 2000). By 2005, 2335 varieties were released through mutagenesis in the world, in which ornamental crops and decorative crops are 552 varieties (IAEA, 2005). So, introduction of valuable variation through tissue culture of *Gypsophila* plant may help in programmes designed to improve characteristics of the plant. In breeding programs identification of new varieties or mutant lines is very important. This identification is particularly interesting in *Gypsophila*, because in many cases the origin of varieties is unknown. Traditionally, identification has been based on morphological characters; but development of new techniques based on DNA information has made it quicker and precise. The PCR based technique, RAPD (Random Amplified Polymorphic DNA) (Williams et al.,

Table 1. Mean square of analysis of variance for the effect of radiation level, explants and their interaction on callus induction (%), shoot formation (%), number of shoots per explant and shoot length (cm) on *Gypsophila*.

S.O.V.	d.f	Callus induction (%)[a]	Shoot formation (%)[a]	Number of shoots per explant[b]	Shoot length (cm)
Radiation level(A)	4	10849.66**	1446.11[N.S]	34.37 **	8.37 **
Explants(B)	1	496.41[N.S]	479.71[N.S]	9.90 *	15.38 **
A x B	4	1530.17*	396.25[N.S]	7.97**	3.09 [N.S]
Error	90	606.91	637.51	1.93	2.12

**: Highly Significant at 0.01 probability level. *: Significant at 0.05 probability level. [N.S]: Not significant. [a]:Data were transformed to angular transformation. [b]:Data were transformed to square root.

Table 2. Means of Gypsophila callus induction (%), shoot formation (%), number of shoots and shoot length (cm) as affected by radiation level.

Gamma ray dose (Gy)	Callus induction (%)	Shoot formation (%)	Number of shoots per explant	Shoot length (cm)
control	95.01 a	96.67 a	12.30 b	3.01 ab
0.25	83.40 ab	83.33 a	14.15 b	3.09 ab
0.5	28.32 c	75 a	4.90 c	1.80 c
0.75	90 a	98.34 a	30.55 a	2.35 b
1.0	65.00 b	90.00 a	23.00 a	3.40 a

*Means followed by the same letter(s) are not significantly different at 0.05 level of probability.

Table 3. Means of *Gypsophila* callus induction (%), shoot formation (%), number of shoots and shoot length(cm) as affected by explants.

Explants	Callus induction (%)	Shoot formation (%)	Number of shoots per explant	Shoot length (cm)
Lateral bud	75.334 a	85.998 a	19.28 a	2.34 b
Shoot tip	69.328 a	91.334 a	14.68 b	3.12 a

*Means followed by the same letter(s) are not significantly different at 0.05 level of probability.

1990) has been widely used for characterization of plant germplasm (Wilde et al., 1992; Barakat et al., 2010). The aims of the present investigation were to induce mutation in *Gypsophila paniculata* through *in vitro* mutagenesis by treating the explants with four doses of gamma radiation and to apply RAPD analysis for the detection of genetic polymorphism among *Gypsophila paniculata* mutants and their parent.

Materials and methods

Source of plant material

Small seedlings of *Gypsophila paniculata* were obtained from the 3H Company for cut flowers, Orabi Route, Ismailia, Egypt. Lateral buds and shoot tips were collected from the cutting of *Gypsophila paniculata* which were planted in the greenhouse.

In vitro mutagenesis

Effect of gamma irradiation on in vitro culture

Shoot tips and lateral buds of *Gypsophila paniculata* L. were irradiated in a gamma cell with a 60 cobalt source at the National Center of Radiation Research and Technology, Nasr City, Cairo, Egypt, with the doses of 0.25, 0.5, 0.75 and 1 Gy. Shoot tip and lateral bud explants were directly planted on Jar with medium protocol A which was the best medium protocol for shoot formation (Barakat and El-Sammak, 2010).The medium protocol A for shoot formation consisted of MS salts, 100 mg/L inositol, 0.5 mg /L nicotinic acid, 0.5 mg/L pyridoxine-HCL, 2.0 mg/L glycine, 0.1 mg/L thiamine-HCL, O.5 mg/L BAP, 0.5 mg/L NAA, 30 g/L sucrose and 7g/L agar. The medium of the root induction had similar medium of Protocol A but instead of hormones, 3.0 mg/L IBA was added to the medium.

Data of in vitro culture traits

After six weeks of incubation, four *in vitro* culture traits were recorded for each jar (1) callus induction: determined as a percentage of callus produced from each explant. (2) Shoot formation: determined as a percentage of shoots produced from each explant. (3) Number of shoots per explants. (4) Shoot length (cm) derived from the lateral bud or the shoot tip base explants.

Transfer of in vitro grown plantlets to greenhouse

After developments of shoots and roots, regenerated plants were washed with tap water to remove agar from the roots and were transplanted to small pots filled with peat moss, perlite and sand (1:1:1 v/v/v). The pots were incubated under moist conditions in the greenhouse for acclimatization in Irshad nursery, Faculty of Agriculture, Alexandria University, Alexandria, Egypt. After 2-3 weeks of hardening, plants were transferred into the greenhouse for flowering in the same place and were grown under a complete commercial production programme in the greenhouse.

Table 4. Means for radiation level × explants interaction effect on callus induction (%) and number of shoots of *Gypsophila*.

Trait	Explant	Gamma Rays Doses (Gy)				
		0.0	0.25	0.5	0.75	1.0
Callus induction (%)	Lateral bud	90.0	100.0	23.3	96.7	66.7
	Shoot tip	100.0	66.7	33.3	83.3	63.3
Number of shoots	Lateral bud	13.3	21.8	2.3	30.8	28.2
	Shoot tip	11.3	6.5	7.5	30.3	17.8

L.S.D. $_{(0.05)}$ for callus induction = 21.86
L.S.D. $_{(0.05)}$ for number of shoots = 1.2

Table 5. The *Gypsophila paniculata* cv. "Perfecta", and its variants obtained by *in vitro* mutagenesis.

Phenotype/Genotype	Explant / Treatment	Variation
Mother plant	Shoot tip/ control	Normal plant
V1	Shoot tip /0.25 Gy	Compact plant
V2	Shoot tip/0.5 Gy	leaves
V3	Shoot tip/0.75 Gy	Narrow leaves
V4	shoot tip/1.0 Gy	Top branched plant
V5	lateral Bud/0.25 Gy	Dwarf plant
V6	lateral bud/0.25 Gy	Sturdy plant
V7	lateral bud/0.5 Gy	Light green + branched
V8	lateral bud/0.75 Gy	Well branched with large leaves
V9	lateral Bud/1.0 Gy	Tall plant + light green

Statistical analysis

Data were, statistically, analyzed as CRD (Complete Randomization Design) with ten replicates. Data in percentage was subjected to arcsine transformation prior to statistical analysis (Steel and Torrie, 1980). Comparisons among means were made using the Least Significant Differences Test (LSD). The data were analyzed, using SAS program, version 6 (1985).

RAPD analysis

Plant material

PCR analysis was carried out by using the genomic DNA from the *Gypsophila paniculata* cultivar "perfecta", and its variants obtained from *in vitro* mutagenesis. The *Gypsophila paniculata* cultivar as well as its variants lines was grown in greenhouse.

DNA extraction

Frozen young leaves (500 mg) were ground to powder with pestle and mortar in liquid nitrogen. The powder was poured in to tubes, containing 9.0 ml of warm (65°C) CTAB extracting buffer (Sagahi-Maroof et al., 1984). Tubes were incubated at 65°C for 60-90 min. An additive of 4.5 ml chloroform/octanol (24:1) was used and tubes were shaken in mixer for 10 min and centrifuged for 10 min at 3200 rpm.The supernatants were pipette into new tubes and 6 ml isopropanol was added. After 60 min., the tubes were centrifuged for 10 min at 3200 rpm and the pellets obtained were put in sterile Eppendorf tubes, containing 400 µl of TE buffer of pH 8.0 (10 mM Tris – HCl , pH 8.0+ 1.0 mM EDTA, pH 8.0).

PCR amplification

Ten primers (Table 6) were obtained from Pharmacia Biotech. (Amersham Pharmacia Biotech., UK limited, HP79NA, England), and tested in the experiment to amplify the templated DNA. The volume of amplification reaction was 25 µl, each containing 1X PCR buffer with $MgCl_2$ [50 mM KCl ,10 mM tris-HCl (pH=9.0), 2 mM $MgCl_2$ and 1% Trition X-100], 200 µM of each of dNTPs, 50 PM primer, 50ng template DNA and 1.5 µl of *Taq* polymerase. Reaction mixtures were exposed to the following conditions: 94°C for 3 min, followed by 45 cycles of 1 min at 94°C, 1 min at 36°C, 2 min at 72°C, and a final 7 min at 72°C as final extension. Amplification products were visualized with DNA marker on 1.6% agarose gel with 1X TBE buffer and were detected by staining with an ethidium bromide solution for 30 min. Gels were, then distained in deionized water for 10 min and photographed on Polaroid films under U.V. light.

Data handling and cluster analysis

Data were scored for computer analysis on the basis of the presence of the amplified products for each primer. If a product was present in a genotype, it was designated as "1", if absent, it was designated as "0", after excluding the unreproducible bands. Pair-wise comparisons of *Gypsophila paniculata* cultivar

Table 6. Number of amplification and polymorphic products, using ten primers in *Gypsophila paniculata* and its variants.

Primer number	Nucleotide sequence (5' ⟶ 3')	Number of amplification[a]	Number of polymorphic[b]	Polymorphism [b/a] (%)
OPU06	ACCTTTGCGG	7	5	71.43
OPC04	CCGCATCTAC	15	8	53.3
OPA02	TGCCGAGCTG	16	11	68.75
OJ10	AAGCCCGAGG	11	3	27.27
OPH13	GACGCCACAC	9	3	33.3
Pr 7	GGTCCCTGAC	13	7	53.85
Pr 6	GAAACGGGTG	7	6	85.7
Pr 13	CAGCACCCAC	13	9	69.23
Pr 16	AGCCAGCGAA	14	8	57.14
OPE20	AACGGTGACC	7	5	71.4

Table 7. Simple Matching Coefficient of similarity matrix for *Gypsophila paniculata* CV. Perfecta" and its somaclones determined from RAPD analysis using 10 different primers and analyzed by UPGMA program.

	Parent	V1	V2	V3	V4	V5	V6	V7	V8	V9
Parent	1									
V1	0.74	1								
V2	0.72	0.70	1							
V3	0.76	0.85	0.74	1						
V4	0.76	0.82	0.74	0.91	1					
V5	0.78	0.84	0.75	0.94	0.97	1				
V6	0.75	0.81	0.73	0.88	0.88	0.90	1			
V7	0.73	0.75	0.72	0.81	0.74	0.76	0.76	1		
V8	0.64	0.64	0.59	0.67	0.61	0.63	0.62	0.65	1	
V9	0.70	0.72	0.64	0.78	0.72	0.73	0.73	0.68	0.74	1

"Perfecta", and its mutants, based on the presence or absence of unique and shared polymorphic products was used to determine similarity coefficients, according to Jaccard (1908). The similarity coefficients were then used to construct dendograms, using the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) employing the SAHN (Sequential, Agglomerative, Hierarchical and Nasted clustering) from the NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System), version 1.80 (Applied Biostatistics) program (Rohlf, 1993).

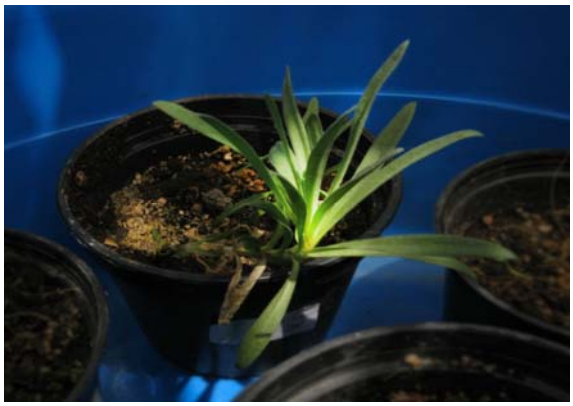
Results and discussion

Induction of mutations based on ionizing radiations is one of the major breeding approaches for plant improvement. More than 2,200 mutant varieties have been released using irradiation mutagenesis (Jain, 2005) and among them 566 represent ornamental plant. (http://www.mvd.iaea.org). A combination of *in vitro* technique and radiation induced mutagenesis has been recommended to improve cultivars of vegetatively propagated plants (Maluszynski et al*.,* 2000). Some of the selected important agronomical traits of mutant ornamental plants were flower colour, flower morphology, flowering time and resistance to abiotic and biotic stress (Das et al*.,* 2000; Misra et al., 2003). The present investigation designed to find out the best gamma ray dose linked with the best explant used on the medium protocol (A) for the induction of mutation in *Gypsophila paniculata* cultivar "perfecta" (Barakat and El-Sammak, 2010). Because of the high frequency of shoot formation on medium protocol A for *Gypsophila paniculata* cultivar "perfecta", this medium protocol enabled us to established an effecient *in vitro* mutagenesis system.

Effect of gamma irradiation on in vitro culture

The basic requirement for an effective use of mutation induction in plant breeding programmes is the analysis of radio sensitivity of the explant material (Walther and Saure, 1986). Predieri and Gatti (2001) reported that one of the first steps in mutagenic treatments is the estimation of the most appropriate dose to apply. Analysis of variance (Table 1) revealed that radiation levels caused highly significant variations in callus induction (%), number of shoots per explant and shoots length (cm), whereas explants type caused significant variations in number of shoots per explant and shoot length (cm). Radiation level × explant interaction significantly affected all studied characters except shoot formation (%) and shoot length (cm). Means of the *in vitro* traits as affected by gamma radiation levels are presented in Table (2). Shoot formation (Fig.1), though insignificantly affected by radiation level, gave high values at control and 0.75 Gy followed by 1.0 and 0.25 Gy, whereas the lowest value was recorded for 0.5 Gy radiation level. Regarding callus induction, the highest value was obtained at control (95.01 %) and was insignificantly different from 0.25Gy (83.40%) and 0.75Gy (90%). However, control and 0.25 Gy were significantly superior to 1.0 Gy (65.00%) which was, in turn, significantly higher than 0.5 Gy (28.32%). Comparisons of radiation levels on number of shoots per

A



B

Fig 1. Micropropagation of *Gypsophila paniculata* cv. "Perfecta".

A: Callus differentiated into shoots.

B: Propagated plants established on pots under green house.

explant indicated that 0.75 Gy and 1.0 Gy have significantly higher values for that character compared to control and 0.25 Gy, which were, in turn, significantly superior to 0.5 Gy. The highest value for shoot length was obtained at 1.0 Gy radiation level (3.40 cm), which was statistically similar to control (3.01cm) and 0.25 Gy (3.09 cm), followed by 0.75Gy (2.35 cm). The significantly lowest shoot length was obtained at 0.50 Gy (1.80 cm). Type of explant had insignificant effect on shoot formation and callus induction (Table 3). On the other hand lateral bud explants gave significantly higher number of shoots (19.28) compared to shoot tip explants (14.68). Meanwhile, the shoot tip explants were significantly superior to lateral bud explants in shoot length (3.12 vs. 2.34 cm). Regarding with callus induction, both explants performed similarly, at the different radiation levels (Table 4) with different magnitudes at each level, except at 0.25 Gy where lateral bud explants gave significantly higher callus induction than shoot tip explants. With regard to number of shoots per explants, both explants gave similar and highest values at 0.75 Gy. Shoot tip explant was significantly superior to lateral bud explant at 0.5 Gy radiation level (Table 4). Several studies have been conducted on the radiosensitivity of *in vitro* cultures of several crops (Hell, 1983; Walther and Sauer, 1986; Wang et al*.*, 1988; Cheng et al*.*, 1990; Shen et al*.*, 1990; Charbaji and Nabuls, 1999; Predieri and Gatti, 2000; Barakat et al., 2010). They studied the effect of gamma irradiation on *in vitro* cultures in breeding applications, with an objective of developing suitable *in vitro* mutagenic system for the induction and selection of desirable mutants.

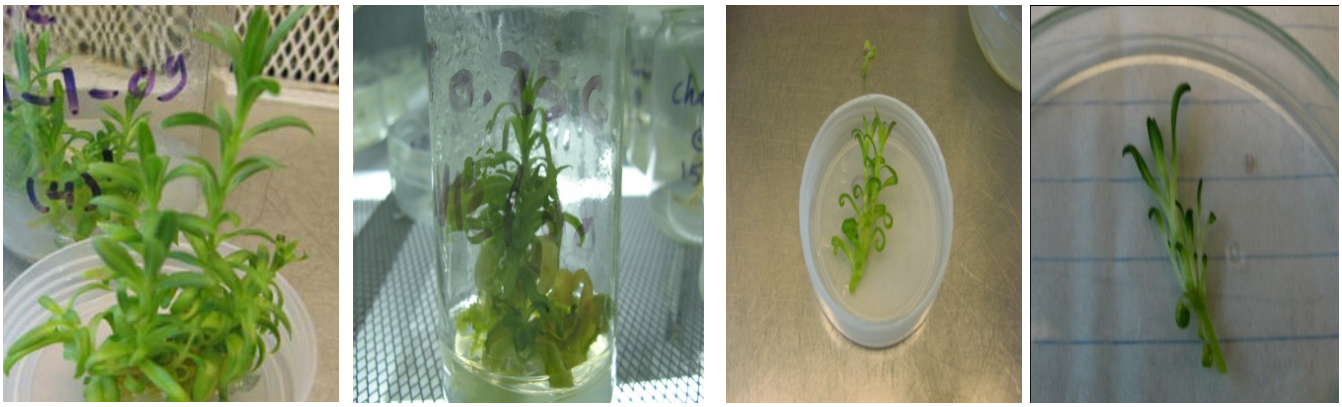
Effect of gamma irradiation on morphological characters

Plants in jars and in the greenhouse (after rooting and acclimatization) were screened for variation in the morphological characters. From the observation, there were abnormalities in leaves. Some leaves were sticked to each other (Fig.2). The number of shoots per explant increased clearly by radiation level of 1.0 Gy which gave the highest number of shoots per explant, while 0.5 Gy gave the smallest number of shoots per explant. Radiation also had a great effect on shoot length. Shoot length was higher in the radiated plants than parents. The highest value for shoot length was obtained at 1.0 Gy radiation level (3.40 cm). On the other hand, the lowest shoot length was obtained at 0.5 Gy. Nine variants were recorded (Table 5 and Fig. 2). RAPD analysis was used to find out the genetic relationship between the mother plant (*Gypsophila paniculata* cv. Perfecta) and the variants.

RAPD Analysis

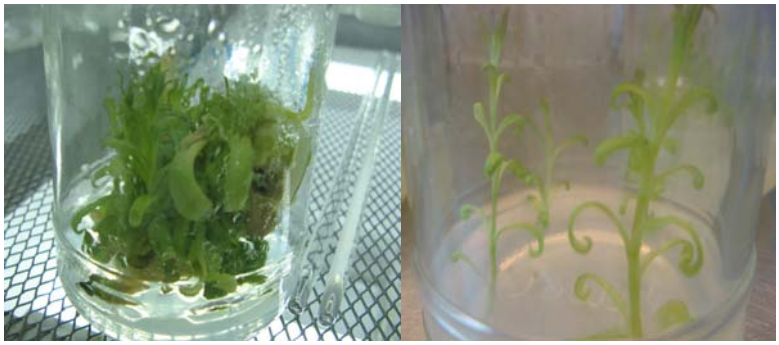
Screening for polymorphic primers in Gypsophila paniculata cv. "Perfecta" and its mutants

Ten primers were screened for their ability to amplify the genomic DNA of the *Gypsophila paniculata* cultivar "Perfecta", and its variants. The number of amplified DNA fragments ranged from 7 to 16, depending on the primer and the DNA sample with a mean value of 10.5 bands per primer (Table 6). The sizes of fragments ranged from 100 to 3000 bp. A total of 105 fragments were produced by the ten primers. Of these 105 amplified fragments, (38.1 %) were not polymorphic; whereas, the remaining bands (61.9%) were polymorphic in one or other of the ten genotypes (one cultivar and nine variants). However, Figure 3. shows the amplification profiles, generated by primer 16 across the *Gypsophila paniculata* cultivar "perfecta" and its variants. The 8 scroble bands of the primer 16 were polymorphic across the *Gypsophila paniculata* genotypes. RAPD analysis was used in ornamental breeding to characterize genotypes (Yamagishi, 1995; Scott et al*.*, 1996), and for identification of genes controlling important traits. Also RAPD markers tightly linked to the locus controlling the flower type of *Dianthus caryophyllus* were identified by Scovel et al*.* (1998). RAPD analysis has been used to characterize the propagated plants derived from *in vitro* culture of *Gypsophila paniculata* (Rady, 2006). He reported that 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, 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. Genotype identification of ornamental species by RAPD had been reported by Benedetti et al. (2001). Likewise, RAPD analysis had been used also to identify DNA



Mother plant V1: Shoot tip/ 0.25 Gy V2: Shoot tip /0.5Gy V3: Shoot tip / 0.75Gy

V4: Shoot tip / 1.0 Gy V5: Lateral bud / 0.25Gy V6: Lateral bud / 0.25Gy V7: Lateral bud / 0.5Gy



V8: Lateral bud/ 0.75 Gy V9: Lateral bud / 1.0 Gy

Fig 2. Variants of *Gypsophila paniculata* cv. "Perfecta" obtained by *in vitro* mutagenesis.

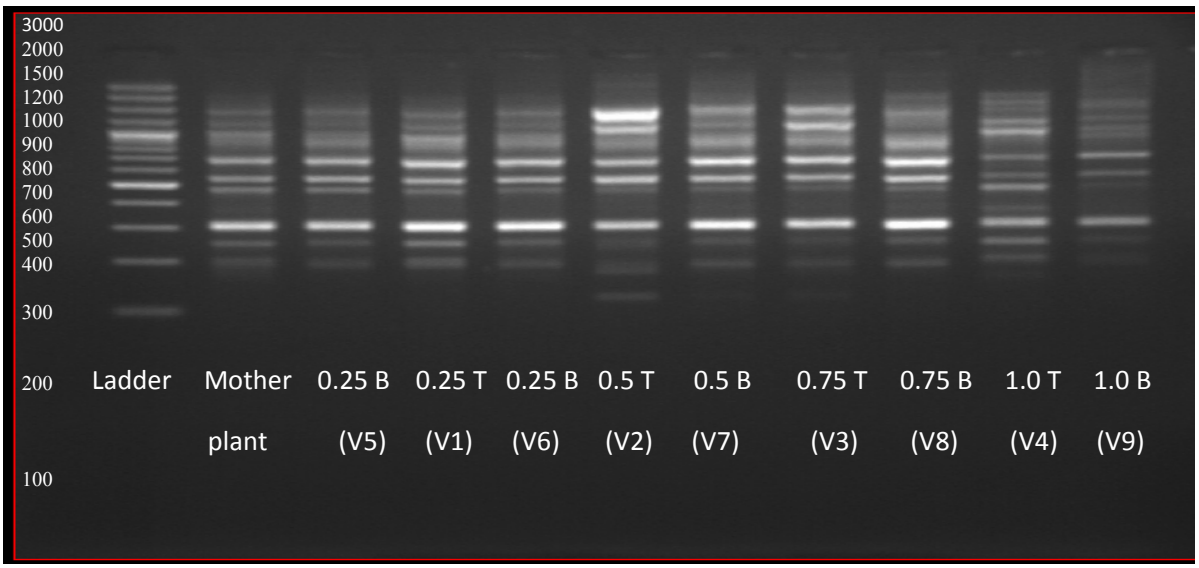


Fig 3. RAPD polymorphism in *Gypsophila paniculata* cv. "Perfecta" and its variants using primer 16. T: Shoot Tip, B: Lateral bud

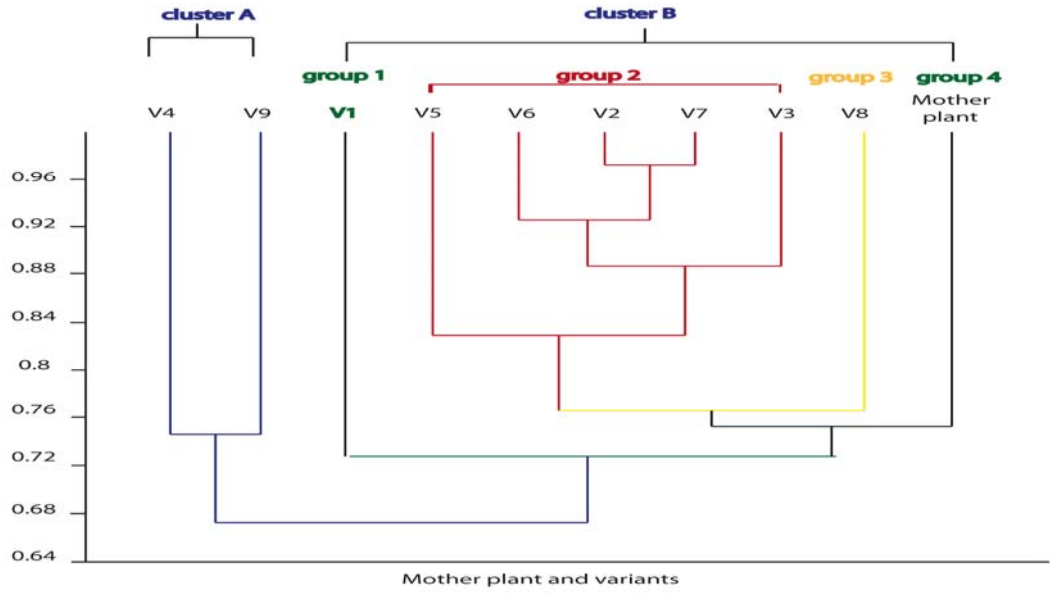


Fig 4. Phylogenic tree illustrating the relationship among the parent and its 9 somaclones / variants (v1-v9) using RAPD data and the UPGMA method of clustering to show DNA similarity

markers correlated to *Fusarium oxysporum* resistance in the greenhouse condition (Scovel et al., 2001). Recently, RAPD analysis has been used for the detection of genetic polymorphism among chrysanthemum mutants and their parent (Barakat et al., 2010). They reported that five RAPD primers were used to amplify DNA segments from the genomic DNA of chrysanthemum and its 13 somaclones. The genetic similarity among the fourteen genotypes ranged from 0.43 to 0.95. The chrysanthemum cultivar and its 13 somaclones were classified into five clusters. Williams et al. (1990) reported that polymorphism among individuals could arise through nucleotide change that prevented amplification by introducing either a mismatch at one priming site, deletion of a priming site, insertions that rendered priming sites to support amplification and insertions or deletions that change the size of the amplified product. RAPD has proved to be a very useful and rapid method to detect variation between different Gypsophila genotypes, even when not a very high number of markers have been scored. Wolff (1996) reported that the choice of the primers may be an important factor in obtaining a rapid discrimination between samples.

Cluster analysis

One of the goals of the present study was to investigate the efficiency of RAPD markers in accurately determining, the genetic relationship between somaclones and the parent. The RAPD markers, produced by ten primers, were used to construct a similarity matrix (Table 7). Simple matching coefficient, ranged from 0.59 to 0.97, suggested a broad genetic base for Gypsophila and its variants. Table (7) indicates the genetic similarity estimates of the pairwise comparisons among the *Gypsophila paniculata* cultivar" Perfecta" and its variants, based on 65 polymorphic bands. Figure 4 represents the clustering of *Gypsophila* cultivar generated by UPGMA analysis of the parent; namely, *Gypsophila paniculata* cv." Perfecta" and its nine variants. The results of characterization analysis revealed a high diversity between the cultivar and its somaclones. Two clusters (A and B) could be observed. The first cluster included the variant 4 (V4) and variant 9 (V9), while the second cluster included four groups. The first group included only the variant1 (V1). The second group included five variants i.e. V5, V6, V2, V7 and V3. The third group included the variant 8 (V8), while the fourth group included the mother plant (Figure 4). This figure shows the shortest genetic distances or the highest similarity value (0.97) between variant 2 and variant 7 (V2 and V7), whereas the highest distance or the low similarity value (0.59) was observed between the variant 1 and the variant 4 (V1 and V 4). These results indicate that RAPD technique could successfully be applied to ornamental crops. RAPD analysis in characterization of the Gypsophila and their somaclones allows the detection of mutations produced during *in vitro* culture and also, with samples taken subsequently through the entire process, to detect at which time the alteration has been produced (Martin et al., 2002). The phenotypic changes that these variations could produce must be checked subsequently in order to select those favorable phenotypic modifications that can be applied for commercial propagation. Recently, Barakat et al. (2010) reported that the similarity among the chrysanthemum cultivars and mutants varied from 0.43 to 0.95, using RAPD analysis. Mutant with different flower colour could be identified at the molecular level using RAPD technique holding promise to identify unique genes as SCAR markers. In conclusion, gamma rays irradiation can induce mutation. If desired mutations were obtained, they can be carefully acclimatized and commercially propagated under suitable condition. Radiation level affects shoot length and number of shoots per explant. The shoot length and number of shoots per explant also increased by radiation levels. 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 phenotypic characteristics which will be investigated in a further study. Also, these results suggested that by using RAPD markers, the newly *Gypsophila* variants can be easily differentiated from their parents.

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