Regeneration, *in Vitro* Mutation and Evaluation of Genetic Stability of Gardenia Somaclones *Via* Ssr Markers

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ABSTRACT

The aim of this research was to study the possibility of creating genetic differences in Gardenia plant to obtain a new variety with high economic value. To achieve this goal, three laboratory experiments were conducted as follows:

1- ***In vitro* propagation: The results indicated that the axillary buds when cultured on medium consisting of MS + 0, 5 of BA+ 0.1 mg l^{-1} of Kin. gave the highest significant results for leaves number 25.4, shoot number 3.28 and shoot length 3.1 cm. The highest significant values of root number and length 6.65 and 1.3 cm, respectively were recorded when shoots were transferred to MS medium combined with 0.75 mg l^{-1} of IBA.**

2- ***In vitro* mutation: Results indicated that the degree of variegated leaves increased by increasing the concentration of the mutagen Ethylmethanesulphonate (EMS) up to 0.4 %, but treatment with a concentration of 0.5% led to explants death or deformation of the leaves.**

3- **Molecular markers Experiment: An experiment was conducted to examine the applicability of SSR markers to discover the polymorphisms of mutations caused by EMS and their genetic relationships for the first time. Thirteen SSR primers were used. Plants were divided into five groups, namely the original plant and the mutant plants resulting from growth on the five EMS concentrations (0.0, 0.1, 0.2, 0.3 and 0.4%). Cluster analysis was done for all the genotypes under study. Results indicated that 24 bands were obtained, their size ranged between 50: 250 pb, and all of them were 100% Polymorphic. PIC values were calculated, which ranged between 0.32: 0.48 for each primer. H_0 was also calculated and the values ranged between 0.32: 0.50. Results in the present study showed the effectiveness of EMS to induce *in vitro* mutation of Gardenia and this way can be used to develop breeding programs for ornamental plants. However, the use of more than one molecular marker in this type of studies may be more useful to cover multiple regions of the genome.**

Keywords: Gardenia – Micropropagation - E.M.S - in vitro mutation - SSR markers.

INTRODUCTION

Gardenia jasminoides (Ellis) is considered one of the beautiful aromatic plants of economic value The nice perfumed panned out from the astonishing white flowers and is generally known as Gardenia or Cape jasmine. It is an evergreen and belongs to the Rubiaceae family with chromosome number = 22 (Zhichao Xu *et al*., 2020). Gardenia plant is of great economic value and usually cultivated indoors or outside in gardens. It is also counted a therapeutic plant as it has many biological activities for both *in vitro* and *in vivo* conditions. It has antidiabetic, antioxidant, anti-depression, anti-inflammatory parcels. (Xiao *et al*., 2017 and Kesavan *et al*., 2018). Among the means of developing plant species and enriching their characteristics is that genetic diversity that can be created through convinced chemical mutations to gain new plants with better characteristics. (Suprasanna *et al*., 2015; Gaber and Barakat, 2019). Ethyl Methane Sulphonate (EMS) is one of the most robust chemical mutagens used in plants. Its usage on the plant is easy and affordable to create mutations, to enrich their traits and raise its economic value. To our knowledge, there are no reports so far on the effect of EMS in Gardenia; consequently the aim of this study was to determine the prospect of applying this mutagen to create mutations in the *in vitro*- grown Gardenia moreover, evaluate the genetic differences via SSR markers.

Abbreviations: Ethylmethanesulphonate (EMS) - Murashige and Skoog (MS) -
benzyl *adenine* (BA) – Kinetin (Kin.) - 3- Indole butyric acid (IBA) -
Short sequence repeats (SSR) – Base pair (bp).

MATERIAL AND METHODS

The present work was carried out at the Tissue Culture Laboratory, Horticulture Research Institute (HRI), Agricultural Research Center (ARC), Antoniades Gardens, Alexandria,Egypt, during the period from 2015 to 2022.

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Plant material and *in vitro* culture: Leaf base explants (0. 5 cm), axillary buds and shoot tips were separated from their mother plants. Explants were sterilized externally by immersing in 70% ethanol, for 1 min. followed by immersing in 0.1% Mercuric chloride for 15 min then, washed with six changes of sterile distilled water. Explants were placed in jars (300 ml) containing 40 ml of culture medium. The medium used in the present study consisted of MS salts and vitamins (Murashig and Skoog ,1962), solidified with 0.8% w/v agar. MS media were supplemented with different concentrations of BA 0.00, 0.25, 0.50, 0.75 and 1.00 mg l^{-1}, Kin. 0.00, 0.25, 0.50, 0.75 and 1.0 mg l^{-1} and their combinations. Juvenile shoots (4 - 6 leaves) were transferred to jars containing M.S medium with different concentrations of IBA 0.00, 0.25, 0.50, 0.75 and 1.00 mg l^{-1} for root induction. Cultures were incubated in a growth chamber at 25±2°C under 16 / 8 hrs light / dark cycle. (200 lux, daylight fluorescent tubes). After development of shoots and roots, regenerated plants were washed with faucet water to remove agar from the roots and implanted to plastic pots 12 cm in size, filled with perlite and peat moss (1:1 v/v). Pots were incubated under humid conditions in the greenhouse for adaptation till blooming under a complete commercial production program. Temperatures ranged from 24 to 28°C and the humidity was over 60% throughout the acclimation period.

***In vitro* mutation treatments**: From the previous experiment, the best medium protocol linked with the best explant to produce the highest shoots number was determined. Therefore, the *in vitro* resulted shoots 3- 4 leaves were soaked in EMS solution for 0.0, 30, 60 and 90 min. then, cultured on MS medium supplemented with different concentrations of EMS (0.0, 0.1, 0.2, 0.3, 0.4, and 0.5%) for 8 weeks. After developing shoots and roots, plantlets were washed with tap water to remove agar from the roots and were transplanted into plastic pots 12 cm in diameter filled with peat moss and perlite (1: 1 v/v). Subsequently, pots were incubated under moist conditions in the greenhouse for acclimatization as mentioned above.

Statistical analysis for *in vitro* propagation and mutation experiments: Data were, statistically, analyzed in a completely randomized design with 20 replicates. All data, except the callus weight, were subjected to arcsine transformation prior to statistical analysis (Steel and Torrie, 1980). The Least Significant Differences test was used to compare the means (LSD). The SAS (1985) program was used to examine the data.

DNA extraction: Ten somaclones' immature leaves (500 mg) resulted from plantlets grew on each previously mentioned EMS dose; in addition to the main mother plants' leaves were ground to a powder in a mortar with liquid nitrogen after frozen. DNA was isolated from 60 mg of the grinded tissues using i-genomic plant DNA extraction mini kit (iNTRON Biotechnology, Inc, catalog No. 17371) according to their manufacturer instruction. DNA concentration was adjusted at 25 ng/µl.

SSR analysis: To amplify the templated DNA, thirteen primers from Pharmacia Biotech. (Amersham Pharmacia Biotech., UK Limited, HP79NA, England) were tested in this work. Each amplification solution contained 25.0 µl of 1X PCR buffer with MgCl2 [50 mM KCl, 10 mM tris –HCl (pH =9.0), 2.0 mM MgCl2, and 1% Trition X – 100], 200.0 M of each of dATP, dGTP, dCTP, and dTTP, 50.0 PM primer, 50.0 ng template DNA, and 1.5 l of Taq The reaction mixtures were heated to 94°C for 3 minutes, then subjected to 45 cycles of 1 minute at 94°C, 1 minute at 36°C, 2 minutes at 72°C, and a final 7-minute extension at 72°C. On a 1.6 percent agarose gel with 1X loading, amplification products were detected using DNA marker TBE buffer and were detected after 30 minutes of staining with an Ethidium Bromide solution. Gels were then dyed in deionized water for 10 minutes before being taken using Polaroid films under ultraviolet light.

Data handling and Cluster analysis: The existence of amplified products for each primer was used to score the data for computer analysis. After removing the un reproducible bands, a product was labelled as "1" if it was present in the sample, and "0" if it was not. According to Jaccard (1908), similarity coefficients were calculated using pair-wise comparisons of cultivars based on the presence or absence of unique and shared polymorphic products. The similarity coefficients were then used to create dendrograms using the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) and the SAHN (Sequential, Agglomerative, Hierarchical, and Nested clustering) from the NTSYS–PC (Numerical Taxonomy and Multivariate Analysis System), version 1.80 (Applied Biostatistics) program from the NTSYS–PC (Numerical Taxonomy and Multivariate Analysis (Rohlf, 1993).

RESULTS AND DISCUSSION

RESULTS

***In vitro* culture response:** Leaf base explants produced callus but failed to develop into shoots. On MS media with different combinations of the used plant growth regulators BA and Kin, shoot development from either axillary buds or shoot tips was detected in all other treatments. However, the displayed results in table (1) clearly showed that medium protocol No.17 that composed of MS + 0.5 mg l^{-1} of BA and 1.0 mg l^{-1} of Kin. combined with the axillary bud explant was superior across the all other tested protocols and

explants under study. The maximum result for leaves number was 25.4. The highest number of shoots achieved was 5.40 and the highest significant value of shoot length was 3.25. Table (2) revealed that root system was observed on medium protocol No. (4) that was consist of MS + 0.75 mg l^{-1} of IBA .It recorded the best value for percentage of roots number and length across axillary bud 1.30 and 6.65 cm, respectively. Figure (1) presents the *in vitro* propagation process results.

(C) (B) (A)

Fig.1. *In vitro* propagation of *Gardenia jasminoides*

(A) Form shoots on rooting medium.
(B) Plantlet after rooting.
(C) Whole plant regeneration established in the greenhouse.

***In vitro* mutation:** From the obtained data of the previous mentioned experiment, we could determine the best medium protocol which linked with the best explant to give the highest significant mean value of shoots number and length. Axillary bud explant was soaked for four periods 0.00,30.0,60.0 and 90.0 min., respectively in 0.5% EMS solution then, cultured on medium composed of MS + 0.50 mg l^{-1} of BA and 1.00 mg l^{-1} of kin., augmented with five concentrations of EMS. Soaking in 0.5% of EMS for 90 min. was lethal and caused explants death while the rest soaking periods caused shoots and leaves malformation. The obtained results for shoot number and length, leaves number, root number and length / explant are presented in table (3)**.** The lowest significant value of shoots number 0.38 resulted when lateral buds soaked in 0.4% of EMS for 90 min. Best results obtained when explants soaked in either 0.1 or 0.2% of EMS for 30 or 90 min. (1.33, 1.34, 1.32 and 1.33, respectively). For shoot length, the lowest significant means also resulted from soaking lateral buds in 0.5% of EMS for 30 min. (0.51 cm) while the highest data obtained (4.03 cm) when explants soaked in 0.1% of EMS for 60 min. Root system (Fig.2 A) and subsequently plant establishment (Fig.2B) was observed with different rates according to treatments under study. The highest significant means of roots number obtained when explants soaked in 0.1% EMS for either 30 or 90 min (1.41 and 1.39, respectively) while the lowest data recorded with 0.4% of EMS for 90 min. (0.34). Root length ranged from 5.15 cm (when explants directly cultured without soaking in medium containing 0.3% of EMS) to 1.05 cm when explants soaked for 90 min. then cultured on medium containing 0.4% of EMS. Different EMS treatments revealed a gradual reduction in Chlorophyll content as the concentration of EMS increased. Significant differences were found when data from EMS treatments were compared with the control. Solutions with 0.1% of EMS caused reductions of 44.7 % (26.63 x 100/ 48.1). With 0.2 % of EMS chlorophyll decreased to 56.9 %. The reduction with 0.3% of EMS reached 72.1% and was 77.8% with 0.4% of EMS when compared with plantlets obtained from the treatment of immersion in water (control = 100%). Results in this study agree with what was found by previous researchers.Deepthi and Remesh (2016) reported that Chlorophyll studies provide one of the most dependable indices of mutagenic treatments. They stated that Chlorophyll diminished proportionately with increasing doses of EMS where EMS treatment diminished the total chlorophyll content in Safflower. Moreover, the incidence of Chlorophyll mutation induced by EMS was mentioned by Natrajan and Upadhya (1964). The effect of mutation on Chlorophyll was also studied by Rodrego *et al.* (2004) and they found less Chlorophyll of Chrysanthemum plants.

(B) **(A)**

Fig.2: *In vitro* mutagenesis of *Gardenia jasminoides*

(A) Rooted *in vitro* mutant plantlets established on rooting media.
(B) Whole variant plants grow in the greenhouse.

Table1. Effect of Benzyl adenine (BA) and Kinetin (Kin.) treatment on *in vitro* culture of *Gardenia Jasmenoides*

MS media + BA and/or Kin.(mg l^{-1})	explant	Leaves number	Shoot number	Shoot length
Free MS	1	4.60±0.73	1.30±0.70	1.40±0.14
	2	2.30±0.58	0.80±0.13	0.88±0.22
0.25 BA	1	4.50±1.2	1.10±0.17	1.08±0.15
	2	4.20±1.02	1.00±0.14	1.38±0.22
0.50 BA	1	8.10±0.94	1.50±0.16	1.68±0.15
	2	3.30±0.82	0.70±0.14	0.97±0.24
0.75 BA	1	11.17±2.04	1.30±0.14	2.15±0.23
	2	7.00±1.24	1.10±0.17	1.60±0.22
1.00 BA	1	12.70±2.80	2.20±0.37	2.42±0.36
	2	9.40±1.32	1.40±0.15	2.80±0.46
0.25 Kin.	1	0.40±0.38	0.10±0.094	0.20±0.19
	2	0.40±0.38	0.10±0.94	0.10±0.94
0.50 Kin.	1	0.40±0.38	0.20±0.12	0.14±0.09
	2	0.0	0.00	0.00
0.75 Kin.	1	0.20±0.28	0.10±0.09	0.10±0.09
	2	0.0	0.00	0.00
1.00 Kin.	1	1.0±0.19	0.40±0.20	0.30±0.16
	2	1.2±0.67	0.30±0.14	0.35±0.2
0.25 BA+0.25 Kin	1	0.80±0.58	0.20±1.13	0.30±0.24
	2	0.80±0.58	0.20±0.13	0.30±0.20
0.25 BA+0.50 Kin.	1	0.0	0.00	0.00
	2	0.0	0.00	0.00
0.25 BA+0.75 Kin.	1	0.0	0.00	0.00
	2	0.0	0.00	0.00
0.25 BA+1.00 Kin.	1	0.20±0.28	0.10±0.09	0.04±0.03
	2	0.0	0.00	0.00
0.50 BA+0.25 Kin.	1	1.60±0.68	0.50±0.12	0.48±0.23
	2	1.80±0.82	0.40±0.15	0.60±0.24
0.50 BA+0.50 Kin	1	4.90±1.42	1.30±0.42	1.15±0.32
	2	3.20±0.90	0.70±0.20	0.93±0.26
0.50 BA+0.75 Kin.	1	14.0±2.88	3.50±0.67	2.04±0.40
	2	8.30±1.56	1.30±0.23	2.19±0.42
0.50 BA+1.00 Kin.	1	25.4±3.48	5.40±0.91	3.25±0.51
	2	7.80±2.2	0.80±0.24	2.15±0.60
0.75 BA+0.25 Kin.	1	6.00±4.06	1.40±0.74	1.20±0.51
	2	0.60±0.40	0.20±0.13	0.20±0.13
0.75 BA+ 0.50 Kin.	1	6.80±1.06	0.90±0.094	3.83±0.64
	2	3.80±1.66	0.40±0.15	1.30±0.55
0.75 BA + 0.75 Kin.	1	5.40±1.13	1.00±0.21	2.56±0.52
	2	7.20±1.9	1.10±0.22	2.40±0.45
0.75 BA+ 1.00 Kin.	1	3.00±1.03	0.60±0.15	1.14±0.55
	2	0.00	0.00	0.00
1.00 BA+0.25 Kin.	1	1.40±0.80	0.30±0.14	0.60±0.39
	2	0.80±0.42	0.30±0.15	0.30±0.15
1.00 BA+0.50 Kin.	1	2.80±1.36	0.70±0.31	0.73±0.39
	2	1.60±0.73	0.40±0.15	0.70±0.31
1.00BA+0.75 Kin.	1	0.00	0.00	0.00
	2	0.00	0.00	0.00
1.00 BA+1.00 Kin.	1	0.00	0.00	0.00
	2	0.00	0.00	0.00

1: Axillary bud, 2: Shoot tip

Table 2. Effect of Indole butyric acid (IBA) treatment on *in vitro* rooting of *Gardenia Jasmenoides*

MS media+ IBA(mg l^{-1})	explant	Root number	Root length
Free	1	0.00	0.00
	2	0.00	0.00
0.25	1	0.00	0.00
	2	0.20±0.13	0.15±0.10
0.50	1	0.30±0.14	1.10±0.60
	2	0.40±0.15	0.43±0.20
0.75	1	1.30±0.28	6.65±1.55
	1	0.60±0.20	1.15±0.44
1.00	1	0.00	0.00
	2	0.00	0.00

1: Axillary bud, 2:Shoot tip

Table 3. Effect of EMS treatment on *in vitro* mutation of *Gardenia jasminoides*

EMS concentration	Soaking time (min)	shoot number	shoot length	leaves number	Root number	Root length	Chlorophyll content (mg/g)*
0.0%	0.00	1.19±0.31	3.30±0.27	3.17±1.27	1.18±0.19	4.22±0.45	48.0
	30.0	1.35±0.22	3.55±0.36	3.13±1.27	1.33±0.27	4.72±0.29	48.3
	60.00	1.27±0.25	3.78±0.22	3.17±1.25	1.39±0.20	4.85±0.38	48.0
	90.0	1.34±0.26	3.92±0.30	3.32±1.26	1.39±0.20	4.20±0.31	48.1
0.1%	0.00	1.17±0.35	3.35±0.43	3.22±1.77	1.21±0.28	3.60±0.55	32.1
	30.0	1.33±0.17	3.35±0.26	3.13±1.27	1.41±0.29	4.83±1.89	31.4
	60.0	1.25±0.24	4.03±0.30	3.14±1.24	1.31±0.23	5.00±0.45	22.7
	90.0	1.34±0.26	3.85±0.30	3.31±1.27	1.39±0.20	4.35±0.30	20.3
0.2%	0.00	1.43±0.36	3.75±0.36	3.30±1.50	1.23±0.20	3.80±0.69	20.2
	30.0	1.32±0.33	3.30±0.42	3.35±1.55	1.27±0.25	3.40±0.55	20.6
	60.0	1.12±0.14	1.95±0.19	3.13±1.95	1.24±0.15	4.15±0.66	29.2
	90.0	1.33±0.41	2.45±0.43	3.27±3.07	1.02±0.20	3.15±0.64	13.0
0.3%	0.00	1.33±0.30	3.00±0.39	3.15±0.80	1.18±0.25	5.15±2.2	15.6
	30.0	1.03±0.62	2.04±0.37	3.42±2.27	1.06±0.20	3.60±0.65	11.5
	60.0	0.90±0.09	3.83±0.64	2.43±1.06	1.02±0.19	2.90±0.47	13.3
	90.0	0.88±0.20	2.56±0.52	2.03±1.13	0.88±0.20	2.65±0.50	13.2
0.4%	0.00	1.18±0.21	3.32±0.25	3.43±1.15	1.12±0.15	2.65±0.23	15.0
	30.0	0.95±0.29	2.61±0.20	3.14±1.95	1.32±0.19	2.85±0.37	9.0
	60.0	0.68±0.24	1.61±0.44	1.45±1.0	0.60±0.15	1.40±0.41	10.8
	90.0	0.38±0.25	1.05±0.51	0.99±1.60	0.34±0.20	1.05±0.51	8.0
0.5%	0.00	0.92±0.22	2.30±0.41	2.23±1.07	0.80±0.13	2.15±0.33	-
	30.0	0.58±0.25	0.51±0.51	1.25±1.06	0.50±0.16	1.25±0.40	-
	60.0	0.72±0.26	0.81±0.49	1.63±1.35	0.40±0.15	1.30±0.60	-
	90.0	0.00	0.00	0.00	0.00	0.00	-

* Data were not analyzed.

SSR analysis*:* The ability of 13 primers to amplify the genomic DNA of Gardenia and its somaclones was tested. Depending on the primer and the DNA sample, the number of polymorphic DNA fragments ranged from 1 to 5, with an average of 1.85 bands per primer (Table 4). When compared to the typical number of polymorphic bands, these values for SSR amplification are quite high.

Genetic diversity using SSR markers

Cluster analysis using SSR data grouped parent plant and their somaclones into group with Jaccard's similarity coefficient ranging from 0.00 to 0.20 (fig.3 and table 4).The highest similarity was found between somaclone from control and somaclone from 0.2% EMS (0.20) and the lowest was between control and somaclone from 0.2% EMS , 0.1% across 0.2% and 0.3% EMS (0.00). The results indicated that cluster analysis can be used to isolate mutant somaclones from apart sensitive genotypes if screen is done carefully.

Level of genetic information generated by SSR primers

The size of fragments ranged from 50 to 250 bp. A total of 24 fragments were produced by the 13 primers. 100 % were polymorphic in the five genotypes (original plant and four somaclones). However, Figure (4) shows the polymorphic profiles, generated by primer 13 across parent plant and their somaclones. The 24 scorable bands were polymorphic across the Gardenia genotypes.

Polymorphism information content (PIC) provides information on allele diversity and frequency of Gardenia, PIC differed greatly for 13 SSR markers studied in parent plant and it is somaclones in the present study. The PIC per primer ranged from 0.32 to 0.48 with an average of 0.35 (table 4). The lowest value was recorded for primers No. 2, 4, 5, 6, 7, 8, 9, 10, 11, and 13 (0.32) and the highest was recorded in primers No. 3 and 12 (0.48). Observed heterozygosity (H0) were also calculated for each primer and ranged from 0.32 to 0.50 with an average of 0.35 (table 4). The lowest (H0) was recorded for primers No. 2, 4, 5, 6, 7, 8, 9, 10, 11, and 13 (0.32) and the highest value was recorded for primer No.3 (0.50).

Table 4. The number of polymorphic products, using 13 primers in *Gardenia jasmenoides* and its somaclones

Primer number	Allele size range(bp)	No. of alleles	No. of polymorphic products	Polymorphism (%)	PIC	H0
1	50-200	3	3	100	0.40	0.42
2	50	1	1	100	0.32	0.32
3	50-100	5	5	100	0.48	0.50
4	200	1	1	100	0.32	0.32
5	50	1	1	100	0.32	0.32
6	250	1	1	100	0.32	0.32
7	50-150	2	2	100	0.32	0.32
8	50-200	2	2	100	0.32	0.32
9	50-250	2	2	100	0.32	0.32
10	200	1	1	100	0.32	0.32
11	50	1	1	100	0.32	0.32
12	50	2	2	100	0.48	0.48
13	50-150	2	2	100	0.32	0.32
Total		24	24	1300	4.56	4.56
Average		1.85	1.85	100	0.35	0.35

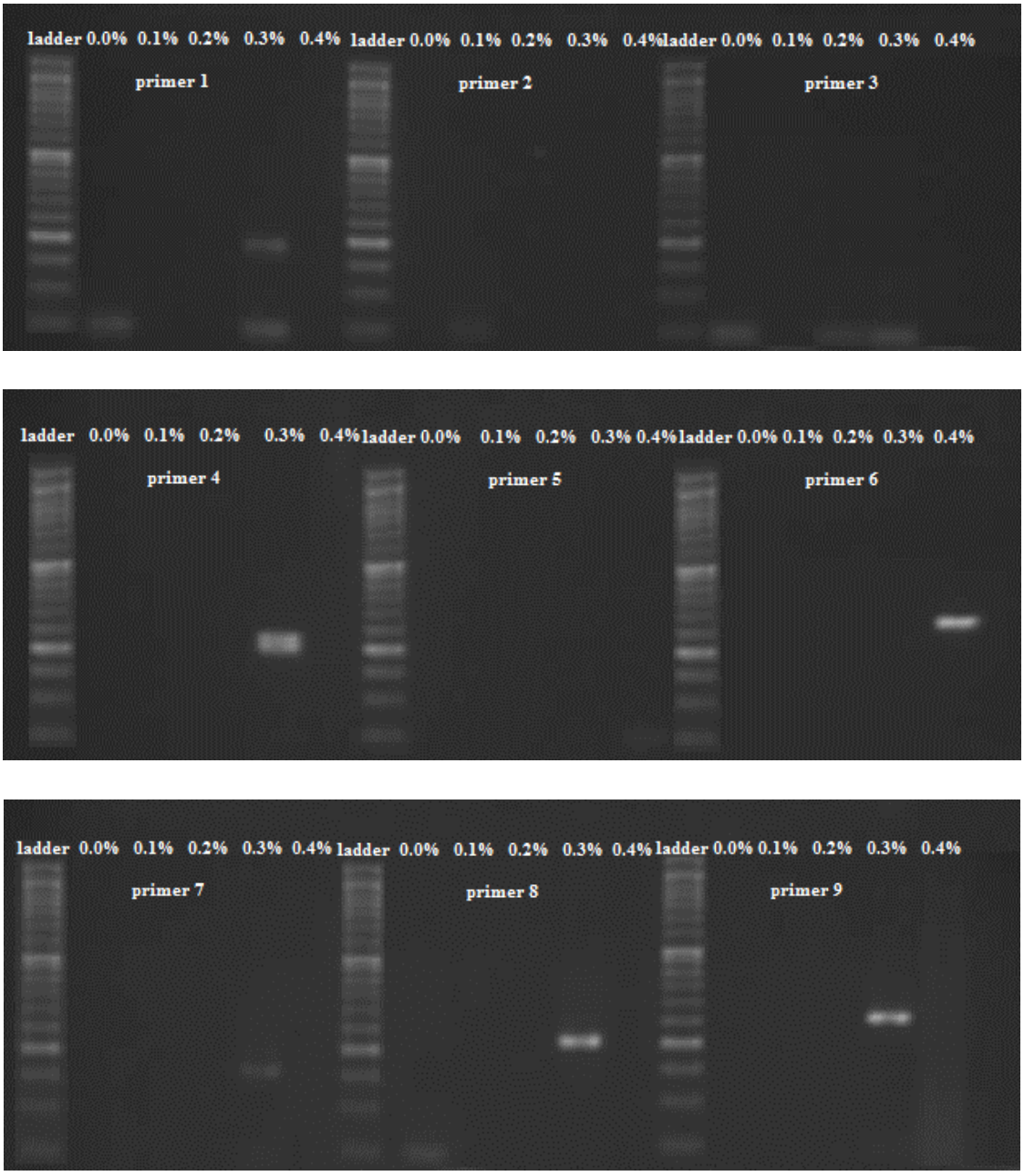

Fig. 3. Examples for the polymorphism obtained from products of SSR primers used to amplify genomic DNA purified, from Gardenia and their somaclones

Table 5. Similarity coefficients among Gardenia and its somaclones calculated according to Jaccard coefficient based on SSR markers

	Control	EMS concentration (%)		
		0.1	0.2	0.3
Control	1.00			
0.1% EMS	0.00	1.00		
0.2% EMS	0.20	0.00	1.00	
0.3% EMS	0.11	0.00	0.18	1.00

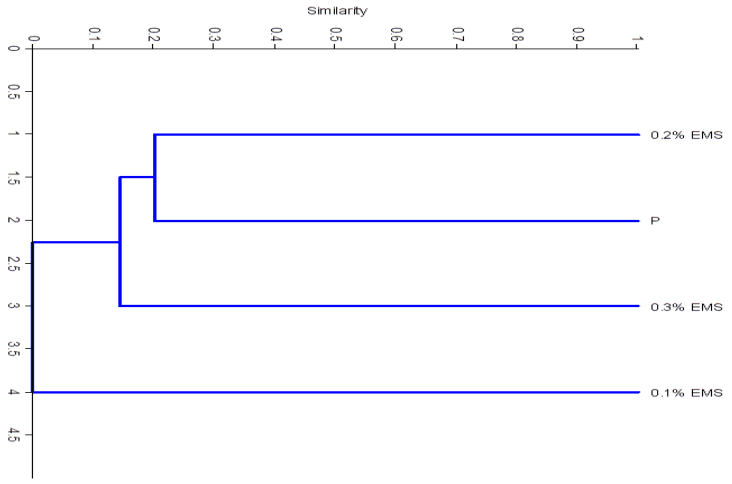

Fig. 4. Genetic distance dendrogram constructed using SSR data and UPGMA clustering method showing DNA similarity between somaclones and their parents

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الملخص العربي

الإكثار والتطفير المعملى للجاردينيا وتقييم الثبات الوراثى للسلالات الناتجة بواسطة دلائل ال SSR

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اجرى البحث بهدف دراسة مدى إمكانية إحداث إختلافات وراثية فى نباتات الجاردينيا للحصول على صنف جديد ذو قيمة إقتصادية أكبر. ولتحقيق هذا الهدف تم إجراء ثلاثة من التجارب المعملية كما يلى:

١- تجربة الإكثار المعملى: وقد أفادت النتائج أن البراعم الجانبية عند تنميتها على بيئة مكونة من 1.0 mg l^{-1} .kin of +M.S BA 0.5 mg l^{-1} + قد أعطت أفضل النتائج بالنسبة لصفات عدد الأوراق (٢٥،٤) وعدد وطول الأفرع الخضرية (٣،٢٨ – ٣،١ على التوالى). وبالنسبة لصفات عدد الجذور وطول الجذور فقد تم تسجيل أعلى القيم معنوية عند تنمية الأفرع الخضرية على بيئة مكونة من M.S + 0.75 mg l^{-1} of IBA حيث سجلت قيم (٦،٦٥و١،٣) على التوالى.

٢- تجربة التطفير المعملى : أشارت النتائج الى أن درجة تبرقش الأوراق يزيد بزيادة تركيز المطفر حتى 0.4 ولكن المعاملة بتركيز0.5 أدى الى موت الاجزاء النباتية أو تشوه الأوراق .

٣- تجربة الدلائل الجزيئية : تم عمل تجربة لفحص قابلية تطبيق دلائل ال SSR لإكتشاف تعدد أشكال الطفرات التى يسببها ال EMS وعلاقاتها الوراثية لأول مرة. حيث تم إستخدام ١٣ Primer و تم تقسيم النباتات الى خمس مجموعات و هى النبات الأصلى والنباتات الطافرة الناتجة من النمو على الخمسة تركيزات من ال EMS (٠،٠% – ٠،١%– ٠،٢% – ٠،٣% ٠،٤%). تم عمل Cluster analysis لجميع التراكيب الوراثية تحت الدراسة وأشارت النتائج الى أنه قد تم الحصول على ٢٤ band تراوح حجمها بين ٥٠: ٢٥٠ pb وكانت جميعها Polymorphic بنسبة ١٠٠% و تم حساب قيم ال PIC حيث تراوحت بين ٠،٣٢: ٠،٤٨ لكل من البادئات المستخدمة و تم أيضا حساب H_0 و كانت القيم تتراوح بين ٠،٣٢: ٠،٥٠ . على أى الأحوال فان إستخدام أكثر من واحد من الدلائل الجزيئية فى هذا النوع من الدراسة قد يكون أكثر فائدة لتغطية أجزاء متعددة من الجينوم.

الكلمات المفتاحية: الجاردينيا ، الاكثار المعملى، E.M.S، التطفير المعملى ، دلائل ال SSR.