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ORIGINAL ARTICLES

Effects Of Chemical Mutagenic Agent In Phytoconstituents On *Gardenia Jasminoids*, Ellis, Through Tissue Clture Technique

[1]Sawsan, S. Sayed, [2]Omnia, F. Abou El Lil and [2]S.S. Saleh

[1]*Ornamental Dep. Hotri. Research Institute Agric. Research Center*
[2] *Medical and Aromatic Plants Dep. Hotri. Research Institute Agric. Research Center*

ABSTRACT

The experiment has been carried out to investigate the effect of chemical mutagenic on shooting behaviour, chemical composition and rooting behaviour of *Gardenia jasminoids*, Ellis. The results revealed that the explant were cultured on control medium, colichicine and EMS with different concentrations produced the highest survival %, Shootlet number/explant, Shootlet length (cm) and leaf number. On the other hand, increasing the concentrations of sodium azid from 10 to 20 mg/l increased the inhibition of shooting behaviour to the lowest value. For chemical composition antioxidant activity and total phenols were higher in shootlet cultured in medium contain 20 mg/l colichicine. While total flavonoids were higher in shootlet cultured in medium contain 15 mg/l sodium azid. The results of leaves SDS-PAGE revealed total number of 13 bands with molecular weights (MW) ranging from about 13 to 74 KDa. The analysis of data showed no difference in banding pattern which they present in all treatments and control as common bands. But there is a big difference with banding pattern of mother plant. For rooting, the shootlet produced from control and 20 mg/l colichicine and transfer to rooting medium recorded the longest root. While shootlet produced from 15 and 20 mg/l colichicine recorded the largest number of roots.

Key words: *Gardenia jasminods***,** Ethyl Methan Sulphonat (EMS), Sodium Azid, Colichicine

Introduction

Gardenia (*Gardenia jasminoi,des* Ellis), belonging to family Rubiaceae. It is an evergreen shrub cultivated in many temperate regions. The plant bears oval-shaped fruits, which attain a reddish yellow color when ripe in late autumn and are used in traditional Chinese herbal medicine for treating a number of ailments (He *et al.* 2006). The extracts of gardenia fruit can exhibit yellow, red and blue colors, and are widely used as natural colorants in the food industry (Hendry and Houghton 1996 and Yamada *et al*. 1996). Successful utilization of *in vitro* techniques for propagation, maintenance and manipulation of plant germplasm has been possible for a great number of plant species (Suprasanna and Bapat, 2005). The ability to culture and manipulate a large number of totipotent cells provides a greater opportunity for *in vitro* selection of useful mutations at cellular level (Suprasanna and Souza, 2007). There are many molecules which can interact with DNA and cause mutations. Some of these are alkylating agents such as imines (ethylene imine), sulphates and sulphonates e.g. Ethyl Methane Sulphonate (EMS), Methan Methane Sulphonate (MMS) and other compounds such as nitrous acid, hydroxyl amine and sodium azide. EMS, MNH (N-Methan-N-nitroso urea) and ENH (N-ethyl-N-nitroso urea) are some of the commonly used mutagens in plant research (Sharma *et al*., 2000). Antioxidant compounds in food play an important role as health protecting factor. The main characteristic of antioxidant is its ability to tap free radical. Highly reactive free radical and oxygen species are present in biological systems from a wide variety of sources. Antioxidant compounds like phenolic acid, polyphenols and flavonoids inhibit the oxidative mechanisms that lead to degenerative disease (chen *et al*., 2006). Laemmli (1970) reported that protein electrophoresis becomes the technique of choice for laboratory assessment for the identification and characterization of different cultivars. He added that sodium dodecyle sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is considered a low cost, reproducible and rapid method for quantifying, comparing and characterizing proteins. Gorinstein *et al.* (1999) confirmed that electrophoretic patterns of the protein fractions are directly related to the genetic background of the protein and can be identified and used to certify the genetic makeup of wild cultivated or newly derived cereals. The experiments has been carried out to investigate the effect of chemical mutagenic on shooting behaviour, chemical position and rooting behaviour

Corresponding Author: Sawsan S. Sayed, Horticulture Institute Agriculture Research Center
PO box,: 9 El-Gmaa St. Egypt
Tel: 00202 01224637956; E-mail: sawsansamei@yahoo.com

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Material And Methods

The experiments have been conducted on *Gardenia jasminoides*, Ellis at Tissue Culture and Germplasm Conservation Researches Laboratory, Horticulture Research Institute-Agricultural Researches Center-Giza, Egypt; during the years of 2010 and 2011. The experiments has been carried out to investigate the effect of chemical mutagenic on shooting behaviour, chemical position and rooting behaviour

Plant materials:

One-month-old *in vitro* shootlet of *Gardenia jasminods* (5-7cm long), resulted from the previous experiments carried out by Sawsan *et al*., (2010), were applied as a source of vegetative materials.

Culture conditions:

The cultures were incubated in growth chamber at 24±1°C temperature and 3 K lux light intensity for 16 hours photoperiod.

Shooting behaviour:

The basal MS-medium was the formulation of Murashige and Skoog (1962) enriched with benzylaminopurine (BAP) (2.0 mg/l), sucrose (30 g) and Anachemia agar (7 g) Sawsan *et al*., (2010). This culture medium used alone (control) or which provided with different concentrations (10, 15 and 20 mg/l) for each one of colchicine, sodium azide and Ethyl Methan Sulphonate (EMS) and adjusted to pH 5.7 ±0.1 and pured at 25 ml in 200 ml-capacity glass jars before autoclaving at 121°C and 1.2 kg/cm^2 for 15 min. Two-three nodal explants (microcutting of 2-3 cm long) were aseptically sectioned and used for serving the colchicines, Ethyl Methan Sulphonate (EMS) and sodium azide treatments. In each treatment twenty five explant with five replicates were cultured for one month, the developed shootlets were aseptically divided into nodal explants and subculture into control medium (MS with 2 mg/l BAP) for four weeks.

At the end of culture period, the data of micropropagability of explants (including survival capacity of explants, number and length of the developed shootlet, number of leaves were recorded and determination of total protein, antioxidant activity, total flavonoids and phenolics).

Chemical composition:

- *Sample preparation and extraction:*

For methanolic, the extraction process was carried out by grounding (0.1 to 1.5 g) plants in a pestle with 10 ml of 80% methanol. The homogenate was filtered to obtain methanolic extraction colorless.

- *Antioxidant activity (%):*

The free radical scavenging activity of methanol extracts was measured by 1, 1-diphenyl-2- picryl-hydrazyl (DPPH) using the methods described by Band-Williams *et al*., (1995). The absorbance was measured at 517nm.

Antioxidant activity % = [(Control OD-Sample OD)/ControlOD] x 100

- *Total flavonoids:*

Total flavonoids were estimated using method of Woisky and Salation (1998) using aluminum chloride, the absorbance was measured at 420 nm.

- *Total phenols*

The total phenolics content of methanolic extract was determined according to the method described by Singleton *et al* (1999) by folin-ciocalteu reagent. The absorbance was recorded at 725nm.

Protein Electrophoresis:

Leaf storage protein:

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SDS-polyacrylamide gel electrophoresis was performed in 12 % acrylamide slab gels following the system of Laemmli (1970**)** to identify their protein profiles.

Gel Analysis:

Gels were photographed scanned, analyzed using Gel Doc Vilber Lourmat system

Rooting behaviour:

The shootlets produced from the previous treatment were transfer to rooting medium (half MS salt strength with 1 g/l activated charcoal) (Sawsan *et al*., 2010) for four weeks. In each treatment twenty five explant with five replicates were cultured. At the end of this period shootlet length (cm), leaf number, root number/shootlet and root length (cm) were recorded.

Acclimatization:

The shootlet with roots produced from the previous were culture on peat moss and sand (1:1 v:v) (Sawsan *et al*., 2010) for three months. All treatments recorded the highest percentage of survival ranged from (95-100%).

Experimental design and data analysis:

The lay-out of the experiments was designed one factor in completely randomized design and the test of LSD was used for comparison among means according to Steel and Torrie (1980).

Results And Discussion

Effect of chemical mutagenic on shooting behaviour on Gardenia jasminoides, Ellis:

As shown in Table (1) a significant fluctuation in the behaviour of shooting of explant (Viz, survival percentage, shootlet number/explant, shootlet length and leaf number/shoot) attributed to different kinds and concentrations of chemical mutagenic). The explants were cultured on control medium (only growth regulators), colichicine and EMS with concentrations (10, 15 and 20 mg/l) produced the highest survival percentage, shootlet number/explant, shootlet length (cm) and leaf number/shootlet. On the other hand adding sodium azid reduced survival percentage, shootlet number/explant, shootlet length (cm) and leaf number. Increasing the concentrations of sodium azid from (10, 15 and 20 mg/l) were increased the inhibition of shooting behaviour to the lowest value. The finding goes on line with youssef (2003) on *Yucca elephantips*

Table 1: Effect of chemical mutagenic on shooting behaviour on *Gardenia jasminoides*, Ellis

	Survival	Shoot number	Shoot length (cm)	Leaf number
Control	100.00	2.76	1.90	8.40
Col. 10 mg/l	96.00	2.19	1.40	8.40
Col. 15 mg/l	84.00	2.19	1.80	8.20
Col. 20 mg/l	96.00	2.60	1.40	7.20
S. A. 10 mg/l	40.00	1.27	0.64	5.20
S. A. 15 mg/l	36.00	1.00	0.40	3,40
S. A. 20 mg/l	16.00	0.80	0.34	2.80
EMS 10 mg/l	88.00	2.11	1.60	8.20
EMS 15 mg/l	80.00	2.08	1.60	7.60
EMS 20 mg/l	84.00	1.78	1.26	7.20
LSD 5%	26.43	0.82	0.56	2.35

Col. = Colchicine S.A.= Sodium Azid EMS= Ethyl Methan Sulphonate

Effect of chemical mutagenic on chemical composition of Gardenia jasminoides Ellis:

The results in Table (2) demonstrated that, the antioxidant activity and total phenols were higher to (83.69% and 70.26 mg/g DW) in the shootlets cultured in medium contain 20 mg/l colichicine in compared to mother plant which gave (69.64% and 15.65 mg/g dw). On the other hand, total flavoniods was higher to (0.97 mg/g dw) in shootlet cultured in medium contain 15 mg/l sodium azid as compared to mother plant which contain (0.61 mg/g DW). These results manifested the correlation between the efficince of antioxidant activity and the accumulation of phenolic compounds. In this consistent with our results, the relationship between antioxidant activity and phenolic content has been also evaluated (Sun and Ho, 2005 and Guo *et al*., 2008) where a positive correlation between antioxidant activated and phenolic content were found.

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Table 2: Effect of chemical mutagenic on chemical composition of *Gardenia jasminoides*, Ellis

	Antioxidant activity (%)	Total flavonoids mg/g DW	Total phenols mg/ g DW
Mothe plant	69.46	0.16	15.65
Control	82.11	0.28	17.19
Col. 10 mg/l	82.02	0.39	24.04
Col. 15 mg/l	83.75	0.83	70.26
Col. 20 mg/l	81.81	0.23	18.62
S. A. 10 mg/l	82.82	0.66	44.93
S. A. 15 mg/l	83.69	0.97	60.74
S. A. 20 mg/l	83.20	0.90	55.09
EMS 10 mg/l	82.68	0.49	18.69
EMS 15 mg/l	82.03	0.41	19.87
EMS 20 mg/l	81.97	0.57	30.44
LSD 5%	0.0537	0.054	0.054

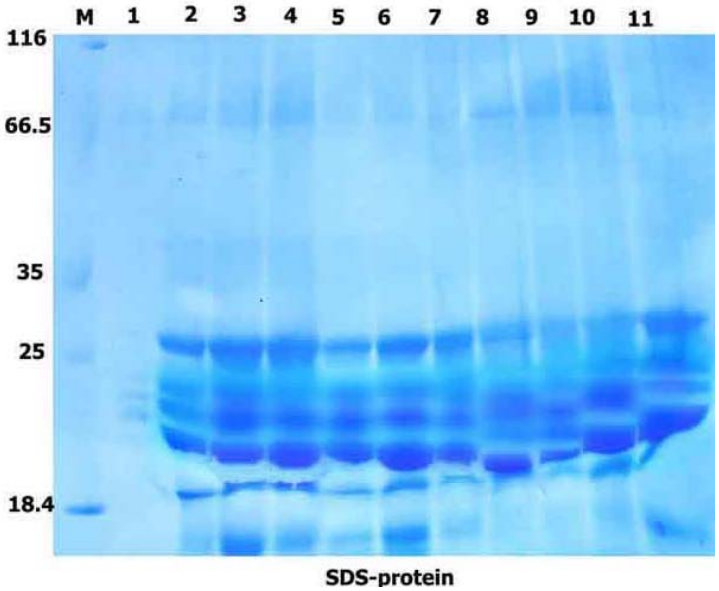
Col. = Colchicine S.A.= Sodium Azid EMS= Ethyl Methan Sulphonate

SDS-Protein electrophoresis in leaves:

The electrophoretic banding patterns of proteins extracted from leaves of different treatments of *Gardenia jasminoides*, Ellis; mother plant and control are shown in Figure (1). Their densitometric analyses are illustrated in Table (3). The presence and absence of bands were assessed with one (1) and zero (0), respectively. The results of leaves SDS-PAGE revealed a total number of 13 bands with molecular weights (MW) ranging from about 13 to 74 KDa. The analysis of data showed no difference in banding pattern which they present in all treatments and control as common bands but there is a big difference with banding pattern of mother plant. The chemical mutagenic adding to medium lead to absence some bands in some treatments. The lowest number of bands appears on shootlet culture in 15 and 20 mg/l sodium azid and 20 mg/l EMS. However, all bands are found in control (medium without chemical mutagenic).

A total of 3 common bands (monomorphic bands) were detected while, the remaining 9 bands were polymorphic with 94.23% polymorphism. Our study found that all the chemical mutagenic have a great affect on protein contents which appeared larger number of protein subunits in the treatments than the mother plant.

The data revealed that, the number of protein bands in all treatments are bigger than which in the mother plant. It is explain why the concentration of antioxidant in all treatments is higher than the mother plant. The protein bands which present in the treatments and absent in mother plant might be responsible of total flavonoids and total phenols as expressions of chemical mutagenic affects.



1. Mother plant 2. control 3. Col. 10 mg/l
4. Col. 15 mg/l 5. Col. 20 mg/l 6. S. A. 10 mg/l
7. S. A. 15 mg/l 8. S. A. 20 mg/l 9. EMS 10 mg/l
10. EMS 15 mg/l 11. EMS 20 mg/l

Fig. 1: Effect of chemical mutagenic on SDS-protein banding patterns of leaf proteins (KDa) for *Gardenia jasminoides*Ellis.

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Table 3: Effect of chemical mutagenic on SDS-protein banding patterns of leaf proteins (KDa) for *Gardenia jasminoides*Ellis as the presence and absence of bands were assessed with one (1) zero and (0).

Band No.	M.W KDa	*Gardenia jasminoides* Ellis,mother										
		Mother plant	C	T1	T2	T3	T4	T5	T6	T7	T8	T9
1	74.0	1	1	1	1	0	0	0	1	1	1	1
2	44.0	0	1	0	0	0	0	0	0	0	0	0
3	38.0	0	1	1	1	0	0	0	0	0	0	0
4	30.0	1	1	1	1	1	1	1	1	1	1	1
5	29.0	1	1	1	1	1	1	0	0	0	0	0
6	25.0	1	1	1	1	1	1	1	1	1	1	1
7	23.0	1	1	1	1	1	1	0	0	0	1	0
8	22.3	1	1	1	1	1	1	1	1	1	1	1
9	22.2	1	1	1	1	0	1	1	1	1	0	0
10	21.0	1	1	1	1	1	0	0	0	0	1	1
11	19.0	0	1	1	1	1	1	1	1	1	1	0
12	16.0	0	1	1	1	1	1	1	1	1	1	0
13	13.0	0	1	1	1	1	1	1	0	0	0	1
Total		8	13	12	12	9	9	7	7	7	8	6

Effect of chemical mutagenic on rooting stage on Gardenia jasminoides Ellis:

From the explants were cultured on medium containing chemical mutagenic and transfer to medium for rooting in Table (4). The data clear that, for shootlet length, the explant produced from medium containing 10 mg/l EMS gave the longest shootlet (3.90 cm). While the explant produced from medium containing 20 mg/l sodium azid reduced the shootlet length to the minimum length (1.74 cm).

For leaf number, all explant produced from all treatments of mutagenic except 20 mg/l sodium azid which gave the lowest number of leaves (6.60 leaf/explant) gave no significant difference on leaf number which ranged (11.00 to 14.20 leaf/shootlet).

In the root length, the explant was grown on control and 10 mg/l colchicines and transfer to rooting medium recoded the longest root length (4.10 and 4.00 cm). On the other hand the explants were cultured on medium containing 20 mg/l sodium azid produced the shortest root length (1.20 cm).

For root number, the explant which cultured on medium containing colchicines with the concentration 15 and 20 mg/l recorded the largest number of roots (10.40 and 11.60 root/shootlet). But the explants produced from medium containing 20 mg/l sodium azid depletion root number to the latest number (0.80 root/shootlet).

Table 4: Effect of chemical mutagenic on rooting behaviour on *Gardenia jasminoides*, Ellis

	Shoot length (cm)	Leaf number	Root length (cm)	Root number
Control	3.20	12.40	4.10	8.20
Col. 10 mg/l	2.80	11.00	4.00	6.60
Col. 15 mg/l	3.00	12.20	3.10	10.40
Col. 20 mg/l	3.80	13.00	3.10	11.60
S. A. 10 mg/l	2.00	11.20	2.30	9.00
S. A. 15 mg/l	2.80	11.20	2.30	3.60
S. A. 20 mg/l	1.74	6.60	1.20	0.80
EMS 10 mg/l	3.90	13.00	3.60	9.80
EMS 15 mg/l	3.00	14.20	3.00	8.60
EMS 20 mg/l	3.40	12.80	3.40	9.20
LSD 5%	1.02	3.40	1.36	3.51

Col. = Colchicine S.A.= Sodium Azid EMS= Ethyl Methan Sulphonate

Conclusion:

Using coliochicine and EMS increased shooting and rooting behaviour. For chemical composition antioxidant activity and total phenols were higher in shootlet cultured in medium contain 20 mg/l colichicine. While total flavonoids were higher in shootlet cultured in medium contain 15 mg/l sodium azid.

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