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CALLUS INDUCTION, REGENERATION AND ACTIVE PRINCIPLE PRODUCTION FROM *Urginea maritime* (L.) BAKER

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ABSTRACT

The effect of different benzyl adenine (BA) concentrations and bulb explant zones on regeneration from *Urginea maritime* bulbs was studied. Results showed that Z2 on BA conc. 4.0 mg/l gave the highest mean number of shoots after four months.

The effect of different 2, 4-D concentrations and explant zones on the production of both somatic embryos and calli showed that callus production was highest on 2.0 mg/l 2,4-D when using explants of Z2 and Z5.Highest frequency of embryo production on 0.25 mg/l 2,4-D, using Z4. The highest mean number of embryos was produced when explants of Z1 were treated with 4.0 mg/l2, 4-D after 4 months.

Using different naphthalene acetic acid (NAA) concentrations, Callus was produced with high frequency on 2.0 mg/l when Z2 explants were used and also on 4.0 mg/l when Z1and Z4 explants were used.

Mean number of embryos was highest on 1.0 mg/l NAA from Z1 explants. Analysis of variance for the effect of NAA on explant zones showed significant differences between NAA concentrations, explant zones, and also for their interaction.

Proscillaridin A (PsA) was purified from field collected material using silica gel columns and the structure was determined and identification carried out by means of EI-MS, ^{1}H-NMR and ^{13}C-NMR.

In vitro culture were initiated on different concentrations of BA and 2, 4-D. Both calli and regenerated plants were tested for the presence of cardiac glycosides using thin layer chromatography (TLC) and quantified for PsA using HPLC. In calli PsA ranged from 0.10-0.18%DW in samples positive for PsA.

Regenerated plants produced through organogenesis were positive for cardiac glycosides but negative for PsA except bulbs grown for 15 months from Z1 on media supplemented with 4.0 mg/l BA and 0.1 mg/l NAA which was 0.20% dry weight PsA.

INTRODUCTION

Many plants known to contain cardiac glycosides have long been used as arrow poisons (e.g. *Strophanthus*) or as heart drugs (e.g. Digitalis). They are used to strengthen a weakened heart and allow it to function more efficiently; the safety margin of these classes is very narrow.

White variety of *Urginea maritima* (formerly *Scilla maritima*; also it is known as *Drimia maritima*) (*Liliaceae*) with the common name squill. It is growing on sea shores around the Mediterranean, contains bufadienolides (up to 4%), principally scillaren A and proscillaridin A. Squill is not usually

used for its cardiac properties, as the glycosides have a short duration of action. Instead, squill is employed for its expectorant action. Large doses cause vomiting and a digitalis-like action on the heart (Dewick, 1997).

The main drug lanoxin (digoxin) used in Egypt for the treatment of congestive heart failure is produced from the plant *Digitalis* that is not cultivated in Egypt. The bioactive ingredient is all imported.

Bufadienolides from *Urginea maritima* may provide an alternative for digoxin, but the plant has been heavily uprooted from its natural habitat in Egypt thus making it difficult to rely upon for production of bioactive compound on any large or continuous scale.

The use of different *in vitro* cell and tissue culture techniques provide a potential and useful tool for propagating the plant and supplying bioactive compounds through mass culture technology.

In vitro, culture technology has been extensively used with *Digitalis* (Greidziak *et al.*, 1990; Theurer *et al.*, 1998 and Framm *et al.*, 2000).

Urginea indica has been studied to a lesser extent for micropropagation and for bufadienolide production *in vitro* (Jha *et al.*, 1984; Jha and Sen, 1986; Jha and Sen, 1987 and Jha, 1989).

Urginea maritima have been poorly studied for its culture *in vitro* (El Grari and Backhaus, 1987; Guimaraes and Montezuma, 1987 and Stojakowska, 1993)

The present work aimed for:

1. Studying different factors that affect organogenesis from *in vitro* cultures of *U. maritima*.
2. Production of callus cultures and testing some factors that may affect their frequency.
3. Purification and structural determination of Proscillaridin A from bulbs.
4. Testing the regenerated plants and callus produced *in vitro* for the presence of cardiac glycosides and the quantitation of Proscillaridin A in the cardiac glycoside-containing materials.

MATERIALS AND METHODS

1. Plant material

The bulbs of *Urginea maritima* (L.) Baker (*Liliaceae*) were collected from Matrouh province during July of 2001 and 2002. Identification of the species was carried out using Tackholm (1974) and Townsend *et al.* (1985).

2. Media

The basal media used was Murashige and Skoog (1962). Phytagel (2%) was used to solidify the medium. The pH of the medium was adjusted to 5.8 before autoclaving. Autoclaving was carried out at 121°C and pressure of 1.1kg/cm^2 for 20 minutes.

3. In vitro culture of Urginea maritima

1. Bulb zones description

The bulb of *U. maritima* (before sterilization) was cut into 7 zones (Fig 1). The outer fleshy leaves of the bulb were cut into 3 zones, the lower zone attached to discoid stem called Z1 (1.0 x 1.0cm^2), followed by Z2, then the

upper part called Z3. The inner bulb scales were cut into 2 zones, the lower part attached to the stem called Z4 followed by the upper part called Z5. The green leaf of the bulb was cut into 2 parts, the lower part Z6, then the upper Z7. Explant zones 2, 3,4,5,6 and 7 all were 0.5 x0.5 cm^2 in size. In preliminary work zones Z3, Z6and Z7 were always contaminated. Therefore all experiments were carried out using zones Z1, Z2, Z4 and Z5.

Fig. (1): Zones of *U. maritima* bulbs (Z1, Z2, Z3, Z4, Z5, Z6) as described in materials and methods.

2. Sterilization of fleshy leaves

The following steps were carried out:

a)Washing of fleshy leaves by tap water.
b)Immersion in ethyl alcohol 95% for 1 minute.
c)Immersion of leaves in 1 % fungicide for 1 hour with stirring.
d)Immersion of leaf parts in 5.52% NaOCl bleach solution for 30 minutes.
e)Washing the leaves with sterile ddH_2O 3 times, each 15 minutes.
Sterile leaves were kept in laminar flow in closed sterile Petri-dishes (15 cm) until culture.
Steps d and e were carried under aseptic conditions.

4. Effect of BA concentrations on Morphogenesis

Fleshy leaves of *Urginea maritima* were cut into 4 zones (Z1, Z2, Z4 and Z5) and sterilized as described before. The explants were cut from different zones and aseptically cultured. Each was placed on adaxial surface of the leaf, on full strength MS media, with different concentrations of BA (0.25 mg/l, 0.5, 1.0, 2.0 and 4.0 mg/l) in addition to 0.1 mg/l NAA. The pH of the medium was 5.8 before sterilization. The medium was supplemented with 3 % sucrose and solidified by adding 2 g/l phytagel.

Four replication plates and 5 explants/ plate were cultured for each concentration. Plates were incubated at 25°C in 8/16 light/dark photoperiod for 4 months. Three subcultures were carried out, each after one month. First two subculturs were on the same initiation medium while the 4th month was on MS medium supplemented with 0.1 mg/l BA. Cultures were microscopically examined every month to notice the formation of

morphogenesis or callus. The frequency of meristem formation was recorded and the number of meristems / explant was recorded after 3 and 4 months.

5. Effect of 2, 4-D concentrations on Embryo and Callus production

Fleshy leaves of *Urginea maritima* were cut into 4 zones (Z1, Z2, Z4 and Z5) and sterilized as described before. The explants were cut from different zones and aseptically cultured on MS media supplemented with different concentrations of 2, 4-D (0.25, 0.5, 1.0, 2.0 and 4.0 mg/l) and 0.5 mg/l BA. The pH of the medium was 5.8 before sterilization. The medium was supplemented with 3 % sucrose and solidified by adding 2 g/l phytagel.

Three replication plates and 5 explants/ plate for each concentration were carried out. Plates were incubated at 25°C in 8/16 light/dark photoperiod for 4 months. Three subcultures were carried out each after one month. The first two subcultures on the same initiation medium, while 4th month the first three concentrations were subcultured on MS medium supplemented with 0.1 mg/l BA and the other two concentrations were subcultured on MS medium supplemented with 0.1 mg/l 2, 4-D and 0.05 mg/l BA. Cultures were examined every month to notice the formation of callus and embryos. The frequency of embryo formation was recorded for all concentrations. Number of embryos / explant was recorded after 3 and 4 months.

6. Effect of NAA concentrations on callus production

Culture was carried out following the above- mentioned protocol, with changing the concentrations of NAA (0.25, 0.5, 1.0, 2.0 and 4.0 mg/l) and 0.1 mg/l BA. The pH of the medium was 5.8 before sterilization. The medium was supplemented with 3% sucrose and solidified by adding 2 g/l phytagel.

Three replication plates and 5 explants/ plate for each concentration were used. Plates were incubated at 25 °C in 8/16 light/dark photoperiod for 4 months. Three subcultures were carried out each after one month. First two subcultures on the same initiation medium while 4th month on MS medium supplemented with 0.1 mg/l NAA and 0.02 mg/l BA. Cultures were examined every month to notice the formation of roots or callus. The frequency of roots and embryo formation was recorded. Number of embryos and roots / explants was also recorded.

7. Statistical analysis

Results were statistically analyzed by a factorial analysis of variance, in completely randomized design according to the procedure by Snedecor and Cochran (1981) and means were compared by multiple range tests (Duncan, 1955).

8. Determination of cardiac glycosides

Materials:

Silica gel $60F_{254}$, **Alufolien** (E. Merck, Germany) for TLC, silica gel G (E. Merck, Germany) 70-230 mesh for CC, RP-8 Silica gel 5µm (Merck, Germany) for HPLC.

Solvent system; chloroform: methanol 9:1 (S_1). The chromatograms were sprayed with 10% sulphuric acid in methanol (Stahl, 1969) or vanillin/sulphuric acid reagent (Geeff, 1981) and heated at 120°C till maximum colour was obtained. NMR Varian mercury, VXR-300 NMR spectrometer, ^{1}H-NMR and ^{13}C-NMR spectra were recorded in CD3OD at

300 MHz and at 75 MHz, respectively. Chemical shifts were related to that of the solvent. HPLC Perkin Elmer series 200, Autosampler: Perkin Elmer series 200, Detector: Perkin Elmer series 200 UV/VIS wavelength detector. Stationary phase: Rp-8 (5 µm). Column dimension: 100x4.6 mm., Mobile phase: water – acetonitrile, gradient (10 % acetonitrile-90% acetonitrile in 136 minute), flow rate: 1.0 ml/min.

9. Isolation and purification of PsA

Chloroform extract of the fresh bulbs of *Urginea maritima* (500 g) was distilled off under reduced pressure till dryness and the residue (5 g) was fractionated on column chromatography packed with silica gel and eluted with chloroform followed by an increasing proportion of methanol. Fractions were collected, 15 ml each. Tubes 13, 14 and 15, eluted by the polarity chloroform/methanol (9:1v/v) were found to contain the same compound, then were collected together to give 1.9 g which was re-chromatographed on silica gel column chromatography, using gradient chloroform and methanol as mobile phase. Fractions eluted by the chloroform/methanol (9:1) were tested on TLC and the tubes containing the same compound were collected to give 40 mg. The residue was purified using PTLC technique. The plates were developed, using the solvent system (S_1) and the pure compound was visualized under short wave UV light at wavelength 256nm. The detected zone was scratched and extracted by methanol to give pure compound (8 mg).

10. Determination of cardiac glycosides *in vitro* cultures

1. Callus initiation

The calli were initiated on different concentrations of 2,4-D (0.25, 0.5, 1.0, 2.0 and 4.0 mg/l)+ 0.5 mg/l BA and on different concentrations of NAA (0.25, 0.5, 1.0, 2.0 and 4.0 mg/l) +0.1 mg/l BA.

2. Extraction and estimation of cardiac glycosides

A half gram dry weight of each sample of tissue culture (2 pieces of callus or 3 *in vitro* regenerated bulbs) was exhaustively extracted by hot methanol, the methanol extract was dried under reduced pressure, and the residue was then dissolved in 10 ml methanol and filtered through filter paper (0.5µm) and finally 10µl from the clear solution was injected in HPLC to determine the quantity of proscillaridin A by means of a previously prepared standard curve plotted by program called "Origin 5 Program".

RESULTS AND DISCUSSION

1. Effect of different concentrations of BA on shoots production in four zones of *U. maritima* bulb

In this experiment the effect of different BA concentrations (0.25, 0.5, 1.0, 2.0 and 4.0 mg/l) in addition to 0.1 mg/l NAA on shoot production in four zones of *Urginea maritima* bulb (Z1, Z2, Z4 and Z5) were studied. Screening was carried out every month for 4 months with subcultures as described before in materials and methods.

Explants were healthy and became green in color after the first week. Very week callus appeared in the second month. Meristems appeared on some explants at 2.0 and 4.0 mg/l BA in the first weak of the second month (Fig. 2 a).

Analysis of variance for mean number of shoots produced after 3 months using different concentrations of BA showed significant differences between BA concentrations at 5 and 1% levels and between explant zones, but at probability levels 5% only. No significant differences were obtained for the interaction of BA concentrations and explants zones. Fourth month culture results showed significant differences between BA concentrations (5 and 1%), but there was neither significant difference between explant zones nor between concentrations and explant zones.

The mean number of shoots formed after 3 month on MS medium suplemented with different concentrations of BA in 4 explants zones of *Urginea* (Table 1) showed that across the different concentrations of BA, the highest mean value was 3.37 obtained in treatment with 4.0 mg/l followed by 1.56 with 2.0mg/l, which were significantly different. Concentrations of 0.25 gave 0.93 shoots/ explants, which were not significantly different from those recorded by any of 0.5, 1.0 and 2.0 BA concentration. The lowest mean of 0.62 obtained in treatment with 0.5 and 1.0 mg/l.

Across the different 4 zones tested, there were different values of the mean number of shoots. The highest mean value was 2.55 obtained in Z4 followed with 2.4, obtained at Z1 and both values were not significantly different. Both mean values of 0.7 and 0.05 in zones Z5 and Z2, respectively, which were also not significantly different and these two mean values were significantly different with the mean values obtained by Z4 and Z1.

In Z2 highest mean number was 0.25 obtained in treatment with 4.0 mg/l. No shoots were formed in treatments 0.25, 0.5, 1.0 and 2.0 mg/l.

In Z4 highest mean number was 4.0 obtained in treatment with 4.0 mg/l.Treatment with 2.0 mg/l showed the following rank,giving 3.75. Concentrations 0.25 and 0.5 mg/l gave mean values of 2.75 and 1.25, respectively.The lowest mean value was 1.0 obtained in treatment with 1.0 mg/l.

In Z5, the highest mean number was 3.5 in treatment with 4.0 mg/l. No shoots were obtained in treatments with 0.25, 0.5, 1.0 and 2.0 mg/l.

The mean number of shoots formed after 4 month on MS medium supplemented with different concentrations of BA (Table 1) showed that across the different concentrations of BA, the highest mean value was 11.12 obtained in treatment with 4.0 mg/l which was significantly different with the other mean values. A mean number of 1.87 was obtained with 2.0 mg/l and 1.56 with 0.25 mg/l, which were not significantly different. Concentrations of 0.5 and 1.0mg/l gave 0.57 and 0.68 shoots/ explants, respectively which were not significantly different.

Across the different 4 zones tested, there were different values of the mean number of shoots. Highest value was 5.25 obtained in Z1 followed by 4.0 which obtained by Z2 and the two value were not significantly different. Both mean values of 2.65 and 0.95 in zones Z4 and Z5, respectively.

In Z1, the highest mean was 17.0 shoots/ explant obtained as a result treatment with 4.0 mg/l. A mean number of 2.75 was obtained in treatment

with 0.25 mg/l. Concentration 2.0 mg/l gave 2.5. The lowest mean value was 2.0 obtained in treatments with 0.5 and 1.0 mg/l.
In Z2, the highest mean number was 18 obtained at treatment 4.0 mg/l. Treatment with 0.25 mg/l followed giving 1.0. Concentration of 1.0 and 2.0 mg/l gave the mean value 0.75 and 0.25, respectively. No shoots were formed in treatment with 0.5 mg/l.
In Z4 highest mean number was 4.75 obtained at treatments with 4.0 and 2.0 mg/l. Concentrations 0.25 and 0.5 mg/l gave mean value of 3.5 and 0.29, respectively. No shoots were formed in treatment with 1.0.
In Z5 highest mean number was 4.75 obtained in treatment with 4.0 mg/l. No shoots were formed with concentrations 0.25, 0.5, 1.0 and 2.0 mg/l of BA.

Jha *et al.* (1984) showed that the growth of shoot primordia increased in media containing less auxins and vitamins while the rooted bulbous plantlets obtained were maintained on MS medium with 0.5% sucrose. Adventitious shoots were induced from adaxial epidermal cells of outer scales of regenerated bulbs used as secondary explants in the presence of 1 mg/l 2,4-D with a slightly higher concentration of the three MS vitamins. From each scale leaf, approximately 400 bulblets were produced in 18 weeks in liquid culture. About 90% of the plants transferred to potted soil survived.

Guimaraes and Montezuma (1987) found that the most suitable medium for shoot production contained 1 mg/l BA+ 0.1 mg/l NAA. Rooting of shoots was obtained on medium containing 0.5 mg/l NAA. Repeated subculturing increased incidence of tetraploid plants.

El Grari and Backhaus (1987) induced bulblets from bulb scales of red squill *U. maritima* cultured in the dark on a MS medium supplemented with 0.5 or 1.6 µM NAA and 0.4 or 1.3 µM BA. Bulblets induced *in vitro* were rooted in a medium containing 0.5 or 1.6 µM NAA and planted in vermiculite

Stojakowska (1993) developed a method of micropropagating *U. maritima* by adventitious shoot formation. Bulb scales and leaf fragments were used as primary and secondary explants, respectively. The most favorable media for shoot regeneration were MS supplemented with 2.0-4.0 mg/l BA or 8.0 mg/l kinetin (bulb scales) and half-strength MS containing 2.0 mg/l NAA +2.0 mg/l BA (leaf explants). No difficulties in rooting and adaptation of plants to greenhouse conditions were observed.

The present results showed that there were significant differences between BA concentrations at probability levels 5 and 1%, but there were no significant differences between explant zones. A similar conclusion was also shown for the interaction between concentrations and explant zones at probability levels 5 and 1%. Results showed that Z2 on BA concentration 4.0 mg/l gave the highest mean number of shoots (18) after four months.

Fig. (2a) Meristem initials from Z1 on MS medium supplemented with 4.0 mg/l BA after 4 months from culture initiation.

Fig. (2b) Five-month-old *de novo* regenerated meristem induced from media supplemented with 4.0 mg/l BA and placed on hormone-free media for root initiation.

Table (1) : Mean number of shoots formed after 3 and 4 months on MS medium supplemented with different concentrations of BA in four zones of squill bulbs

	Explants Zones	BA Concentration (mg/l)					Mean Zones
		0.25	0.5	1.0	2.0	4.0	
Third month	Z1	1.00	1.25	1.50	2.50	5.75	2.4a
	Z2	0	0	0	0	0.25	0.05b
	Z4	2.75	1.25	1.0	3.75	4.0	2.55a
	Z5	0	0	0	0	3.5	0.7 b
	Mean Conc.	0.93 b	0.62b	0.62b	1.56b	3.37a	
Forth month	Z1	2.75	2.0	2.0	2.5	17.0	5.25a
	Z2	1.0	0	0.75	0.25	18.0	4.0a
	Z4	3.5	0.29	0	4.75	4.75	2.65ab
	Z5	0	0	0	0	4.75	0.95 b
	Mean Conc.	1.56b	0.57b	0.68 b	1.87b	11.12a	

Third month LSD=1.6458(conc.) LSD=5.306 (Conc.)
Forth month LSD=4.022 (Zone) LSD=1.5326(zone)

2. Effect of different 2, 4-D concentrations on callus production and embryo induction in *U. maritima* bulb

In this experiment we studied the effects of different concentrations of 2,4-D (0.25, 0.5, 1.0, 2.0 and 4.0 mg/l) +0.5 mg/l BA on callus production and embryo induction in explant zones Z1, Z2, Z4 and Z5. Explants were screened every month for 4 months with three subcultures, two on the same medium and the third on a different medium, as described before in materials and methods.

Callus production occurred in the last week of first month but was not clearly differentiated to embryogenic or non-embryogenic. Embryos appeared in the third week of the second month. Embryogenic callus was creamy compact and nodular, but the non-embryogenic callus was watery in its appearance. In the third month, embryos were distinguished and usually present in clusters. From the 4 months screen, the frequency of

embryogenesis was calculated and the number of embryos on each explant was counted, from which the mean number of embryos was calculated.

The frequency (%) of total calli (TC) formation and embryogenic callus (EC) production after 4 months in culture on MS media containing different concentrations of 2,4-D is presented in Fig(3).

Throughout the different zones, the mean frequency of (TC) formation was different. Highest mean frequency was 82.66% obtained in Z2, followed by Z4 and Z1 which gave 74.62% and 71.96%, respectively. The lowest frequency was 66% in Z5. Embryogenic callus (EC) frequency was also different in the tested zones, the highest mean frequency was 48% obtained in Z4, followed by Z1 and Z2 which gave 46% and 41.98% respectively. The lowest frequency was 33.96% in Z5.

Production of total callus also was different throughout the different concentrations of 2,4-D. Highest value was 87.5% in treatment with 2.0 mg/l, followed by 86.65% at 4.0 mg/l, followed by 67.47% at 1.0 mg/l, while concentration 0.5 mg/l gave 64.94%. The lowest frequency of 62.48% was obtained on treatment with 0.25 mg/l.

The frequency of EC was also different throughout the different concentrations of 2,4-D. The highest frequency was 48.33% obtained with 0.25 mg/l, followed by 44.98% at 1.0 mg/l, whereby concentrations 2.0 mg/l gave 41.65% and 4.0 mg/l gave 42.08. The lowest frequency of 35.4% was obtained on treatment with 0.5 mg/l.

In Z1, the frequency of TC ranged from 83.3 to 53.3%. Highest value obtained by treatment with 4.0 mg/l and2.0 mg/l followed by 0.25 mg/l which gave 73.3%. Concentrations of 0.5 mg/l gave 66.6%. The lowest value was 53.3% obtained in treatment with 1.0%.

The frequency of EC was also different, thus the highest value of 50% was obtained in treatment with 1.0 and 4 mg/l, followed by 46.7% on treatment with 2.0 mg/l, while 0.25mg/l gave 43.3%. The lowest value was 40% obtained in treatment with 0.5 mg/l.

In Z2, the frequency of TC ranged from 96.7 to 53.3% in treatment with 2.0 and 4.0 mg/l, followed by 0.5 and 1.0 mg/l, which gave 83.3%. The lowest value was 53.3% obtained in treatments with 0.25mg/l. The frequency of EC was also different throughout the different concentrations of 2,4-D. The highest value (53.3%) was obtained in treatment with 1.0 mg/l followed by 46.7% in treatment with 4.0 mg/l, then 0.25 gave 43.3%. The lowest value was 33.3% obtained with 0.5 mg/l.

In Z4, the frequency of TC ranged from 83.3 to 66.6%. The highest value was obtained in treatment with 1.0 and 4.0 mg/l followed by 73.3% at 2.0 mg/l, while 0.25 and 0.5 mg/l gave 66.6%. The frequency of EC was also different throughout the different concentrations of 2,4-D. The highest value of 56.7% was obtained in treatment with 0.25 mg/l followed by 50% on treatments 4.0 mg/l. Concentration 0.5mg/l followed giving 46.7%. The lowest value was 43.3% obtained in treatment with 1.0 and 2.0 mg/l.

In Z5, the frequency of TC ranged from 96.7% to 43.3%. The highest value was obtained in treatment with 2.0 mg/l followed by 4.0 mg/l which gave 83.3%. Concentrations 0.25mg/l and 1.0mg/l gave 56.7% and 50%, respectively. The lowest value was 43.3% obtained in treatment with 0.5%.

The frequency of EC was also different throughout the different concentrations of 2,4-D. The highest value of 50% was obtained in treatment with 0.25 mg/l followed by 43.3% on treatments 2.0, and concentration 1.0 mg/l followed giving 33.3%. The lowest value was 21.6% obtained in treatment with 0.5 and 4.0 mg/l.

For the mean number of embryos produced after 3 months using different concentrations of 2,4-D with different explant zones , analysis of variance showed non-significant differences between 2,4-D concentrations, between explant zones and also the interaction between concentrations of 2,4-D and explant zones at 5 and 1% levels. Fourth month culture results showed no significant differences between 2,4-D concentrations, but there were significant differences between explant zones at probability level 5% only. No significant differences for the interaction between concentrations and explant zones at probability levels 5 and 1%.

The mean number of embryos formed after 3 and 4 months on media supplemented with different concentrations of 2, 4-D in 4 explant zones of *Urginea* are shown in Table 2.

After three months culture, results showed that across the different concentrations of 2,4-D, the highest mean value was 6.65 obtained at treatment with 0.25 mg/l followed by 2.4 obtained in 1.0mg/l and 2.16 at 4.0 mg/l which were non significantly different. Concentrations of 0.5 and 2.0mg/l gave 1.16 and 1.24 embryo/ explants, respectively which were not significantly different.

Across the different 4 zones tested, there were different values of the mean number of embryos. The highest value was 5.45 obtained in Z4. The three mean values of 2.26, 2.25 and 0.92 were obtained in zones Z1, Z2 and Z5, respectively.

In Z1, highest mean was 3.3 embryos/ explant obtained from treatment with both 1.0 mg/l. A mean number of 2.67 was obtained in treatment with 0.5 mg/l. Concentration of 2.0 and 4.0mg/l gave 2.0. The lowest mean number was 1.33 obtained in treatment 0.25 mg/l.

In Z2, the highest mean number was 6.0 obtained in treatment with 0.25 mg/l. Treatment with 1.0 and 4.0 mg/l followed giving 2.3. The concentration 2.0 mg/l gave the mean value 0.67. No embryos were formed in treatment with 0.5 mg/l.

In Z4, the highest mean number was 19.0 obtained in treatment with 0.5 mg/l. Treatment with 4.0 mg/l followed giving 3.67. The concentration 1.0 mg/l gave a mean value of 2.0. The lowest mean value was 1.3 obtained in treatment with 0.5 and 2.0 mg/l.

In Z5, the highest mean number was 2.0 in treatment with 1.0 mg/l. The concentration 2.0 mg/l gave mean value of 1.0. Treatment with 0.5 and 4.0 mg/l gave a mean value of 0.67. The lowest mean value was 0.3 obtained in treatment with 0.25.

After 4 month of culture, across the different concentrations of 2.4-D, the highest mean value was 25.34 obtained in treatment with 4.0 mg/l followed by 9.97 obtained at 0.25 mg/l and 9.57 at 1.0 mg/l which were non significantly different. Concentrations 0.5 and 2.0mg/l gave 4.74 and 8.74 embryo/ explants, respectively which were significantly different.

Across the different 4 zones tested, there were different values of the mean number of embryos. The highest value was 25.65 obtained in Z1 followed by 13.32 obtained by Z4 and the two values were significantly different. The two mean values of 5.79 and 1.92 in zones Z2 and Z5, respectively which were not significantly different at probability levels 1 and 5%.

In Z1, the highest mean was 55 embryos/ explant obtained as a result of treatment with 4.0 mg/l which was the highest mean all over the treatments and zones. A mean number of 26.3 was obtained in treatment with 2.0 mg/l. Concentration of 0.25 and 1.0mg/l gave 19.3 and 22.0, respectively. The lowest mean value was 5.67 obtained in treatment with 0.5 mg/l.

In Z2, the highest mean number was 10.3 obtained in treatment with 0.25 mg/l. Treatment with 1.0 mg/l followed giving 7.0. The concentrations 0.5 and4.0 mg/l gave the mean values 5.0 and 5.67, respectively. The lowest mean number was 1.0 obtained in treatment with 2.0 mg/l.

In Z4, the highest mean number was 40.67 obtained in treatment with 4.0 mg/l. The concentrations 0.25 and 0.5 mg/l gave mean value of 10.3 and 8.0, respectively. Treatment with 1.0 mg/l gave a mean number of 5.0. The lowest mean value was 2.67 obtained in treatment with 2.0 mg/l.

In Z5, the highest mean number was 5.0 obtained at treatment with 2.0 mg/l. The concentrations 0.5 and 1.0 mg/l gave mean values of 0.3 and 4.3, respectively. No embryos were formed at concentrations 0.25 and 4.0 mg/l of 2.4-D.

Jha *et al.* (1984) established callus cultures from bulb explants of *Urginea indica* on a modified MS basal medium supplemented with either 2 mg/l 2,4-D + 15% (v/v) coconut milk or 4 mg/l 2,4-D +2 mg/l mg/l NAA + 2 mg/l kinetin + 1 g/l yeast extract

Jha and Sen (1986) showed that one-year-old friable calli which derived from bulb scale explants, were grown in the presence of 2 mg/l 2,4-D and subcultured at 6 month intervals. They found that 40% formed embryogenic clumps between the second and fourth year after callus induction.

Jha (1989) induced friable calluses from bulb scale explants of diploid (2n=20) *U. indica*. Embryogenic calluses were formed when one-year-old friable calluses were allowed 999to remain on the high 2,4-D medium.

Our results showed that there was no significant differences between 2,4-D concentrations, but there was significant differences between explant zones at probability level 5% only. No significant differences for the interaction between concentrations and explant zones at both probability levels 5 and 1%. Callus production was highest (96.6 %) on 2.0 mg/l 2,4-D when using explants from 2 zones; Z2 and Z5. Highest frequency of embryo production was 56.7 % on 0.25 mg/l 2,4-D, using Z4 explants. The highest mean number of embryos (55.0) was produced when explants from zone Z1 were treated with 4.0 mg/l 2,4-D after 4 months.

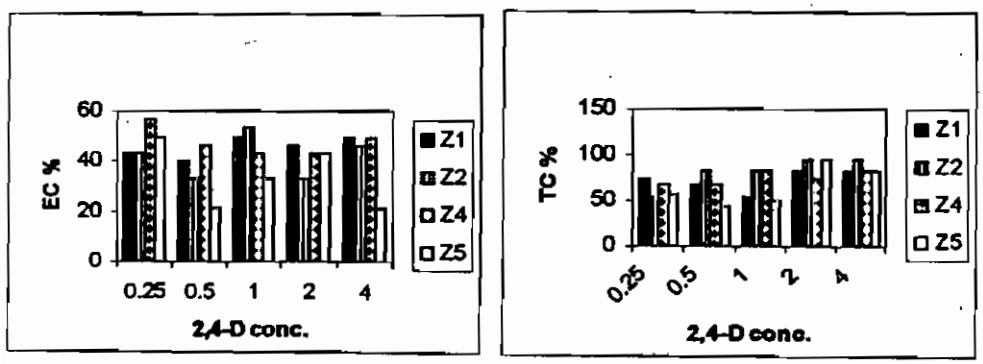


Fig (3):Frequency (%) of total callus (TC) and embryogenic callus (EC) after 4 months on MS medium containing different concentrations of 2,4-D (0.25, 0.5, 1.0, 2.0 and 4.0 mg/l) in explant zones Z1, Z2, Z4 and Z5.

Table (2): Mean number of Embryos formed after 3 and 4 months on MS medium supplemented with different concentrations of 2,4-D in four zones of squill.

	Explant Zones	2,4-D Concentration (mg/l)					Mean Zones
		0.25	0.5	1.0	2.0	4.0	
Third month	Z1	1.33	2.67	3.3	2.0	2.0	**2.26 a**
	Z2	6.0	0	2.3	0.67	2.3	**2.25 a**
	Z4	19.0	1.3	2.0	1.3	3.67	**5.45 a**
	Z5	0.3	0.67	2.0	1.0	0.67	**0.92 a**
	Mean Conc.	**6.65 a**	**1.16 a**	**2.4 a**	**1.24a**	**2.16 a**	
Forth month	Z1	19.3	5.67	22.0	26.3	55.0	**25.65 a**
	Z2	10.3	5.0	7.0	1.0	5.67	**5.79 b**
	Z4	10.3	8.0	5.0	2.67	40.67	**13.32ab**
	Z5	0	0.3	4.3	5.0	0	**1.92 b**
	Mean Conc.	**9.97 a**	**4.74 b**	**9.57 a**	**8.74 a**	**25.34a**	

Third month LSD=6.815(conc.) LSD=5.201(zone)
Forth month LSD=19.166(conc.) LSD=17.1433(zone)

3.Effect of different NAA concentrations on callus and embryo induction *U. maritima*:

The effect of different concentrations of NAA (0.25, 0.5, 1.0, 2.0 and 4.0 mg/l) +0.1 mg/l BA on callus and embryo induction in explant zones Z1, Z2, Z4 and Z5 was studied. Explants were screened every month for 4 months with three subcultures two on the same medium and the third on a different medium as described in materials and methods.

From the results we noticed that callus production occurred in the last week of first month but was not clearly differentiated to embryogenic or non-embryogenic. Embryos appeared in the third week of second month. Embryogenic callus was creamy compact and nodular, but the non - embryogenic callus was watery in its appearance. In the third month, embryos were distinguished and usually present in clusters. From the 4

months screen, we calculated the frequency of embryogenesis and count the mean number of embryos.

The frequency (%) of total callus (TC) formation and embryogenic callus (EC) production after 4 months in culture on MS media containing different concentrations of NAA is shown in Fig (4).

Throughout the different zones, the mean frequency of (TC) formation was different. Highest mean frequency was 80.6% obtained in Z4, followed by Z1 and Z2, which gave 78.6% and 77.3%, respectively. The lowest frequency was 46.6% in Z5. The frequency of embryogenic callus (EC) was also different in the tested zones. The highest mean frequency was 44.6% obtained in Z4, followed by Z1 and Z2 which gave 39.3% and 35.3%, respectively. The lowest frequency was 33.3% in Z5.

Production of total callus was also different throughout the different concentrations of NAA. The highest value was 85.83% in treatment with 4.0 mg/l, then 75.83% at 2.0 mg/l, followed by 70.8% with 1.0 mg/l. The concentration 0.25 mg/l gave 61.6%, while the lowest frequency of 59.95% was obtained in treatment with 0.5 mg/l.

The frequency of EC was also different throughout the different concentrations of NAA. The highest frequency was 46.68% obtained with 4.0 mg/l, followed by 46.23% at 1.0 mg/l. The concentration 0.5 mg/l gave 40.85% and 0.25mg/l gave 34,17. The lowest frequency of 33.7% was obtained on treatment with 2.0 mg/l.

In Z1, the frequency of TC ranged from 96.7 to 66.6%. The highest value was obtained by treatment with 4.0 mg/l followed by 1.0 mg/l which gave 83.3%. The concentrations 0.25 mg/l and 2.0 mg/l gave 73.3%. The lowest value was 66.6% obtained in treatment with 0.5%.

The frequency of EC was also different. Thus the highest value of 50% was obtained in treatment with 4 mg/l followed by 43.3% in treatment 1.0 mg/l, then 0.25 and 0.5mg/l which gave 40% and 36.7%, respectively. The lowest value was 26.6% obtained in treatment with 2 mg/l.

In Z2, the frequency of TC ranged from 96.7 to 66.6%. The highest value was obtained in treatment with 2.0 mg/l and 4.0 mg/l which gave 83.3%. The concentration 1.0 mg/l gave 73.3%. The lowest value (66.6%) was obtained in treatment with both 0.25 and 0.5mg/l. The frequency of EC was also different throughout the different concentrations of NAA. The highest value of 46.7% was obtained in treatment with 0.5 mg/l followed by 36.7% on 4.0 mg/l then 2.0 mg/l gave 33.3%. The lowest value was 30% obtained in treatments with either 0.25 or 1.0 mg/l.

In Z4, the frequency of TC ranged from 96.7 to 66.6%. The highest value was obtained by treatment with 4.0 mg/l followed by 1.0 and 2.0 mg/l which gave 83.3%. The concentration 0.5mg/l gave 73.3%. The lowest value was 66.6%, obtained in treatment with 0.25%. The frequency of EC was also different throughout the different concentrations of NAA. The highest value (50%) was obtained in treatment with 4 mg/l followed by 46.7% in treatments with 0.5 and 1.0 mg/l. The concentration 2.0mg/l gave 43.3%. The lowest value was 36.7%, obtained in treatment with 0.25 mg/l.

In Z5, the frequency of TC ranged from 66.6% to 33.3%. The highest value was obtained by treatment with 4.0 mg/l followed by 2.0 mg/l which

gave 50%. The concentrations 0.25mg/l and 1.0mg/l gave 40% and 43.3%, respectively. The lowest value was 33.3% obtained in treatment with 0.5 mg/l. The frequency of EC was also different throughout the different concentrations of NAA. The highest value of 50% was obtained in treatment with 4 mg/l followed by 33.3% on treatment with 0.5 followed by 2.0mg/l, which gave 31.6%. Treatment with 0.25mg/l gave 30%. The lowest value was 21.6% obtained in treatment with 1.0 mg/l.

Both the third and fourth month analysis of variance for the mean number of embryos produced from different explant zones on NAA treatments, showed significant differences between NAA concentrations, explant zones, and also for the interaction between concentrations of NAA and explant zones, at both probability levels 5 and 1%.

The mean number of embryos formed after 3 month on MS medium supplemented with different concentrations of NAA in 4 explant zones of *Urginea* (Table 3) showed that across the different concentrations of NAA the highest mean value was 1.16 obtained in treatment with 4.0 mg/l followed by 1.15 obtained at 1.0mg/l and 0.97 at 0.25mg/l which were non-significantly different. Concentrations 0.5 and 2.0mg/l gave 0.49 and 0.72 embryo/ explants, respectively and were not significantly different.

Across the different 4 zones tested, there were different values of the mean number of embryos. Highest value was 1.64 obtained in Z1. The three mean values 0.71, 1.18 and 0.06 ere shown with zones Z2, Z4 and Z5, respectively.

In Z1, the highest mean number was 3.3 embryos/ explants obtained from treatment with both 0.25 mg/l and 4 mg/l which was the highest mean all over the treatments and zones. A mean number of 1.0 was obtained on treatment with 1.0 mg/l. Concentrations of 0.5 and 2.0 mg/l gave 0.3.

In Z2, the highest mean number was 1.3 obtained in treatment 1.0mg/l. Treatment with 0.5 mg/l followed giving 1.0. Concentration 4.0 mg/l gave the mean value 0.67. The lowest mean number was 0.3, obtained with two treatments 0.25 and 2.0 mg/l.

In Z4, the highest mean number was 2.3 obtained in treatment with 1.0 mg/l. The second highest mean was 2.0 obtained in treatment with 2.0 mg/l. Concentrations 0.5 and 4.0 mg/l gave a mean value of 0.67. The lowest mean value was 0.3 obtained in treatment with 0.25 mg/l.

In Z5, concentration 2.0 mg/l gave mean value of 0.3 whereas no embryos were formed with all other concentrations.

The mean number of embryos formed after 4 month on MS medium supplemented with different concentrations of NAA (Table 3) showed that across the different concentrations of NAA, the highest mean value was 11.49 obtained in treatment with 1.0 mg/l followed by 10.74 obtained at 4.0mg/l and 8.49 at 0.5mg/l which were significantly different. Concentrations 0.25 and 2.0mg/l gave 2.06 and 1.99 embryo/ explants, respectively which were significantly different.

Across the different 4 zones tested, there were different values of the mean number of embryos. The highest value was 12.85 obtained in Z4. The three mean values of 12.18, 3.05 and 0.52 were obtained with zones Z1, Z2

and Z5, respectively. These values were significantly different at probability levels 1 and 5%.

In Z1, the highest mean was 31 embryos/ explant obtained from treatment with 1.0 mg/l which was the highest mean all over the treatments and zones. A mean number of 17.67 was obtained on treatment with 4.0 mg/l. Concentrations 0.25 and 2.0mg/l gave 7.67 and 3.3, respectively. The lowest mean value was 1.3 obtained in treatment 0.5 mg/l.

In Z2, the highest mean number was 5.67 obtained in treatment with 4.0mg/l. Treatment with 0.5 mg/l followed giving 3.67. Concentrations 2.0 and1.0 mg/l gave the mean values 1.0 and 0.67, respectively. The lowest mean number was 0.3 obtained in treatment 0.25 mg/l.

In Z4, the highest mean number was 29 obtained in treatment with 0.5 mg/l. The second highest mean was 19.3 obtained in treatment 4.0 mg/l. Concentration 1.0 mg/l gave a mean value of 14.Treatment with 2.0 mg/l gave1.67.The lowest mean value was0.3,obtained in treatment with0.25 mg/l.

In Z5, the highest mean number was 2.0 obtained in treatment with 2.0 mg/l. Concentrations 1.0 and 4.0 mg/l gave a mean value 0.3. No embryos were formed with concentrations 0.25 and 0.5 mg/l of NAA.

Guimaraes and Montezuma (1987) cultured hypocotyl explants of *Urginea maritima* (L.) Baker on MS medium supplemented with NAA and BA. BA in combination with NAA had a marked synergistic effect on shoot induction but an antagonistic effect on callus and root initiation.

Jha *et al.* (1984) established callus cultures from bulb explants of a high cardiac bufadienolides yielding diploid *Urginea indica* on a modified MS basal medium supplemented with either 2 mg/l 2,4-D + 15% (v/v) coconut milk or 4 mg/l 2,4-D +2 mg/l mg/l NAA + 2 mg/l kinetin + 1 g/l yeast extract.

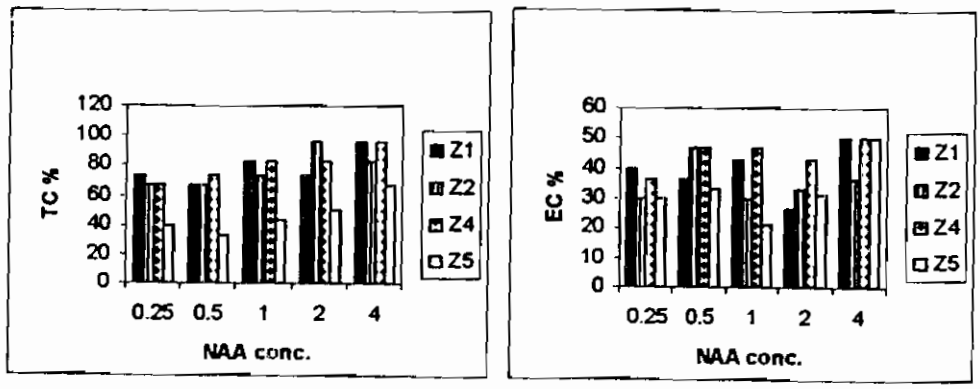


Fig (4) Frequency (%) of total callus (TC) and embryogenic callus (EC) after 4 months on MS media containing different concentrations of NAA in explant zones Z1, Z2, Z4 and Z5.

Our results showed that there were significant differences between NAA concentrations, explant zones and also for the interaction between concentrations of NAA and explant zones, at probability levels 5 and 1%.

Callus was produced with highest frequency 96.7% on 2.0 mg/l NAA when Z2 explants were used and also on 4.0 mg/l when Z1 and Z4 explants were used. Embryo mean number was highest on 1.0 mg/l NAA from Z1 explants.

Table (3): Mean number of roots formed after 3 and 4 months on MS medium supplemented with different concentrations of NAA in four zones of squill bulbs.

	Explant Zones	NAA concentration (mg/l)					Mean Zones
		0.25	0.5	1.0	2.0	4.0	
Third month	Z1	3.3	0.3	1.0	0.3	3.3	1.64a
	Z2	0.3	1.0	1.3	0.3	0.67	0.71c
	Z4	0.3	0.67	2.3	2.0	0.67	1.18b
	Z5	0	0	0	0.3	0	0.06d
	Mean Conc.	0.97a	0.49b	1.15a	0.72ab	1.16a	
Forth month	Z1	7.67	1.3	31	3.3	17.67	12.18b
	Z2	0.3	3.67	0.67	1.0	5.67	3.05c
	Z4	0.3	29	14	1.67	19.3	12.85a
	Z5	0	0	0.3	2.0	0.3	0.52d
	eanConc.	2.06 d	8.49 c	11.49 a	1.99d	10.74b	

Third month LSD=0.3985 (conc.) LSD=0.3564(zone)
Forth month LSD=0.5209(conc.) LSD=0.465(zone)

4.Purification and structural analysis of Proscillaridin A from *Urginea maritima* bulbs

The NMR and MS data of the isolated pure compound were in complete accordance with the published data of proscillaridin A (Kopp and Danner, 1983).

5. Determination of cardiac glycosides in *U. maritima in vitro* cultures

All samples of *in vitro* cultures were examined on TLC using chloroform: methanol (9:1v/v) as developing system and sprayed with spray reagent acetic anhydride followed with conc. sulphuric acid.

All calli induced from different bulb explants at different culture ages were negative for cardiac glycoside except those as illustrated in table 4.

6. Quantitative estimation of PsA for *in vitro* culture material

1. From callus materials

These results showed that some cultures of Z1 and Z2 of 5-months-old were positive for cardiac glycosides as determined using TLC and the same cultures gave the same results for PsA as determined using HPLC. Concentrations of PsA ranged from 0.1082 at Z1 on a media supplemented with 4.0 mg/l 2, 4-D with 0.5 mg/l BA after 5 months age, whereas Z2 gave 0.1728 on a media supplemented with 0.25 mg/l 2, 4-D with 0.5 mg/l BA after

5 months age. Concentration 0.1341 was given on a media supplemented with 0.5 mg/l 2, 4-D with 0.5 mg/l BA after 5 months age then finally conc. 0.1873 at Z2 on media supplemented with 1.0 mg/l 2, 4-D with 0.5 mg/l BA after 5 months age.

Jha *et al.* (1991) revealed that shoot forming cultures showed the presence of trace amounts of PsA, while bufadienolides were undetectable not only in undifferentiated calli and cell suspensions but also in rhizogenic calli and during various stages of embryoid development.

Our results showed that there is a detectable amount of PsA in calli produced on media supplemented with different concentrations of 2,4-D and the best result was obtained with those initiated from Z2 on medium supplemented with 1.0 mg/l 2,4-D. No reports in the literature for the production of PsA from calli were produced from bulb explants of *U. maritima*.

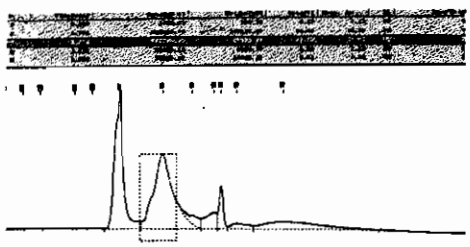
2. From plants regenerated through organogenesis:

All bulbous plants produced by organogenesis gave negative results for PsA except bulbs grown after 15 months age from Z1on media supplemented with 4.0 mg/l BA and 0.1 mg/l NAA, which gave 0.2083 % DW PsA (Table 4).

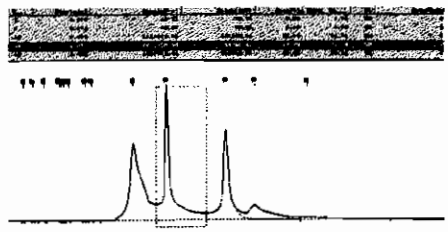
Sumita Jha *et al.* (1991) showed that all bulbous plants derived from shoot buds or from somatic embryoids were found to produce both PsA and ScA. Both bufadienolides were detected in newly formed bulblets as well as in maturing bulbs. The content of PsA and ScA increased with the age of the bulbs. In one month old bulblets, the PsA and ScA contents were 0.011% and 0.009%, respectively in diploid; 0.002% in triploid and 0.004% and 0.013% in tetraploid. In one year old bulblets, the PsA and ScA contents were 0.043% and 0.024%, respectively in diploid; 0.013% and 0.012% in triploid and 0.15% and 0.21% in tetraploid.

Stojakowska (1990) found that shoot primordia and adventitious shoots (up to 0.5 cm long) synthesized small amounts of bufadienolides (proscillaridin A content up to 0.02%). That level remained unchanged throughout the period when unrooted shoots were growing in the dark. When the shoots were transferred to a hormone-free medium in the light (for 16 h) for rooting, content of proscillaridin A in the rooted plants after 12 weeks increased up to a level comparable to that in plants from the greenhouse (0.06% DW of whole plant). Bulbs and roots contained 0.06% and 0.10% of proscillaridin A, respectively. This level showed no tendency to increase with the age of plants.

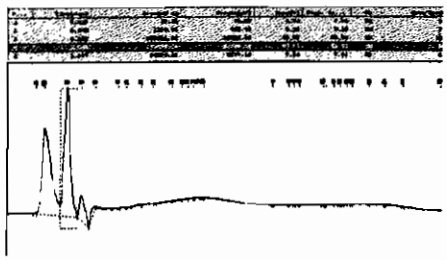
Our results showed that PsA was detected *in vitro* regenerated bulblets produced after 15 months from Z1 on medium supplemented with 4.0 mg/l BA and the content of PsA was 0.20% DW, whereas Sumita Jha *et al.* (1991) detected PsA in one year old bulblets and its content was 0.15% DW from tetraploid bulb. Stojakowska (1990) also showed that the content of proscillaridin A in the rooted plants after 12 weeks increased up to a level comparable to that in plants from the greenhouse (0.06% DW of whole plant). Bulbs and roots contained 0.06% and 0.10% of proscillaridin A, respectively.



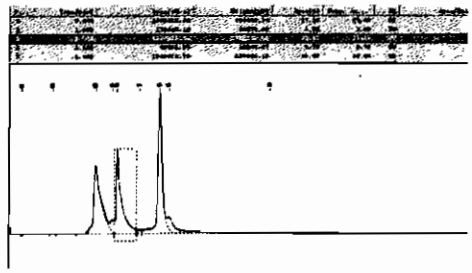
from Z1 by 2,4-D (4.0 mg/l).



from Z2 by2,4-D (0.25 mg/l).



from Z2 by 2,4-D (0.5 mg/l).



from Z2 by 2,4-D (1.0 mg/l).

Fig. (5) : Chromatogram of callus induced after 5 months.

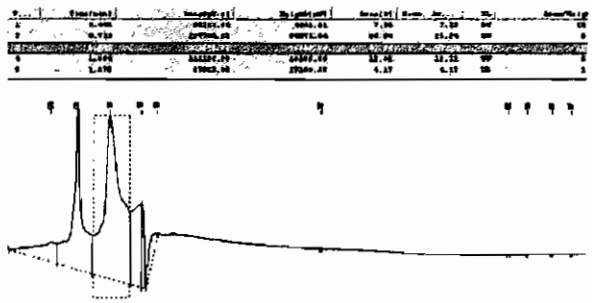


Fig. (6): Chromatogram of shoots produced after 15 from Z1 by BA (4.0mg/l).

Table (4): Concentration Of PsA % from calli and regenerated plants different bulb explants at different culture ages.

	Sample No.	Months	Zones	Growth regulator Conc. mg/l	Cardiac glycoside	Conc. % of PsA
callus	1	5	Z1	2,4-D = 0.25	-	
	2			0.5	-	
	3			1.0	-	
	4			2.0	-	
	5			4.0	+ ve	0.1082
	6		Z2	2,4-D = 0.25	+ve	0.1728
	7			0.5	+ve	0.1341
	8			1.0	+ve	0.1873
	9			2.0	-	
	10			4.0	-	
	11			NAA = 0.5	-	
	12			1.0	-	
	13			4.0	-	
	14					
Regenerated plants	1.	4	Z2	BA = 0.5	+ve	
	2.		Z2	1.0	+ve	
	3.	14	Z1	NAA = 0.5	+ve	
	4.		Z4	0.5	+ve	
	5.	15	Z1	BA = 0.5	+ve	
	6.			4.0	+ve	0.2083

REFERENCES

Dewick, P. M. (1997). Medicinal natural products. Jhon Willy and Sons Ltd Chichester. New York., Weinheim, p. 466.

Duncan, D. D. (1955). Multable range of multible F tests. Biometrics.11: 1-42.

El- Grari, R. and Backhaus, R. A. (1987). *In vitro* propagation of red squill, *Urginea maritima* Baker. Plant cell, Tissue and Organ culture. 10(1): 65-71.

Framm, I. J.; Peterson, A.; Thoeringer, C.; Pongert, A.; Hornung, E.; Feussner, I.; Luckner, M. and Leidemann, P. (2000). Cloning and functional expression in *Escherichia coli* of a cDNA encoding cardenolide 16'-O-glucohydrolase from *Digitalis lanata*. Plant and Cell Physiology. 41: 1293-1298.

Gamborg, O. L.; Miller, R. A. and Ojima, K. (1968). Nutrient requirements of suspension culture of soybean root cells. Exp. Cell Res., 50:151-158.

Geeff, K. (1981). Crdiac glycoside, experimental pharmacology., part 1, Springer Verlag., Berlin Hedelberg.

Guimaraes, M. L. and Montezuma, J. (1987). *In vitro* culture of *Urginea maritima* (L.) Baker, for rapid clonal propagation. Bol. da Soc. Broteriana. 60: 365-376.

Greidziak, N.; Diettrich, B. and Luncker, M. (1990). Batch cultures of somatic embryos of *Digitalis lanata* in gaslift fermenters. Development and cardenolide accumulation. Planta Medica. 56: 175-178.

Jha, S. (1989). Cytological analysis of embryogenic callus and regenerated plants of *U. indica* kunth., Indian squill. Caryologia. 42(2): 165-173.

Jha, S. and Sen, S. (1986). Development of Indian squill (*Urginea indica* Kunth.) through somatic embryogenesis from long term culture. Plant Physiology 124: 431-439.

Jha, S. and Sen, S. (1987). Karyotype variability in regenerated plants of *Urginea indica* Kunth. Cytologia. 52: 615-626.

Jha, S.; Sen, S.; Sumita, J. and Sumita, S. (1984). Comparative qualitative analysis of bufadienolides in different types of *Urginea indica* Kunth. By TLC. Journal of the Indian Botanical Society. 63: 252-260.

Kopp, B. and Danner, M. (1983). Structure of the bufadienolides of *Urginea India* (Roxb.) Kunth ('Indian squill'). Scientia Pharmaceutica. 51: 227-237.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassay with tobacco tissue culture. Plant Physiol. 15: 473- 497.

Snedecor, G. W. and W.G. Cochran (1981). Statistical Methods, Iowa state, university press, USA . 569-571.

Stahl, E. (i969). Thin layer chromatography. 2nd ed. Springer Verlag., Berlin Hedelberg, New York, USA.

Stojakowska, A. (1990). Changes in the proscillaridin A content during micropropagation of *Urginea maritima*. Planta Medica. 56: 549-550.

Stojakowska, A. (1993). Micropropagation of *Urginea maritima* (L.) Baker s. str. Planta Medica. 58: 284-285.

Sumita-Jha, Sahu, N. P. and Mahato, S. B. (1991). Callus induction, organogenses and somatic embryogenses in three chromosomal races of *Urgineaindica* and production of bufadienolides. Plant cell, Tissue and Organ culture. 25 (2): 85-90.

Täckholm, V. (1974). Students' flora of Egypt.2nd edition, Cairo Univ.,Cooperative printing, Beirut.

Townsend, C. C.; Guest, E.; Omar, S. A. and Al-Khayat, A. H. (1985) Flora of Iraq. Vol. 8 Baghdad, Ministry of Agriculture and Agrarian Reform.

Theurer , C.; Kreis, W. and Reinhard, E. (1998). Effect of digotoxigenin, digoxigenin and various cardiac glycosides on cardenolide accumulation in shoot cultures of *Digitalis lanata*. Planta Medica. 64: 705-710.

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تحفيز انتاج الكلس و نباتات جديدة مع انتاج المادة الفعالة الاساسية من نبات بصل العنصل الابيض

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٢.قسم العقاقير- كلية الصيدلة- جامعة حلوان.
٣.قسم البيوتكنولوجيا النباتية-معهد الهندسة الوراثية و التكنولوجيا- جامعة المنوفية- مدينة السادات.

يهدف البحث الى الإكثار الدقيق لبصل العنصل الابيض بإستعمال مزارع الانسجة مع دراسة انتاج العقار المهم صيدلانيا من انواع مختلفة من مزارع الأنسجة و مقارنتة بانتاج النباتات البرية و الى دراسة العوامل المختلفة المؤثرة من مزارع الأنسجة و قد أوضحت الدراسة وجود فروق معنوية بين التركيزات المختلفة لBA و لكن لا يوجد فروق معنوية بين مناطق الزراعةالمختلفة من البصلة حيث استخدمت ٤ مناطق رئيسية للزراعة و كذلك لا يوجد تفاعل معنوى بين التركيزات و مناطق الزراعة.

لا يوجد فروق معنوية بين التركيزات المختلفة ل 2,4-D و لكن وجدت فروق معنوية بين مناطق الزراعة المختلفة من البصلة و كذلك وجد انة لا يوجد تفاعل معنوى بين التركيزات و مناطق الزراعة.

توجد فروق معنوية بين التركيزات المختلفة ل NAA و فروق معنوية بين مناطق الزراعةالمختلفة من البصلة حيث استخدمت ٤ مناطق رئيسية للزراعة و كذلك يوجد تفاعل معنوى بين التركيزات و مناطق الزراعة.

تم فصل و تعريف مركب PsA من بصل العنصل بإستخدام الرنين النووى المغناطيسى لذرتى الهيدروجين و الكربون و ذلك تمهيدا لإستخدامة فى عملية التعيين الكمى للمادة داخل أنسجة النبات.

حيث تم تعيين كمية PsA فى النباتات الناتجة من مزارع الانسجة بواسطة HPLC.