PRODUCTION OF SOME SECONDARY PRODUCCTS FROM DATE PALM TISSUE CULTURES (SEWI CULTIVAR) USING SOME PRECURSOR 2- Embryogenesis stage

*** Hussein A. Bosila[1], Said M. Mohamed [2] Sherif F. El Sharabasy[3] Ibrahim A. Ibrahim [4], Khames A. Refay [5].**

(1) Fac. Agric. Hort. Dept. Alazhar Univ. (2) Medicinal & Aromatic. Plants Dept. Hort. Res. Inst. Agric. Res.Center (3) Central Lab. Res. Development of date palm Agric. Res.Center. (4) Gen. Eng. Biothec.Res. Inst. Menoufia Univ. (5) Fac. Agric. Hort. Dept. Alazhar Univ.

ABSTRACT

Different concentrations (0.0, 0.01, 0.1, 1.0, 10,0 mg/l) from pyruvic acid, squalene, and cholesterol were used as precursors added to the media .The best results of number and weight of embryo derived from shoot tip were achieved with 0.1 mg/l of squalene and 0.1 mg/l cholesterol. The addition of 1.0 mg/l cholesterol showed high response for shoot formation The addition of 0.1 mg/l cholesterol led to obtain the best results of steroid biosynthesis in the embryogenesis stage tissues. The highest value of steroids diffused by the tissue in the medium was that of 1 mg/l squalene. Eight steroids in callus and media were identified ethistron was the dominant compound. The results indicated that the steroids in medium of embryogenesis were higher than those identified in callus. The production of steroids from media is much better than from callus.

INTRODUCTION

Plant tissue cultures have long been regarded as a source of commercially important steroids, alkaloids, and Terpenes for pharmaceutical industry (***Bohm, 1980,Staba 1980; Barz and Eills, 1981, Deus, Zenk, 1982 and Taha 1999***). These compounds, known collectively as secondary products, are mostly obtained from plants grown under tropical condition. The difficulties of obtaining the source plants led to the view that a more convenient and ultimately cheaper source of secondary products would be to grow tissue cultures of the plant sources on a large scale, and then extract the accumulated calli cultures biomass and spent medium for the product.

Yield of specific compounds attempts to increase the contents of secondary metabolites in plant cell cultures may be influenced by environmental factors, such as light, temperature, precursors, and

nutrients, including growth regulators, morphological and chemical differentiation, and biosynthetic capacity **(Barz *et al.*; 1977; Sharp *et al.*, 1979 and Thorpe, 1981).** The present investigation was planned to study the effect of some precursors on the growth, development and secondary metabolite biosynthesis (steroid) biosynthesis from embryogenesis of (*Phoenix dactylifera* L.) Sewi CV.

MATERIALS AND METHODS

In this experiment, embryos of date palm (*Phoenix dactylifera L.)* were used as explants. Embryos were cultured on MS solid medium Murashige and Skoog (1962) treated with 0.0, 0.01, 0.1 and 10.0 mg/L of pyruvic acid squalene, and cholesterol. The MS solid medium and fresh growth regulator were used with the different concentrations. Ten replicates of jars were arranged containing 25-ml medium for each treatment. The pH value was adjusted to 5.7 - 5.8 prior to autoclaving. The cultures were incubated at 27 $\pm$ 2°C temperature and 16 hr. light/day photoperiod. The recorded data were:

1. Number of embryos.
2. Number of shoots per embryo.
3. Weight of embryo (gm).
4. Total steroids were determined by using Spectrophotometer according to the method described by (Pharco 1993). These data were recorded on:
   - A- Embryos tissues of embryogenesis stage.
   - B- Media of embryogenesis stage.

Separation and identification of steroid compounds in the *in vitro* culture of date palm by gas liquid chromatography (G.L.C.)

The steroids were analyzed by Gas Liquid chromatography (PYE UNICAM PRO – GC). The chromatograph was fitted with a capillary column OV 17 (Methyl phenyl silicone) 1.5 m x 4mm. Under the following condition.

* Temperature programming:

Initial : 70°C upper: 270°C Rate: 10°C/ min
Injector : 250°C (N_2) carrier
Detector : 300°C (H_2) flame Ionization (FID).

*** Flow Rate of Gases:**

N_2: 30 ml/min H_2: 33 ml/min
Air: 330 ml/min.

Chart speed: 0.50 cm/min.

- The following parameters were record:

1. Identification of steroid and sterols composition produced in embryo tissues.
2. Identification of steroid composition diffused from embryos in the medium.

RESULTS AND DISCUISSN

Number of embryos

From the presented in table (1) and Fig. (1a) data it appears that embryos formation was of negative correlation responses with increasing pyruvic or/and cholesterol levels from 0.01 mg/l to 10 mg/l. While, increasing squalene level from 0.01 to 0.1 increased embryo formation. More increase in squalene level resulted in negative effect and decreased embryo formation. However, squalene was the most suitable treatments for stimulating embryo formation comparing with the other treatments, and the highest value was recorded for 0.1 mg/l squalene.

The highest average number of embryos between different precursors was (6.08) of average squalene concentrations and lowest average number of embryos was (2.52) for pyruvic acid and cholesterol concentrations.

Number of shoots per embryo

Data of Table (1) show that shoot formation responded differently to the different precursors used in this study. Whereas shoot formation negatively correlated with using squalene levels comparing with untreated explants. In this case using 0.01 mg/l squalene decreased number of shoots/embryo to 2.3 comparing with 4.1 for untreated embryo. Increasing squalene level to 0.1 mg/l increased shoot formation to 2.7/embryo compared with 2.9 / embryo for 1.0 mg/l squalene. The lowest shoot number / embryo was recorded for 10-mg/l squalene. The fluctuation in shoot formation was recorded also for pyruvic acid treatments. The values recorded in this case were 5.6, 2.6, 4.0 and 3.4 shoot/ embryo for 0.01, 0.1, 1.0 and 10 mg/l pyruvic acid, respectively. Although, cholesterol concentrations had no streetlight trend on shoot formation. They were more suitable for shoot formation comparing with the other precursors and control in most cases. The highest shoot number/ embryo (8.2) was formed by embryo which was grown on MS medium contains 1.0 mg/l cholesterol, while 5.2, 3.8 and 4.4 shoots/ embryo were formed from the embryos grown on MS medium contains 0.01, 0.1 and 10 mg/l cholesterol respectively.

Embryo weight

Statistical analysis of variance show that using pyruvic acid at the lowest level (0.01 mg/l) had a significant stimulating effect on embryo weight comparing with the other precursors used at the different rates except of cholesterol 0.1 mg/l (2.829 gm) and 10.0 mg/l (3.376 gm) which had the positive effect (Table 1). The highest average was achieved with cholesterol (2.183 GM), while; the lowest average was obtained with pyruvic acid (1.348 GM). On the other hand, the best concentration was achieved with 0.01mg/l (2.06gm).

Steroid biosynthesis in embryo tissues of embryogenesis stage:

Data of Table (1)show that using 1.0 mg/l of cholesterol on MS medium seemed to be the most suitable precursor to stimulate steroid formation (214% of control) followed by 0.01 mg/l pyruvic acid (160% of control) comparing with the other treatments used which had negative effect on steroid biosynthesis. The fluctuation responses of steroids formation may be due to degradation of steroids by the tissue culture (**Staba, 1980).**

Steroid biosynthesis in the embryo media of embryogenesis stage.

From the results of Table (1) it is clear that pyruvic acid added to the MS media had positive effect on steroids biosynthesis in the embryogenesis media, with a fluctuated trend. The highest value was recorded for 1.0 mg/l corresponding to 6 folds as the control, while, the lowest one was that of 0.10 mg/l concentration comparing with the control. The best results were obtained from MS media supplemented with squalene. In this case steroid formation increased gradually with the increase of the concentrations reaching to the maximum value (1550% of control) for 1.0 mg/l, followed by a decrease when squalene level was 10.0 mg/l but it still more of significant value than control (1183.3% of control).

Using cholesterol precursor resulted in similar effect as that recorded for squalene treatments. Steroid biosynthesis was increased gradually according to in the increase in cholesterol level reaching its maximum value (11183.3 % of control) for 1.0 mg/l. Increasing cholesterol level to 10 mg/l decreased steroid biosynthesis to 0.58 mg/l which is more than control of significant value (483.3% of control). It

could be concluded from the previous results that squalene precursor was of high stimulation potency to produce the steroids regarding the high obtained values which recorded 5.75 to 15.5 folds as the control. Also cholesterol and pyruvic acid come after squalene as they are of considerable of higher potency ranging 4.75-11.87 folds and 3.16-6 folds for cholesterol and pyruvic acid, respectively, compared to the control.

It could be observed that the concentration of 1 mg/l is the most suitable concentration within the studied precursors. Steroid formation in the tissue is of minute values in most cases in comparison with the controls. However, steroid contents in the media after the addition of the precursors obviously showed a marked superiority in such values in comparison with those obtained in the tissues. The observed higher values produced in the media could be explained by that a continuous excretion of steroid synthesis by the tissue took place. So that minute amount of such compounds synthesis by the tissues were remained. Consequently, the observed low values of steroids in tissues do not represent the real value synthesis in the tissues, regarding the continuous excretion of the secondary product in the media.

Accordingly, although culturing the tissues of the different stages of date palm are necessary as a tool to produce secondary products (i.e. steroids). The culture media are the main source to obtain the steroids from the date palm cultured tissues.

Separation and identification of steroid compounds:

A- In embryo tissues

From the presentation in table (2) and chromatograms (1,2,3 and 4) data it appears that culturing date palm Sewi CV. embryos on medium containing 1.0 mg/l squalene or 0.01 mg/l pyruvic acid or 1.0 mg/l cholesterol precursor stimulated the formation process of some steroids in high percentage.

Using squalene (1.0 mg/l) stimulate oestrone formation yielding about 5.06%, ethylenestradiol 57.19% and ostriol 29.89% comparing with pyruvic acid which stimulated the production of ethistron 42.3% only. However, cholesterol precursor show high activity in the production of oestrone 8.17%, ethylenestradiol 7.92%, ethistron 0.12%, ostriol 7.39%, stigmasterol 1.8% and ß-sitosterol 0.3%.

B- Embryogenesis medium.

From the recorded in Table (2) and chromatograms (5,6,7 and 8) data it clear that ethistron is the major steroid diffusated from embryogenesis to the growing medium. Pyruvic acid treatment had the highest effect in this concern. Squalene treatment stimulates the process formation of ethylenestradiol (25.3%), ethistron (42.5%), ostriol (1.99%) and stigmasterol (0.72%). Pyruvic acid treatment led to format the highest value of ethistron (84.8%) beside trace value of cholesterol (0.685%), ethylenestradiol (0.76%) and stigmasterol (0.3%). Cholesterol treatment stimulated steroid process formation to format 0.73% cholesterol, 1.15% Oestrone 18.1% ethylenestradiol, 52.78% ethistron, 0.5% ostriol and stigmasterol.

Table (2): Effect of some precursors on steroid composition produced in embryo tissues and media of date palm Sewi CV.

Compound	Rt	In Embryo Tissues				In Embryo Media			
		Con	SC	PC	CC	Cont	SMC	PMC	CMC
Cholesterol	15.8	0.49	0.00	0.00	0.00	1.20	0.00	0.68	0.73
Oestrone	19.8	0.00	5.06	0.00	8.17	0.00	0.00	0.00	1.15
Ethylenestradiol	21.3	0.00	57.2	0.00	7.92	3.39	25.3	0.79	18.1
Ethistron	22.8	0.00	0.00	42.0	0.22	0.00	42.5	84.8	52.8
Ostriol	23.2	0.00	29.9	0.00	7.39	0.00	1.99	0.00	0.50
Stigmasterol	24.3	0.00	0.00	0.00	1.80	0.28	0.72	0.30	1.38
B-Sitosterol	27.1	0.00	0.00	0.00	0.30	0.00	0.00	0.00	0.00

It is generally accepted that, differentiation in tissue culture is associated with an increase in production of secondary products. Differentiation may occur as increased aggregation, production of specific cell types or initiation of more organized structures such as embryos, roots and shoots. The change in biochemical capacity of the cells may be due to the release of specific gene sequences at the sometime as those responsible for differentiation or may occur because the change in growth levels more substrates available for secondary pathways (***Yeoman et al., 1982***) or because a change in compartmentation of the cells which allows complex of secondary pathway to function **(Neumann, 1983).**

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