PRODUCTION OF SOME SECONDARY PRODUCTS FROM DATE PALM (Phoenix dactylifera) TISSUE CULTURES (SEWI CULTIVAR) USING SOME PRECURSORS *1- Callus stage*

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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 highest volume and weight of callus in Sewi cultivar were obtained with 0.01mg/l of cholesterol and 1 mg/l squalene. All the precursors added did not mostly increase steroids in callus in comparison with the control. The highest value of steroids diffused by the callus in the medium was that of pyruvic acid (10 mg/l). Eight steroids in callus and media were identified namely cholesterol, estrone, ethylenestradiol, ethistron, ostriol, stigmasterol, and B. Sitosterol, 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

Date palm (*Phoenix dactylifera* L.) a plant widely distributed in Egypt, west Asia and North Africa, extensively planted in the Arab countries and also grown to some extent, in southern Europe. It is used as nutritive and therapeutic, its pollen grains are utilized as antisterility agent ***(Ateya, 1975).*** Plants and some animal products are used in folklore medicine for treatment of several diseases e.g. Hypertension, cardiac diseases, kidney disjunction and Diabetes, ... etc. However nothing could be traced concerning drugs which are used in the treatment of sterility except date palm pollen grains, which have been known by the Egyptian and Arabs to be nutritive and used as antisterility agent. Cholesterol,

cholesterol and coprostanol are the animal sterols, while, B-sitosterol, campestral, stigmasterol, ergosterol and brassicasterol are the principal plant sterols, (***Bailey, 1964***). Cholesterol, one believed to be the typical animal sterol, has recently been found to be rather widely distributed among plants. So far, cholesterol has been identified in the pollen of many plants including the date palm, (***Bennett et al., 1966***) and in oil palm (***Slover et al., 1983***).

Higher plants are solar-powered biochemical factors, which manufacture what they need to survive (both primary and secondary metabolites) from air, water, minerals, and their energy from sunlight. Many species of higher plants biosynthesize and accumulate extractable organic substances in quantities sufficient to be economically useful as chemical feeds-tocks or as raw materials for various scientific, technological, and commercial applications. Natural substances are employed, either directly or indirectly, by a large number of industries, and natural plant products (Phytochemicals) figure prominently in several of these. For example, Phytochemicals are utilized to a large extent by the pharmaceutical, cosmetics, food, agrochemical, and chemurgic industries. Economically important plants serve as irreplaceable sources of industrial oils (both volatile and fixed), flavors and fragrances, resins (e.g. rosin and tall oil), gums, natural rubber, waxes, saponins and other surfactants, dyes, pharmaceuticals, pesticides (e.g., insecticides and rodenticides), and many specialty products (***Balandrin and Klocke, 1988).***

The present investigations was planned to study the effect of some precursors on the growth, development and secondary metabolites synthesis (steroids) from callus (*Phoenix dactylifera* L.) Sewi CV.

MATERIALS AND METHODS

In this experiment the optimum callus mass drive from shoot tips was used as explants. The exceeding callus mass divided into small pieces and cultured on MS solid medium supplemented with 100 NAA + 3 mg/l 2ip.Different pyruvic acid, squalene and cholesterol concentrations (0.0, 0.01, 0.1, 1.0, 10.0 mg/l) were used as precursor for steroid biosynthesis from date palm (*Phoenix dactylifera L.*) callus. Ten groups of jars containing 25-ml medium for each treatment were arranged. The pH value was adjusted to 5.7 - 5.8 prior autoclaving. The treatments were incubated in growth chambers at 27$\pm$ 2°C in complete darkness. The following data were recorded after one month on.

A- The callus tissues of callus stage.

B- The media of callus stage.

1. Volume of callus where
   + = 2 = small value.
   ++ = 3 = moderate value.
   +++ = 4 = high value.
2. Callus weight (GM).
3.
4. Total steroids were calculated and determined by spectrophotometer according the methods described by (Pharco 1993).
5.
6. Separation and identification of steroids and sterols compounds in the *in vitro* culture of date palm by gas liquid chromatography (G.L.C.)
7.

The steroid composition of date palm *in vitro* culture treated with some precursors namely squalene, pyruvic acid and cholesterol were identified by gas liquid chromatography (G.L.C.) analysis. The adapted samples were chosen on the basis of their total steroids as well as those of high total steroids content in the media who were considered as promising treatments and subjected to G.L.C. analysis. It should be noticed that the obtained chromatograms represent not only the steroids but also all the compounds in the unsaponifiable fraction in the extracts of each treatment.

*** Identification and determination of steroids composition.**

The steroids and sterols 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.

Unsaponifiable materials separation condition by (GLC).

* 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 recorded:

1. Identification of steroid composition produced in callus tissues.
2. Identification of steroid and sterols composition diffused of callus in the medium.

RESULTS AND DISCUSSION

Volume of callus

Data presented in table (1)) indicated that the best results was achieved with callus grown on medium contained 0.01 mg/l of cholesterol, followed by medium treated with 1.0 mg/l of pyruvic acid and medium contained 1.0 mg/l of squalene. While, the lowest value of callus was observed with callus grown on medium contained 0.01 mg/l of squalene followed by 0.01 mg/l pyruvic acid and cholesterol. Statistical of variance showed that the variations between precursor concentrations and their interactions were of significant value.

Fresh weight of callus

Data of Table (1) clearly show that the highest weight of callus was recorded for MS basal medium supplemented with 1.0 mg/l of squalene followed by 0.01 mg/l of cholesterol. On the other hand, the lowest amount of callus was formed by explants grown on MS basal medium supplemented with 10.0 mg/l of pyruvic acid, followed by the control, with significant difference between precursors, concentrations and their interaction.

The highest average weight of callus was formed by explants grown on MS medium contain squalene, while the lowest average weight of callus was of pyruvic acid regardless of concentrations.

Steroids biosynthesis in callus tissues

It is clear from the recorded data of Table (1) that steroid formation responded differently to the different precursors (pyruvic acid, squalene and cholesterol) levels used in this study. Whereas steroid formation was of negative correlation responses with increasing pyruvic acid levels from 0.01 mg/l, which stimulate steroid formation by about 120% of control, to 0.1 mg/l (60% of control) or 1.0 Mg /l (40% or control) or 10 mg/l (80% of control).

The obtained results show also that MS medium supplemented with 0.1 mg/l squalene stimulated the process of steroid formation and increase it by about 200% of control comparing with 60% for 0.01 mg/l squalene, 120% for 1.0 mg/l squalene or 100% of control for 10.0 mg/l squalene. The recorded data indicated that using 10.0 mg/l cholesterol on MS medium seemed to be the best precursor used in order to stimulate steroid formation resulting in 240% of control or the other cholesterol

levels used which produced 140% of control for 0.1 mg/l and 40% of control for 1.0 mg/l cholesterol.

Steroids biosynthesis in callus media

Data of Table (1) clearly show that the different precursors stimulate steroid biosynthesis processes in callus media comparing with control in the most cases. Steroid formation positively correlated with increasing pyruvic acid levels. However, steroid formation was responded differently to squalene treatments, whereas, it was increased by increasing squalene level from zero mg/l to 0.01 mg/l. increasing steroid content by about (179% of control). The stimulating effect was enhanced when squalene level was increased to 0.10 mg/l, which increased steroid content in the media by about 245% of control. Increasing squalene level to 1.0 mg/l decreased steroid content comparing to the lower squalene levels (0.01 or/and 0.1 mg/l) but it still higher than control by about 218%, other increase in squalene level decreased steroid formation significantly comparing with the other squalene level, but no lower than control.

Increasing cholesterol concentration from 0.01 mg/l to 0.1 mg/l increased steroid content in callus tissues media of significant value comparing with control and the higher cholesterol level (10.0 mg/l) which still higher than control by about 245.4%.

Tissues, which had differentiated roots, produced flavor intensities approaching that of fresh onion, although there were important qualitative differences particularly in respect of lachrymatory potency. Undifferentiated tissues on the other hand contained only very small amounts of flavor components owing to the absence of precursors rather than of the enzyme alliance ***(Freeman et al. (1974).*** Precursor level in callus was only 2-10 % of that found in the intact plant. In undifferentiated callus S-methyl-L-cysteine sulphoxide was present at a low concentration while the major precursor of onion flavor, S-Trans-prop-l-enyl-L-cysteine was absent altogether ***(Selby et al. (1979).*** But, in undifferentiated roots and shoots this precursor was present again. Both crushed roots and shoots had the characteristic odor of onion, however, only crushed roots showed the lachrymatory effect ***(Turnbull et al. 1981).***

Addition of intermediates to the culture medium showed that the callus was capable of the final stages in the synthesis of S-Trans-prop-1-enyl-L-cysteine sulphoxide ***(Selby et al. 1980).*** It has been frequently found that the increased production of onion aromas (monocotyledons) is chiefly attributed to the presence of roots in tissue cultures of Allium

Cepa. ***Freeman et al., (1974); Fridborg et al. (1971) and Turnbull et al. (1981).***

Separation and identification of steroid compounds

A. Callus Cultures

Data of Table (2) and chromatograms (1,2,3 and4) show that squalene at the rate of 0.1 mg/l stimulated biosynthesis the steroid compounds comparing to the other precursor treatments and/or control. It led to format 9.45% cholesterol, 21.98% oestron, 10.46% ethylenestradiol, 2.36% ethistron, 1.29% ostriol and 3.46% stigmasterol. However, cholesterol treatment at the rate of 10 mg/l slightly affected steroid biosynthesis. So it led to format 72.1% oestrome, 0.36, ethylenestradiol and 0.79% stigmasterol. Moreover, pyruvic acid had moderate effect in this order. This identified compounds under the effect of pyruvic acid were cholesterol 1.9%, oestron 1.31%, ethylenestradiol 0.19%, ethistron 13.25% and stigmasterol 0.34%, while, 2.3% cholesterol, 6.46% oestrone, 9.74% ethylenestradiol, 1.81 ethistron and 1.9% stigmasterol were recorded for control.

A. Callus medium:

Data of Table (2) and chromatograms (5,6,7 and 8) show that ethistron is the major steroid formed and diffused from callus to the growing medium regardless of precursor used in this study. In this concern squalene treatment formed 42.11% ethistron, comparing with 86.8% for pyruvic acid, 3.39% for cholesterol and 0.24% for control treatments, respectively. On the other hand squalene treatment led to format 1.05% ethylenestradiol, 0.85 ostriol and trace (0.04%) of stigmasterol in addition to ethistron as major steroid. While, pyruvic acid treatment formed 0.45% stigmasterol beside the major steroid (ethistron) formed in this case. However, cholesterol treatment had the lowest effect on steroid formation resulting in 3.39% ethistron, 1.22-% ostriol, 0.004% stigmasterol and 0.08% ß-sitosterol. Moreover, the diffused steroids from callus to the control medium were in the trace value 0.56% ethylenestradiol, 0.24% ethistron, 0.46% ostriol and 0.37% stigmasterol.

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

Compound	RT	In Callus Tissues				In Callus Media			
		Control	SC	PC	CC	Control	SMC	PMC	CMC
Cholesterol	15.8	2.3	5.45	1.90	0.00	0.00	0.00	0.00	0.00
Oestrone	19.8	6.46	21.98	1.31	72.00	0.00	0.00	0.00	0.00
Ethylenestradiol	21.3	9.74	10.46	0.19	0.36	0.56	1.05	0.00	0.00
Ethistron	22.8	1.81	2.36	13.25	0.00	0.24	42.1	86.8	3.39
Ostriol	23.18	0.00	1.29	0.00	0.00	0.46	0.85	0.00	1.22
Stigmasterol	24.28	1.99	3.46	0.34	0.79	0.36	0.04	0.45	0.04
B-sitosterol	27.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08

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