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# Cell Suspension Culture and Secondary Metabolites Production in African Violet (*Saintpaulia ionantha* Wendl.)

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## ABSTRACT

Initiation of cell suspension culture of African violet (*Saintpaulia ionantha* Wendl.) was studied by inoculating fresh friable fragments of callus on Murashige and Skoog (MS) medium supplemented with different levels of $N^6$-benzyladenine (BA) (4.4, 8.9 or 13.3 µM), kinetin (0.5, 1.4 or 2.3 µM), and 2,4-dichlorophenoxyacetic acid (2,4-D) (2.2, 4.4 or 6.6 µM), or different concentrations and ratios of $NH_4^+$:$NO_3^-$ (30:0, 10:20, 20:10, 0:30) or (0:60, 20:40, 40:20, and 60:0) along with control treatment. The highest cell growth (20.5 mg/ml) was obtained on medium containing 2.3 µM kinetin. Adding 0.54 µM 1-Naphthaleneacetic Acid (NAA) to all growth regulators combinations at certain concentrations resulted in better cell growth compared to those combinations lacking NAA. Using 30 mM total nitrogen at low $NH_4^+$:$NO_3^-$ ratio resulted in the best cell growth. The secondary metabolite, cyanidin, was identified in the leaves of *in vivo* and *in vitro* grown *S. ionantha* microshoots. Cyanidin content for *in vivo* and *in vitro* extracts was 98.16 and 85.04 mg/g dry tissue, respectively. On the other hand, no cyanidin was detected in cell suspension culture.

**KEYWORDS:** African violet, *in vitro*, secondary metabolites.

## INTRODUCTION

*In vitro* production of plant secondary metabolites has been considered with a great enthusiasm (Pierik, 1987; Smith, 1996; Stafford, 1991). However, the key for a feasible economic production of these chemicals is the induction and selection of high-yielding cell cultures as reported by Smith (1996) and Whitaker and Hashimoto (1986). Furthermore, natural plant pigments and pharmaceuticals have been recognized as perfect target compounds for laboratory experiments demonstrating the metabolic pathways for producing these chemicals via *in vitro* culture techniques (Smith, 1996). *In vitro* production of secondary metabolites in plant cell suspension culture systems has been reported from various medicinal plants (Tripathi and Tripathi, 2003). Plant cell culture is often an effective system to study the biological significance of bioactive metabolites under *in vitro* conditions, as well as for producing natural products for bioprocessing applications (Walker et al., 2002).

Plant-derived chemicals have been extracted from *in vivo* grown plants for a long period of time (Pierik, 1987) but the general trend now is to produce these secondary metabolites via *in vitro* culture techniques for several justifications (Hopkins, 1995; Pierik, 1987; Smith, 1996; Stafford, 1991). On the top of these lies the elimination of potential political and geographical boundaries against crop production, and protection from weather fluctuations, diseases, pests or soil problems. By *in vitro* culture techniques, rapid growth of callus and cell suspension cultures, from which secondary metabolites are to be extracted, could be obtained. In addition, plant-derived chemicals can be mass-produced at lower cost over a prolonged period of time. Moreover, *in vitro* production of those products also provides an excellent forum for in-depth investigations of plant metabolic pathways under highly controlled microenvironmental

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conditions. Secondary products can be derived from callus-, cell- and cell suspension cultures (Granicher *et al*., 1995; Pepin *et al*., 1995; Shibli *et al*., 1997 and 1999) as well as from plant leaves and/or flowers. Yet, the precise roles of those metabolites in plants are not clearly known (Verpoorte, 1990).

Several factors have been shown to influence the accumulation of secondary metabolites in callus and suspended cells. The most important are the chemical constitution of the media used with respect to growth regulators (Nawa et al., 1993), carbon source and concentration (Decendit and Merillon, 1996; Mori and Sakurai, 1994) and nitrogen source and concentration (Do and Cormier, 1991; Mori and Sakurai, 1994; Sato et al., 1996). Of all plant secondary compounds, anthocyanins have been investigated most extensively in the areas of chemistry, biochemistry and genetics (Saito and Yamazaki, 2002). Anthocyanins are considered to be economically important naturally-occurring plant products since they make major contributions to the taste, flavor and color of our food and drink (Harborne, 1994). Bomser et al. (1996) have revealed that anthocyanins, in combination with other organic substances, possess an anticarcinogenic activity. Surprisingly, it had been reported that anthocyanins can replace rutin and its derivatives in the treatment of tissue inflammation and capillary fragility (Harborne and Grayer, 1988).

This investigation was initiated to develop a suitable protocol for cell suspension culture and production of secondary metabolites from African violet (*Saintpaulia ionantha* Wendl.).

## Materials and Methods

### *In vitro* Multiplication of Stock Cultures

*In vitro* cultures of African violet (*Saintpaulia ionantha* Wendl.) were obtained from the Plant Tissue Culture Laboratory, College of Agriculture at the University of Jordan (Amman, Jordan). Microshoots were subcultured for six times on MS medium (Murashige and Skoog, 1962) containing 4.4 µM 6-Benzyladenine (BA) and 0.54 µM 1-Naphthaleneacetic Acid (NAA) before we received them. These *in vitro* established cultures were subcultured in our laboratory on the same media (40 ml each) in glass jars. Subculturing was done seven times at 3-week intervals to maintain sufficient mother stock plants for experimentation. Cultures were incubated in the growth chamber where they were maintained under a daily regime of 16-h light/8-h dark photoperiod (Photosynthetic Photon Flux Density (PPFD) of 40-45 µmol $m^{-2}$ $sec^{-1}$) using white fluorescent tubes and 24 ± 1°C.

### Callus Culture

Calli were induced in the dark from the terminal leaves (8 mm diam) of the *in vitro* grown plants. Explants were subcultured on the surface of solid MS media (20 ml in 9.0 cm diameter plastic sterile petri-dishes). Callus induction media were supplemented with 0.5 µM kinetin, 0.54 µM NAA and 88 mM sucrose. Induced calli (5 mm diam) were routinely subcultured on solid MS media supplemented with 2,4-D (2.2 µM) in combination with kinetin (1.4 µM) and sucrose (88 mM) in order to maintain sufficient stock cultures.

### Cell Suspension Culture

Cell suspension cultures were initiated by inoculating fresh friable fragments of calli (2.0 g each) into 50 ml of MS liquid media in 250 ml Erlenmeyer flasks. Media were supplemented with three levels of BA (4.4, 8.9 or 13.3 µM), kinetin (0.5, 1.4 or 2.3 µM) and 2,4-D (2.2, 4.4 or 6.6 µM), and their combinations (Table 2). Benzyladenine combinations with kinetin contained NAA (0.54 µM) in one treatment, but no NAA was added in the other. Control treatment did not contain any growth regulator.

To study the influence of nitrogen source and concentration on cell growth, another experiment was carried out in which MS media were supplemented with different $NH_4^+$:$NO_3^-$ ratios at different concentrations. Ratios (0:30, 10:20, 20:10 and 30:0) were used at 30 mM total nitrogen, whereas ratios of (0:60, 20:40, 40:20 and 60:0) were studied at 60 mM total nitrogen. A combination of 2,4-D (2.2 µM) and kinetin (1.4 µM) was used throughout this experiment with sucrose (88 mM) as the carbon source in the media. Control treatment was carried out without exogenous nitrogen. Suspension cultures were incubated on rotary shaker (100 rpm) for agitation under the dark in the growth room (Shibli *et al*., 1997 and 1999).

Each treatment consisted of four completely randomized replicates without subsamples and growth assessment was performed by recording data every five days during a 30 day period for cells Fresh Weight (FW).

## Anthocyanidin Determination

### Extraction of Anthocyanidin

African violet (*Saintpaulia ionantha* Wendl.) plants were collected from *in vivo* (Greenhouse) and *in vitro* grown plants. Shoots (1.0 g) were dried in the shade at

room temperature for one week and were ground in mortar using liquid nitrogen. Anthocyanidin was then extracted following the procedure detailed by Smith (1996) and Stafford (1991).

**Qualitative and Quantitative Analyses of Anthocyanidin**

**Thin Layer Chromatography (TLC)**

Thin Layer Chromatography (TLC) was carried out on plates of cellulose (the stationary phase) using formic as a mobile phase (conc. HCl: HCOOH: $H_2O$ at 2: 5: 3) for qualitative identification of Cyanidin aglycone. Spots of Cyanidin (standard), *in vivo*, *in vitro* and cell suspension extracts were placed on the cellulose plate after being dissolved with 10 times (w/v) 1 % HCl-methanol. The prepared plate was dipped in the formic mobile phase solution and kept in the glass jar for 3 hours for complete separation of the spotted samples. The cellulose plate was then dried for 30 min. Separation bands of the samples were compared with that of the standard Cyanidin. $R_f$ values were also determined for each spotted sample.

**High Performance Liquid Chromatography (HPLC)**

Analysis of anthocyanidin derived from acid hydrolysis was performed using HPLC, Unicam Liquid Chromatographic System, operated with pumping system (Spectrasystem Gradient Pump-P 4000), detector (Waters 484 Tunable Absorbance Detector) and computing integrator (Unicam 4815) and a 5 µm reverse-phase Hypersil $H_5$ODS analytical column (250 mm x 4.6 mm). The solvent (mobile phase) consisted of MeOH: $H_2O$: HCOOH (75: 24.5: 0.5). Separation was carried out by an isocratic elution at a flow rate of 2.0 ml $min^{-1}$ and 250 nm wavelength.

A 20 ml sample was injected into the column and compared with authentic standards; namely Pelargonidin, Cyanidin, Peonidin, Delphinidin, Petunidin, and Malvidin purchased from Polyphenols AS company (Norway). Retention time ($R_t$) for each sample was recorded.

**Spectrophotometry**

Ultraviolet-Visible absorption spectrophotometer (UV-VIS Recording Spectrophotometer 2401 PC SHIMADZU) was used to detect the absorbance of the injected samples at certain wavelengths in the visible range. Dry *in vivo* plant leaves (0.5 g each) and cells were dissolved in 10 times (w/v) 1 % HCl-methanol, and the spectra of the resulted extracts were measured. Total amounts of Cyanidin in the injected samples were calculated at 535 nm following the procedure of Francis (1982).

**Statistical Analysis**

Each experiment was set up as a completely randomized and analyzed using MSTATC software (Michigan State University, 1988). Means were separated according to the Least Significant Difference (LSD) test at 0.01 probability level.

## Results and Discussion

**Effect of Plant Growth Regulators (PGRs) on Suspended Cell Cultures Growth**

There were no significant differences in FW between the control (0 days), 5-days and 10 days measurements (Table 1). Also, FW was not different between 15 days or more except when 2,4-D was used at 4.4 µM and when Kinetin was used at 0.5 or 2.3 µM. The increase was significant for all hormonal treatments. Kinetin (2.3 µM) had resulted in the highest cells FW followed by 2.2 µM 2,4-D after 30 days incubation (Table 1). Elevated levels of kinetin have resulted in increased cells FW after 15 days. Do and Cormier (1991) were able to maintain cell suspension culture of *Vitis vinifera* L. on MS media supplemented with 1 µM kinetin. On the other hand, increasing 2,4-D concentrations (up to 6.6 µM) reduced cell growth (Table 1). Sahai (1994) reported that low 2,4-D levels were commonly used for good and stable growth of cell suspension cultures. Similar conclusions have been reported by Mori and Sakurai (1994) and Nawa *et al*. (1993) who have found that 2,4-D (4.4 µM) increased the growth of *Fragaria ananassa* cv Shikinari and *Vaccinium ashei* Reade. suspended cells, respectively. In another study conducted by Berlin *et al*. (1986) on *Chenopodium rubrum* L., cell suspension was maintained on MS media supplemented with 2.2 µM 2,4-D for stable growth. The reason standing behind these influences is the ability of 2,4-D, at low levels, to induce cell mitotic activity and promote mRNA synthesis rapidly, as concluded by Allan (1991).

Results showed that the lag phase, in all treatments, had lasted 5 days before the onset of the exponential growth phase. In addition, the time for subculturing the cells into new fresh liquid media was determined to be after 20 days of inoculation. This is because the early stationary phase onsets occur at that time. The best time to perform subculturing had been reported to be at the end of the exponential growth phase and early stationary phase of the

cell growth pattern (Allan, 1991). Furthermore, media become exhausted due to nutrient depletion of the stationary phase. Also, cells will begin to accumulate toxic substances and growth inhibitors (Allan, 1991; Bhojwani and Razdan, 1983; Hopkins, 1995).

A combination of BA and kinetin (with 0.54 µM NAA) resulted generally in higher cells FW values compared to the same combination without NAA (Table 2). Using 13.3 µM BA + 2.3 µM kinetin with 0.54 µM NAA resulted in the highest cell fresh weight (15.57 mg/ml). Several researchers (Mori *et al*., 1993; Rau and Forkmann, 1986; Shibli *et al*., 1997) have emphasized the use of NAA in improving cell growth pattern. Mori *et al*. (1993) found that NAA was better than 2,4-D, IBA or IAA in increasing the cells FW of *Fragaria ananassa* cv Shikinari. Similarly, NAA at high concentrations (5.4–10.4 µM) outperformed 2,4-D and IAA in improving the growth of *Callistephus chinensis* cell cultures (Rau and Forkmann, 1986). Naphthaleneacetic acid (0.54 µM) in combination with 0.45 µM 2,4-D and 20 µM BA was successfully used in initiating and maintaining cell suspension of *Vaccinium pahalae* (Shibli *et al*., 1997).

In contrast, the high levels of 2,4-D (0.5 mg/L and above) in combination with kinetin had a negative influence on cell growth expressed as low cells FW values. Using 2.2 µM 2, 4-D + 1.4 µM kinetin gave the highest cells fresh weight in these combinations (Table 3). No significant variations were observed between treatments with respect to cell growth (Table 4). The same table shows significant increases in cells FW during the first 20 days of incubation. Toivonen *et al*. (1992) reported that cell suspension cultures of *Catharanthus roseus* L. were maintained on B5 medium supplemented with 2,4-D (4.4 µM) and kinetin (0.5 µM ).

**Effect of Nitrogen Source and Concentration**

The best $NH_4^+$:$NO_3^-$ ratio to improve cell growth pattern was (10:20) followed by (20:40) and resulted in 22.5 and 20.25 mg/ml cells FW, respectively after 30 days of incubation (Table 5). Elevated $NH_4^+$:$NO_3^-$ ratios above 10:20 (30.0 mM total nitrogen) and 20:40 (60.0 mM total nitrogen) had significantly reduced cells FW. Similar findings have been reported by Pepin *et al*. (1995) who concluded that extracellular $NH_4^+$ availability may limit cell division when examining the growth kinetics of *Vitis vinifera* L. suspension cultures. On the other hand, increased $NH_4^+$ levels (up to 16 mM) increased the growth of suspended cells of the same variety (Do and Cormier, 1991). In their study, low nitrate concentrations (6.25 mM) had significantly reduced the cell growth. Lower values of cells FW were reported in control treatment compared to all other treatments in the experiment.

The influence of $NH_4^+$:$NO_3^-$ ratio on the kinetics growth of cell suspensions of many plant species had been addressed by Mori *et al*. (1993). High $NH_4^+$:$NO_3^-$ (above 20:10) reduced the density of *Fragaria ananassa* cv Shikinari suspended cells (Sato *et al*., 1996). In their study, low $NH_4^+$:$NO_3^-$ (5:25) were proven to increase the cell density. Similar findings have been reported by Mori and Sakurai (1994) and Decendit and Merillon (1996) who found that low $NH_4^+$:$NO_3^-$ (2:28 and 1:25) have increased the cell growth of *Fragaria ananassa* L. and *Vitis vinifera* L., respectively. A maximal density of suspended cells of *Fragaria ananassa* L. was obtained at 30.0 mM total nitrogen (Sato *et al*., 1996). They have stated that nitrogen levels below or above 30.0 mM can adversely influence the cell growth pattern. All treatments in our study (Table 5) have shown significant increases in cells FW during the first 20 days of incubation.

**Anthocyanidin Analysis**

Anthocyanidin was extracted from leaves of *in vivo* and *in vitro* grown African violet (*Saintpaulia ionantha* Wendl.). Qualitative identification of cyanidin was performed via TLC and HPLC. TLC analysis had shown the presence of cyanidin in both *in vivo* and *in vitro* grown plants at retention factors ($R_f$) of 0.38 and 0.37, respectively compared to the standard cyanidin authentic sample (0.36). Cell suspension cultures had not shown any presence of cyanidin since no separation bands have arisen upon their spotting.

The identity and presence of cyanidin in the *in vivo* and *in vitro* grown *Saintpaulia ionantha* Wendl. leaves were confirmed using HPLC. Chromatograms of the six common aglycones were compared, on the basis of their retention time ($R_t$), with our prepared samples: *in vivo* and *in vitro* extracted pigment and cell suspension derived extracts (under light or dark conditions). Cyanidin was only detected in *in vivo* and *in vitro* pigment extracts. Extracts of suspended cells (light-or dark grown) did not show any presence of cyanidin. Takeda and Abe (1992) have reported a significant increase in anthocyanin level in *Daucus carota* L. suspension cultures with UV-B irradiation. Seitz and

Hinderer (1988) have suggested that light irradiation activates PAL, CHS and CHFI enzymes which are involved in anthocyanin biosynthesis.

The spectrophotometric spectra for the extracts of *in vivo* and *in vitro* plant leaves showed that their were three major absorption peaks characterized the *in vivo* and *in vitro* extracts. Both *in vivo* and *in vitro* extracts have shown high similarity with regards to their absorption spectra. The absorbance at 535 nm for *in vivo* and *in vitro* pigment extracts were (0.964) and (0.835), respectively. Cyanidin for *in vivo* extracts was 98.16 mg/ g of tissue and *in vitro* extracts was 85.04 mg/ g of tissue.

The presence of cyanidin in the leaves of *in vitro* grown *Saintpaulia ionantha* Wendl. suggests that the plant has the capability of producing this valuable pigment under controlled microenvironmental conditions (chemical composition of the media, PGRs, media pH, light irradiation and temperature). Therefore, it can be suggested that the production level of this secondary plant product could be enhanced via adjusting these chemical and physical conditions to meet the optimal requirements.

## Conclusions

In this study, a successful protocol has been set up for cell culture growth in African violet. Furthermore, a report of cyanidin extraction from leaves of *in vivo* and *in vitro* grown African violet (*Saintpaulia ionantha* Wendl.) has been set up for the first time. Several investigations are still needed to study the influence of various chemical and physical factors on the production of anthocyanins from cell suspension culture system and to develop, and commercialize this remarkable and promising venture of plant tissue culture.

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**Table 1. Effect of different levels of BA, 2,4-D or kinetin on cell growth of African violet (*Saintpaulia ionantha* Wendl.) during 30 days of incubation.**

| Time (Days) | C[z] | Cells FW (mg/ml) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | BA (µM) | | | 2,4-D (µM) | | | kinetin (µM) | | |
| | | 4.4 | 8.9 | 13.3 | 2.2 | 4.4 | 6.6 | 0.5 | 1.4 | 2.3 |
| 0 | 6.75 | 7.25 | 7.00 | 7.00 | 7.25 | 6.50 | 6.75 | 6.75 | 6.50 | 6.50 |
| 5 | 7.00 | 7.25 | 7.50 | 7.25 | 7.50 | 6.50 | 6.75 | 6.75 | 6.75 | 6.50 |
| 10 | 8.00 | 8.00 | 8.00 | 7.75 | 8.25 | 7.25 | 7.50 | 7.75 | 7.50 | 7.25 |
| 15 | 10.50 | 12.50 | 12.75 | 12.75 | 14.50 | 11.50 | 11.75 | 12.50 | 12.00 | 14.00 |
| 20 | 14.75 | 17.25 | 18.50 | 18.50 | 19.50 | 16.75 | 15.25 | 19.25 | 19.50 | 21.75 |
| 25 | 15.75 | 17.75 | 19.25 | 18.25 | 20.25 | 17.75 | 16.00 | 19.25 | 19.75 | 20.25 |
| 30 | 14.75 | 17.25 | 19.00 | 18.25 | 20.00 | 17.50 | 15.50 | 18.25 | 19.00 | 20.50 |
| ***LSD*** = 0.946 | | | | | | | | | | |

[z]= Control treatment without PGR.

**Table 2. Combined effects of BA and kinetin (with or without NAA) on cell growth of African violet (*Saintpaulia ionantha* Wendl.) after 30 days of incubation.**

| | Cells FW (mg/ml) | |
|---|---|---|
| **Combinations tested** | **BA + kin (with 0.54 µM NAA)** | **BA + kin (without 0.54 µM NAA)** |
| C [z] | 11.00 | 11.96 |
| 4.4:0.5[*] | 12.96 | 12.93 |
| 4.4:1.4 | 13.64 | 13.54 |
| 4.4:2.3 | 13.57 | 13.71 |
| 8.9:0.5 | 13.75 | 13.82 |
| 8.9:1.4 | 13.93 | 14.32 |
| 8.9:2.3 | 14.68 | 14.43 |
| 13.3:0.5 | 14.86 | 14.43 |
| 13.3:1.4 | 15.46 | 14.64 |
| 13.3:2.3 | 15.57 | 15.18 |
| ***LSD*** = 2.340 | | |

[z]= Control treatment without PGR.

* BA: Kin(**µ**M).

**Table 3. Combined effects of 2,4-D and kinetin (without NAA) on cell growth of African violet .(*Saintpaulia ionantha* Wendl.) after 30 days of incubation.**

| Cells FW (mg/ml) | |
|---|---|
| **Combinations tested** | **2,4-D + kin (without 0.54 µM NAA)** |
| C [z] | 11.50 |
| 2.2:0.5[*] | 13.54 |
| 2.2:1.4 | 15.21 |
| 2.2:2.3 | 14.32 |
| 4.4:0.5 | 14.50 |
| 4.4:1.4 | 14.29 |
| 4.4:2.3 | 14.82 |
| 6.6:0.5 | 12.93 |
| 6.6:1.4 | 13.93 |
| 6.6:2.3 | 13.57 |
| ***LSD* = 2.340** | |

[z]= Control treatment without PGR.

* 2.4-D: Kin (**µM).**

**Table 4. Effect of kinetin in combination with BA (with or without NAA) or 2,4-D on cell growth of African violet (*Saintpaulia ionantha* Wendl.) during 30 days of incubation.**

| | Cells FW (mg/ml) | | |
|---|---|---|---|
| ***Time (Days)*** | **BA + kin (13.3 µM+ 2.3 µM) (with 0.54 µM NAA)** | **BA + kin (13.3 µM+ 2.3 µM) (without 0.54 µM NAA)** | **2,4-D + kin (2.2 µM+ 1.4 µM) (without 0.54 µM NAA)** |
| 0 | 6.700 | 7.175 | 7.225 |
| 5 | 6.800 | 7.175 | 7.225 |
| 10 | 7.700 | 8.125 | 8.375 |
| 15 | 14.150 | 14.130 | 14.800 |
| 20 | 21.580 | 21.480 | 21.300 |
| 25 | 20.300 | 19.700 | 19.380 |
| 30<br>***LSD* = 1.086** | 20.380 | 19.500 | 18.850 |

**Table 5. Effect of different levels of nitrogen with various ($NH_4^+$ : $NO_3^-$) ratios on cell growth of African violet (*Saintpaulia ionantha* Wendl.) during 30 days of incubation.**

| | Cells FW (mg/ml)<br>($NH_4^+$ : $NO_3^-$) ratio [Z] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Time (Days)** | **C [Y]** | **0:30** | **10:20** | **20:10** | **30:0** | **0:60** | **20:40** | **40:20** | **60:0** |
| 0 | 6.25 | 6.75 | 6.50 | 6.50 | 7.25 | 6.50 | 6.25 | 7.75 | 6.75 |
| 5 | 6.25 | 6.75 | 6.75 | 6.75 | 7.25 | 6.50 | 6.50 | 7.75 | 7.00 |
| 10 | 7.50 | 8.00 | 8.00 | 7.75 | 8.50 | 7.75 | 7.50 | 9.00 | 8.00 |
| 15 | 11.75 | 12.25 | 15.75 | 13.00 | 13.00 | 13.25 | 17.00 | 12.50 | 11.75 |
| 20 | 16.50 | 19.75 | 25.00 | 20.25 | 18.00 | 21.25 | 22.25 | 20.00 | 18.75 |
| 25 | 15.25 | 18.50 | 23.00 | 18.75 | 16.25 | 19.50 | 20.75 | 18.75 | 16.50 |
| 30<br>**LSD = 1.080** | 14.50 | 17.75 | 22.50 | 19.25 | 15.50 | 19.00 | 20.25 | 18.75 | 15.50 |

[Z]= Media contained 2.2 µM 2,4-D, 1.4 µM kinetin and 88 mM sucrose.

[Y]= Control treatment in which no exogenous nitrogen was applied to the growth media.

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# زراعة الخلايا المعلقة وإنتاج المواد الثانوية من نبات البنفسج الافريقي (*Saintpaulia ionantha* **Wendl.)**

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## ملخـص

تم استحثاث الخلايا المعلقة لنبات البنفسج الإفريقي بواسطة زراعة الأجزاء النضرة من خلايا الكالوس على وسط غذائي لموراشيج وسكوج مضاف إليه منظمات النمو BA (4.4, 8.9, 13.3 µM ) kinetin (0.5, 1.4, or 2.3µM) و (2.2, 4.4 or 6.6 µM) 2,4-D أو تراكيز ونسب مختلفة من $NH_4^+$:$NO_3^-$ (30:0, 10:20, 20:10, 0:30) أو (0:60, 20:40, 40:20, 60:0). وقد تم أعلى نمو للخلايا على الوسط الغذائي المزود بتركيز kinetin (2.3µM) وأدت إضافة NAA (0.54 µM) إلى كل تراكيب منظمات النمو على تراكيز معينة إلى نمو أفضل للخلايا مقارنة مع تراكيب منظمات النمو الخالية من NAA.؛ بينما أدى استخدام 30 mM من النيتروجين الكلي على نسبة متدنية من $NH_4^+$:$NO_3^-$ إلى نمو أفضل للخلايا. أما المواد الثانوية مثل السيانيدين فقد تم استخلاصها من أوراق البنفسج الإفريقي المزروع خارج وداخل الأنابيب . وكان محتوى السيانيدين المستخلص من أوراق النبات الجافة المزروعة داخل الأنابيب 85.04 mg وكان محتواه في أوراق النبات الجافة المزروعة خارج الأنابيب 98.16 mg . ولم يتم استخلاص السيانيدين من الخلايا المعلقة.

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