

EFFECT OF 2,4-D CONCENTRATION ON ERGOMETRINE ACCUMULATION IN *Ipomoea alba* L. (CONVOLVULACEAE) CELL SUSPENSION CULTURE

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ABSTRACT

In order to find the best conditions for ergometrine accumulation in cellular suspensions of *Ipomoea alba* L., three different combinations of growth regulators were tested to determine their effect on the productivity and kinetic performance of these cells. The combinations studied for this were 0.453 μM 2,4-D + 0.093 μM kinetin, 0.905 μM 2,4-D + 0.093 μM kinetin and 1.357 μM 2,4-D + 0.093 μM kinetin, all of them with the Murashige and Skoog basal salt mixture (1) with 100mg/mL myo-inositol, 10 mg/mL thiamine-HCl and 30 g/l sucrose added. The pH of the medium was adjusted to 5.4 before sterilization (121 °C, 15 psi, 15 min). The first of the three combinations showed to be the one with the more stable and consistent productivity during the experiment; thus, it is recommended as an initial model for the determination of production parameters for scaling-up.

Key words: ergometrine, growing kinetic, *Ipomoea alba* L.

Biotecnología Aplicada 1996:13:98-100

RESUMEN

Con el propósito de obtener las mejores condiciones para la acumulación de ergometrina en suspensiones celulares de *Ipomoea alba* L., se utilizaron tres combinaciones diferentes de reguladores del crecimiento para determinar su efecto sobre la productividad y desarrollo de estas células. Las combinaciones evaluadas fueron: 0,453 μM 2,4-D + 0,093 μM cinetina; 0,905 μM 2,4-D + 0,093 μM cinetina y 1,357 μM 2,4-D + 0,093 μM cinetina, utilizando con todas el compuesto basal de sales de Murashige y Skoog y 100 mg/mL de myo-inositol, 10 mg/mL de tiamina-HCl y 30 g/l sacarosa. El pH del medio se ajustó a 5,4 antes de la esterilización (121 °C, 15 psi, 15 min). La primera de las tres combinaciones resultó ser la de productividad más estable y consistente durante el experimento; por lo que se recomienda ésta como un modelo inicial para la determinación de los parámetros productivos para el proceso de escalado.

Palabras claves: ergometrina, cinética de crecimiento, *Ipomoea alba* L.

Introduction

The use of plants with medical and ritual purposes in several native communities has lead to an intense research on the presence of some of the active principles that can be profitable to modern medicine, as well as their production at an industrial level. A potential alternative in this way, is the cellular, tissue and vegetable organs culture, which tends to accumulate good amounts of secondary metabolites of pharmacological value.

The existence of ergolinique alkaloids in some of the members of the Convolvulaceae family has been demonstrated since the 60's (1, 2), in the same way, the possibility that such compounds accumulate in *Ipomoea violacea* L. (3) and *Ipomoea alba* L. (4) tissue cultures is also a fact.

All ergolinique alkaloids have oxytocic activity in a higher or a lower degree, e.g. ergometrine shows a strong contraction activity on the upper part of the uterus, which is of great therapeutic value for the diminishment of the post-labor hemorrhages and as an adjuvant for uterus contraction in a faster and more efficient way.

When searching for a process for ergometrine production, the possibility of ergolinique alkaloids accumulation must be studied (especially of ergometrine) in *Ipomoea alba* L. cellular suspensions as well as the growth culture characteristics and alkaloid production with time.

Materials and Methods

To establish the starting cellular suspension callus, masses were cultured in semi-solid media, the medium used was the Murashige and Skoog salts base, with 3 % sucrose, 10 mg/mL thiamine, 0.907 μM 2,4 D and 0.0903 μM kinetin (5) added. once the calluses were formed, 1mg of these were submerged in a flask containing 50 mL of liquid medium formulated with the same Murashige and Skoog salts base (4) with 100mg/mL myo-inositol, 10 mg/mL thiamine, 30 g/L sucrose, 1.357 μM 2,4 diclorophenoxyacetic acid (2,4-D) and 0.093 μM kinetin added, adjusting the pH to 5.4. The cellular suspension obtained in this way was called inoculum, and was incubated during 28 days at room

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temperature under continuous light conditions, in a flash shaker at a speed of 120 rpm.

Three growth kinetic studies were accomplished, with the same medium formulation described above, varying only the growth factors in the following way:

	Treatment
1	0.453 μ M 2.4-D + 0.093 μ M kinetin
2	0.905 μ M 2.4-D + 0.093 μ M kinetin
3	1.357 μ M 2.4-D + 0.093 μ M kinetin

The sampling was established for each batch, inoculating each flask with 5 mL of the original cellular suspension, containing 1 mg of cells in suspension (measured in dry weight), the points of the graphs were evaluated at 0, 3, 8, 15, 23 and 31 days, for each point the biomass (measured in dry weight) was evaluated by filtering it through a 0.45 μ Millipore membrane maintained at a constant weight. The alkaloid production was determined by the method described by Reif (6), samples obtained from the inoculum suspensions and other samples from the kinetic experiments were submitted to the technique described by Granados Reyes *et al.* (7) for isolation of ergometrine by HPLC. Each point in the graphs for each combination was evaluated three times.

The graphs of growth, production and amount of alkaloid per dry tissue weight were established with the mean of each point.

Results and Discussion

The presence of alkaloids in each culture was detected by comparing its speed of release in HPLC against a blank of ergometrine maleate (Figure 1).

The growth kinetic studies (Figure 2) show that treatment 1 presents a lower yield in biomass,

reaching a maximum of 11 mg while the other two treatments can reach up to 14 mg in dry weight, on the other hand, treatment 2 reaches its maximum growth in 31 days, while treatment 3 reaches it at day 23 with a decrease in viable biomass at 31 days as biomass decreases to 11 mg.

In general, treatment 2 shows a tendency to have a more stable and longer growth period.

In relation to ergometrine production (Figure 3), treatment 1 is the most productive in comparison to the other two, as it reaches a maximum yield of 0.19 mg in 23 days, while treatment 2 shows its maximum in a similar period of time but with 0.0132 mg of alkaloid and treatment 3 with 0.06 in 31 days. In the first two treatments the maximum yield of growth and alkaloid production do not coincide, but in treatment 3 they correspond.

Productivity, measured in alkaloid weight per unit of tissue weight (Figure 4) shows that treatment 1 is the most productive of the three and it shows the highest consistency, as in the case of the other two, the alkaloid yield seems to be higher in the initial phases than during the growth period, showing also, in the case of treatment 2, a sudden decrease in productivity.

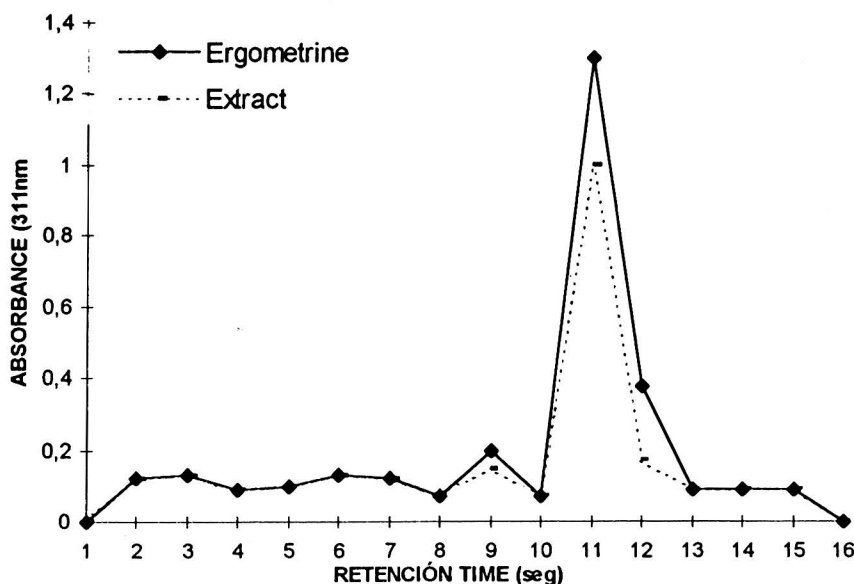
The results described above show that treatment 1 (0.453 μ M 2.4-D + 0.093 μ M kinetin) although it does not produce the highest biomass, it seems to be the most adequate for ergometrine production, as it has a better stability and productivity of the alkaloid. The possibility of improving the process exists, by varying some parameters to increase the cellular production, maintaining the ergometrine production characteristics.

Given the production characteristics shown, this represents an initial experimental model, on which there is still much work to be done, to take it to an industrial scale and for alkaloid production.

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Figure 1. HPLC Graph where the blank is shown for an extract of the cellular suspension vs. an ergometrine maleate blank. In the X axis the retention time is shown and in the Y axis the absorbance at 311 nm.



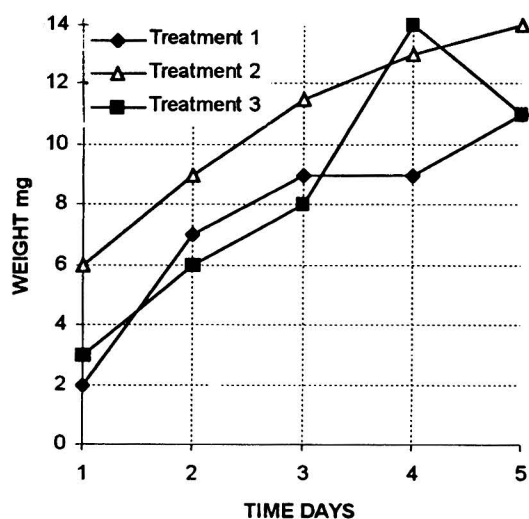


Figure 2. Biomass production accumulated in time vs. the change in 2.4-D concentration. In the X axis the running time of the kinetic is shown (days) and in the Y axis the biomass (mg).

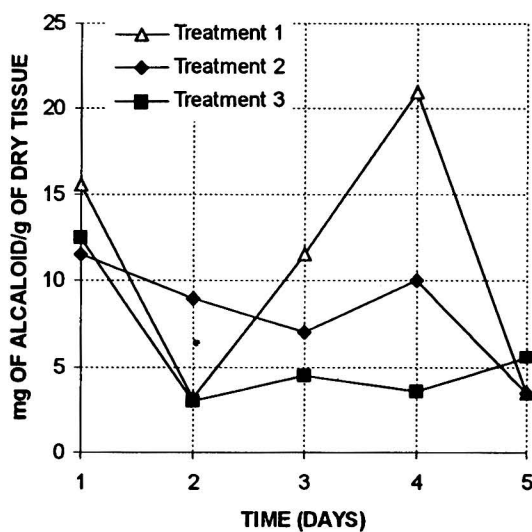


Figure 3. Ergometrine production in cellular suspensions vs. the change in the 2.4-D concentration. In the X axis the running time of the experiment is shown (days) and in the Y axis the ergometrine concentration (mg).

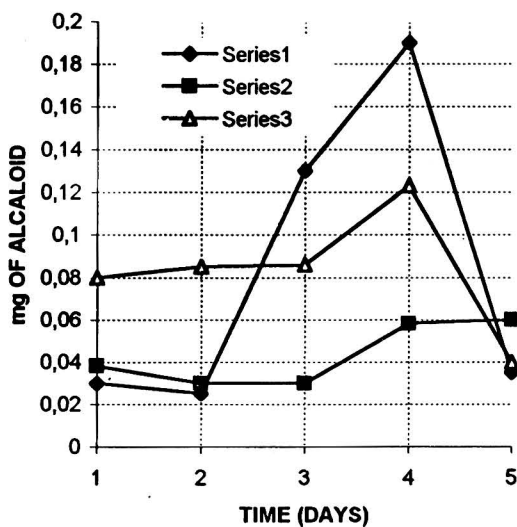


Figure 4. Effect of ergometrine accumulation vs. the suspension biomass for each treatment of variation of 2.4-D concentration. In the X axis the running time of the experiment is shown (days) and in the Y axis, the ergometrine production (mg/g) in dry tissue of the cellular suspension.

Recibido en junio de 1995. Aprobado en noviembre de 1995.