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Research Article

# Kinetics and modeling of cell growth for potential anthocyanin induction in cultures of *Taraxacum officinale* G.H. Weber ex Wiggers (Dandelion) *in vitro*

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ABSTRACT

*Background: Taraxacum officinale* G.H. Weber ex Wiggers is a wild plant used in folk medicine to treat several diseases owing to bioactive secondary metabolites present in its tissue. The accumulation of such molecules in plant cells can occur as a response against abiotic stress, but these metabolites are often deposited in low concentrations. For this reason, the use of a biotechnological approach to improve the yields of technologically interesting bioactive compounds such as anthocyanins is a compelling option. This work focuses on investigating the potential of *in vitro T. officinale* cultures as an anthocyanin source.
*Results:* To demonstrate the suitability of anthocyanin induction and accumulation in calluses under specific conditions, anthocyanin was induced in the *T. officinale* callus. A specific medium of 5.5% sucrose supplemented with 6-benzylaminopurine /1-naphthaleneacetic acid in a 10:1 ratio was used to produce an anthocyanin yield of 1.23 mg $g^{-1}$ fw. An *in vitro* dandelion callus line was established from this experiment. Five mathematical models were then used to objectively and predictably explain the growth of anthocyanin-induced calluses from *T. officinale.* Of these models, the Richards model offered the most suitable representation of anthocyanin callus growth in a solid medium and permitted the calculation of the corresponding kinetic parameters.
*Conclusions:* The findings demonstrate the potential of an *in vitro* anthocyanin-induced callus line from *T. officinale* as an industrial anthocyanin source.
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## 1. Introduction

In the current industrial and scientific research communities, there is a strong trend that focuses on the extraction and production of bioactive chemicals with a high added value, such as anthocyanins, which are natural dyes belonging to the family of polyphenolic compounds [1] that are largely responsible for blue, purple, red, and orange coloration in higher plants. These anthocyanin compounds exhibit free radical scavenging [2,3], anti-carcinogenic [4], antidiabetic, and cardioprotective properties, among other medicinal benefits [5,6,7].

Unlike other phytochemicals present in the biological matrix, anthocyanin biosynthesis can be promoted through external stimulus and stress [8]. This is particularly interesting because it suggests that there is a high industrial potential for anthocyanin stimulation and accumulation in plants or cells cultures under controlled conditions.

However, anthocyanin industrial applications are limited due to low extraction yields and reduced stability. Current research has focused

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on developing a biotechnological anthocyanin source, improving the yields of vegetable-based tissue cell cultures as much as possible. This has been the primary focus because cell culture practices are not limited by seasonal conditions, which allows for stable phytochemical productions under controlled conditions, and *in vitro* cultures permit the development of different biosynthesis induction strategies for metabolites of interest.

Numerous papers have been published related to *in vitro* induction of anthocyanin biosynthesis in species [8,9] such as *Vitis vinifera* [10], *Solanum melongena* [11], *Daucus carota* [12], *Raphanus sativus* [13], and *Ipomoea batatas* [14], among others. Various anthocyanin induction strategies used in cell cultures have also been investigated, including photo-induction, osmotic stress with salts or sugars, changes in temperature or addition of growth regulators, and variation in $NH_4^+$ -N concentration or heavy metals [8,9]. However, anthocyanin production from *Taraxacum officinale* (common dandelion), a plant traditionally considered medicinal, has scarcely been investigated [15]. The effects of radiation [16], growth regulators [17], and heavy metals [18] on dandelion have been studied, and some authors have concluded that a correlation exists between the activity of the enzyme chalcone synthase and anthocyanin production in *in vitro* cultures [19].

Depending on this information, we formulated the hypothesis that callus from hypocotyl explants can be utilized as a potential tissue for induced anthocyanin under controlled culture conditions (for instance, different carbon sources and light regimes). Moreover, it was possible to relate a mathematical model representing callus growth versus anthocyanin concentration. Thus, this work establishes an in vitro culture method from *T. officinale* explants that produce anthocyanin-induced (AI) calluses for biotechnological purposes.

## 2. Material and Methods

### 2.1. Chemicals

All reagents were of analytical or HPLC grade and were purchased from PhytoTechnology Laboratories, Duchefa Biochemie, Sigma Aldrich, or Merck.

### 2.2. Plant Materials

Leaf explants were obtained from 5-month-old *T. officinale* plants maintained in a greenhouse at the Institute of Plant Biology and Biotechnology from the Westphalian Wilhelms–University of Münster. Experiments were conducted there and at the School of Biochemical Engineering of the Pontifical Catholic University of Valparaíso. Leaves were thoroughly washed under running tap water for 3 min. For disinfection, 2 $cm^2$ explants were soaked in 70% ethanol for 30 s and then in 6% sodium hypochlorite (NaOCl) with a few drops of Tween-20 for 10 min. The explants were then rinsed in triplicate with sterilized double-distilled water. All procedures were performed under aseptic conditions.

**Table 1**
Experimental design used for anthocyanin induction of *Taraxacum officinale* callus (a simple factorial design using type and concentration of carbon source and growth regulators as variables in combination).

| Carbon source | | Carbon source concentration (%) | | BAP[a] –NAA[b] (ppm) |
|---|---|---|---|---|
| Glucose | x | 1.0 | x | 1.0–0.225 |
| Sucrose | | 2.3 | | 2.0–0.225 |
| | | 3.2 | | 1.0–0.125 |
| | | 5.5 | | 2.0–0.125 |

Total treatments: $2 * 4 * 4 = 32$
[a] BAP: 6-Benzylaminopurine acid;
[b] NAA: 1-Naphtaleneacetic acid.

### 2.3. Callus cultivation

The callus culture was induced from sterile explants sown in Murashige and Skoog medium supplemented with vitamins (4.4 g $L^{-1}$) [20] and solidified with 0.7% (w/v) agar-agar in petri dishes. The medium was also supplemented with either sucrose or glucose at concentrations ranging from 1.0% to 5.5% (w/v) and both 1-naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BAP), as displayed in Table 1. The pH was adjusted to 5.8 ± 0.1 with 0.1 N KOH. Five explants were placed in each petri dish, with 15 explants per induction medium. Explants were maintained under controlled conditions at 21°C and under a 16/8 h (light/dark) photoperiod with a light intensity of 20 μMol/ $m^{-2}$ $s^{-1}$. Explants were transferred to a fresh medium every 2 weeks until the callus reached a considerable biomass (approx. 5-10 mm diameter).

After induction, the callus was isolated from the explant and initially propagated by transferring the biomass to a fresh medium for 2 weeks. After successive subculture, potential anthocyanin accumulation was evaluated in selected red cell aggregates. To evaluate callus characteristics and quality during the induction and propagation stages, three aspects were considered: friability/compactness, color, and embryogenic capacity. To measure friability/compactness, the callus was distressed with tweezers to remove the easily friable portions, and the mass of the remaining non-friable callus was used to calculate the friability/compactness percentage (0%-33% = low friability, 34%-66% = medium friability, and 67%-100% = high friability). Callus color was defined as yellow, green, dark green, or pink/purple. Embryogenic capacity was observed with a confocal microscope (60x) (Leica Microsystems) (Fig. 1).The control callus was defined as the callus without pink/purple coloration. This callus line was propagated and maintained in a medium supplemented with sucrose 2.3%, 0.225 ppm BAP, and 0.225 ppm NAA.

### 2.4. Growth Parameters

Cultures showing potential anthocyanin induction were harvested to identify possible anthocyanin accumulation. The fresh weight (fw, g) of the callus was recorded every 2 weeks. For dry weight (dw, g) determination, a callus sample was dried at 55°C and moisture (%) was determined gravimetrically. The callus growth index was calculated as $W/W_o$, where W and $W_o$ are the fresh weights at the beginning and the end of the growth period (1 week), respectively. The specific productivities, with regard to secondary metabolite of interest and biomass, are defined as variation in the product obtained (ΔP, mg $g^{-1}$ dw) and the dry cellular matter (ΔX, g dw) at corresponding time intervals (Δt, weeks).

Growth rate (μ) and duplication time ($t_d$) were calculated using [Equation 1] [21]

$$lnX/X_0 = \mu\, t \quad [1]$$

where $X_0$ and $X$ are the initial ($t = 0$) and the final ($t \neq 0$) dry and fresh matter content (g), respectively; $t$ is the time (days or weeks); and $\mu$ is the maximum cellular growth velocity (days$^{-1}$ or weeks$^{-1}$).

The doubling cellular time $t_d$ (days or weeks) was calculated using [Equation 2].

$$t_d = \ln 2/\mu \quad [2]$$

### 2.5. Anthocyanin extraction

The freeze-dried callus sample (approx. 350 mg to 400 mg) was ground with a pestle and mortar. Anthocyanins were extracted twice by mixing 10 ml of methanol acidified with 1.0 N HCl (85:15, v/v) and shaken at 4°C for 6 h. Crude extracts were mixed and filtered