



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 cm² 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_0 , where W and W_0 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]

$$\ln X/X_0 = \mu t \quad [1]$$

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 μ 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

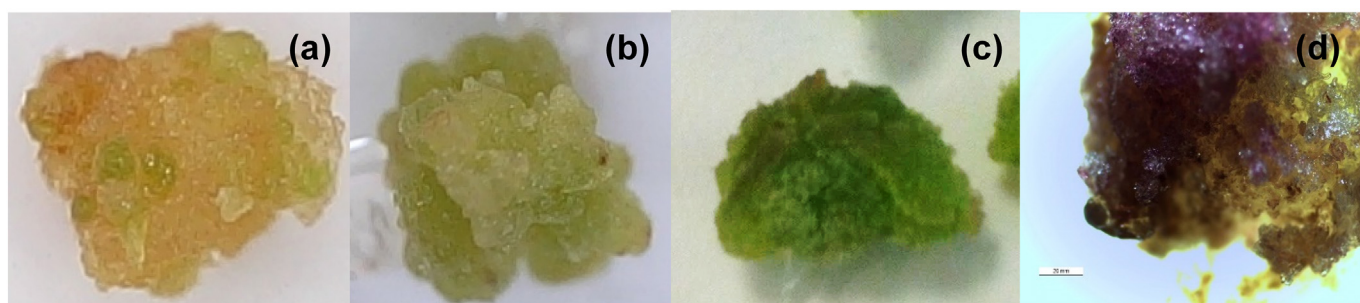


Fig. 1. Visual color scale used during callus growth and anthocyanin induction (-20 mm): (a) Yellow, (b) Green, (c) Dark green, and (d) Pink/Purple.

Table 2

Comparison of the performance and kinetic parameters (values of A, B, k, d, and m) calculated for each growth model fitted to the *in vitro Taraxacum officinale* used in this study.

Model Type	Equation	Kinetic parameters of cell growth				
		A	B	k	d	m
Von Bertalanffy	$y = A * (1 - Be^{-kt})^m + d$	31.37	1.65	0.11	4.59	1.00
Logistic	$y = A * (1 + e^{-kt})^{-m} + d$	30.18	1.00	0.83	2.72	17.656
Logistic modified	$y = A * (1 + Be^{-kt})^{-m} + d$	30.53	$6.68 * 10^{16}$	7.62	-0.52	0.069
Gompertz	$y = A * e^{mBe(-kt)} + d$	30.14	-16.36	0.81	2.80	1.00
Richards	$y = A * (1 - Be^{-kt})^{-m} + d$	30.32	$-2.47 * 10^{12}$	5.66	-0.29	0.096

using Whatman paper No. 1. Sample extractions were replicated in triplicate.

2.6. Total monomeric anthocyanin determination

The monomeric anthocyanin pigment content in dandelion extracts was determined by the pH-differential method [22]. A Jenway 6715 UV-VIS spectrophotometer and 1 cm^{-1} path length cuvettes were used for spectral measurements at 510 nm and 700 nm. Pigment content was calculated as milligrams of cyanidin-3-glucoside per 100 g of fresh weight using the extinction coefficient (ϵ) $26,900 \text{ M}^{-1} \text{ cm}^{-1}$ and a molecular weight of 449.2 g mol^{-1} . The final concentration of anthocyanin was calculated depending on the total volume of the extract and sample weight used. Thin-layer chromatography (TLC) was conducted for anthocyanin identification at an early stage.

2.7. Statistical analysis and modeling of experimental data

Statistical analysis and model approximation of callus growth were performed using Statgraphics® XV Centurion and IBM SPSS v.21 trial software.

Five candidate models were adjusted to the cell growth data using the dry matter, anthocyanin induction, growth period, and growth rate data obtained after 5 weeks of *in vitro* dandelion callus culture. The Von Bertalanffy, Logistic, Logistic modified, Gompertz, and Richards models were used owing to the flexibility displayed when used with other plant crops; however, this work represents the first analysis of *T. officinale*. An optimal model was selected according to the quality of fit, with data statistically examined by the coefficient of determination (R^2), which reflected the total variability explained by the model and considered all the parameters to be estimated, and the sum of squared error (SSE), which analyzed the accuracy of the estimators.

The experimental data were subjected to simple linear regression (RLS), multiple linear regression (RLM), and analysis of variance (ANOVA). Tukey's test was used to compare the means of every treatment to the means of every other treatment. F-values and p-values were used for evaluating statistical significance with a 95% confidence interval for all analyses.

The adjustment of data with nonlinear models to explain callus growth and the possible correlation of kinetic parameters was

performed using the Levenberg–Marquardt algorithm. The kinetic parameters of AI callus growth were defined as follows: A (% w/w), the maximum cell growth potential (the asymptotic value reached by the AI callus cells in terms of dry matter); t and k , the growth period (days or weeks) and velocity (weeks^{-1}), respectively; B and m, the parameters that determined the curve form (sigmoidal); and d, a dimensionless constant. These parameters are summarized in Table 2.

The kinetics curve of AI callus growth was fitted with Solver/Excel Microsoft Platform using GRG nonlinear as the resolution method. The fit criteria were the minimum value of the SSE, and the goodness of fit of each mathematical model was determined using linear regression analysis.

Table 3

Summary of the callus characteristics under the tested induction conditions.

Carbon source	Carbon source concentration ^c	BAP ^a –NAA ^b (ppm)	Anthocyanin induction	Callus characteristics
Glucose	1.0% and 2.3%	1.0 – 0.125	No	Pale green, soft, and friable
		1.0 – 0.225	No	Pale green, soft, and friable
		2.0 – 0.125	No	Pale green, soft, and friable
		2.0 – 0.225	No	Green, mainly soft but with compact spots
	3.2% and 5.5%	1.0 – 0.125	No	Green, compact areas
		1.0 – 0.225	No	Dark green, compact areas
		2.0 – 0.125	No	Green, compact areas
		2.0 – 0.225	No	Dark green, compact areas
Sucrose	1.0% and 2.3%	1.0 – 0.125	No	Green, soft
		1.0 – 0.225	No	Green, mainly soft but with compact spots
		2.0 – 0.125	No	Green, soft, and friable
		2.0 – 0.225	No	Green, mainly soft but with compact spots
	3.2% and 5.5%	1.0 – 0.125	No	Green, compact areas
		1.0 – 0.225	No	Dark green, compact areas
		2.0 – 0.125	No	Green, compact areas
		2.0 – 0.225	Yes	Green, mainly soft but with compact areas. Pink spots appeared with 5.5% sucrose.

^a BAP: 6-Benzylaminopurine; ^b NAA: 1-Naphthaleneacetic acid; ^c Similar results were obtained for 1.0% and 2.3% and for 3.2% and 5.5% of the carbon source.

Table 4

ANOVA for carbon source (type and concentration, CS) and hormone treatments (PGR) regarding the callus characteristics (color and friability).

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
CS treatment	33.52	3	11.17	F(3, 608) = 8.857	$P < 0.0001^*$
PGR treatment	664.5	7	94.93	F(7, 608) = 75.25	$P < 0.0001^*$
CS × PGR	61.73	21	2.940	F(21, 608) = 2.330	$P = 0.0007^*$
Residual	767.0	608	1.262		

CS: Carbon source; PGR: plant growth regulators; *significance values $P < 0.05$.

3. Results and Discussion

3.1. Callus cultivation

An early callus induction and biomass accumulation response was noticed in leaf explants when the MS medium was fortified with glucose and sucrose (indistinctly) at concentrations of 1.0% and 2.3%, respectively, and supplemented with an exogenous application of both growth regulators at concentrations of 1.0 ppm to 2.0 ppm of BAP and 0.125 ppm to 0.225 ppm of NAA. Observations suggested that glucose was slightly better than sucrose for inducing more a friable callus. A more compact callus and an intensely colored biomass were observed in media with higher sugar content. No important embryogenic areas were detected under the microscope for all tested conditions.

Concerning carbon supplementation, despite the extensive usage of sucrose in nutritive media, glucose produced better results in terms of induction, callus proliferation and growth, and organogenesis because of its osmotic potential [23,24]. Michel et al. [25] reported that glucose led to better callus induction in cotton explants than sucrose, fructose, galactose, or maltose. Additionally, better callus proliferation and less browning occurred with glucose (similarly observation observed during this work). These authors also claimed that glucose produced a more friable, voluminous callus than other sugars.

Earlier studies indicated that sugars such as sucrose and glucose are necessary substrates for anthocyanin biosynthesis. The pink cell coloration was possibly produced owing to an osmotic effect caused by sucrose [26]. This sugar can also promote the production of precursors of anthocyanins or phenolics like phenylalanine [27]. However, some authors have indicated that this pigmentation is not permanent and cell coloration was observed regressing to the original hue [27,28].

A summary of the callus characteristics is presented in Table 2. Results showed that all treatments evaluated were suitable for callus induction and proliferation throughout the experimental period. However, possible anthocyanin accumulation (observed as pink spots) was only induced in a highly osmotic medium (sucrose 5.5%) (Table 3).

The use of sucrose as a possible inducer and its positive effect on anthocyanin biosynthesis were previously reported in cellular cultures

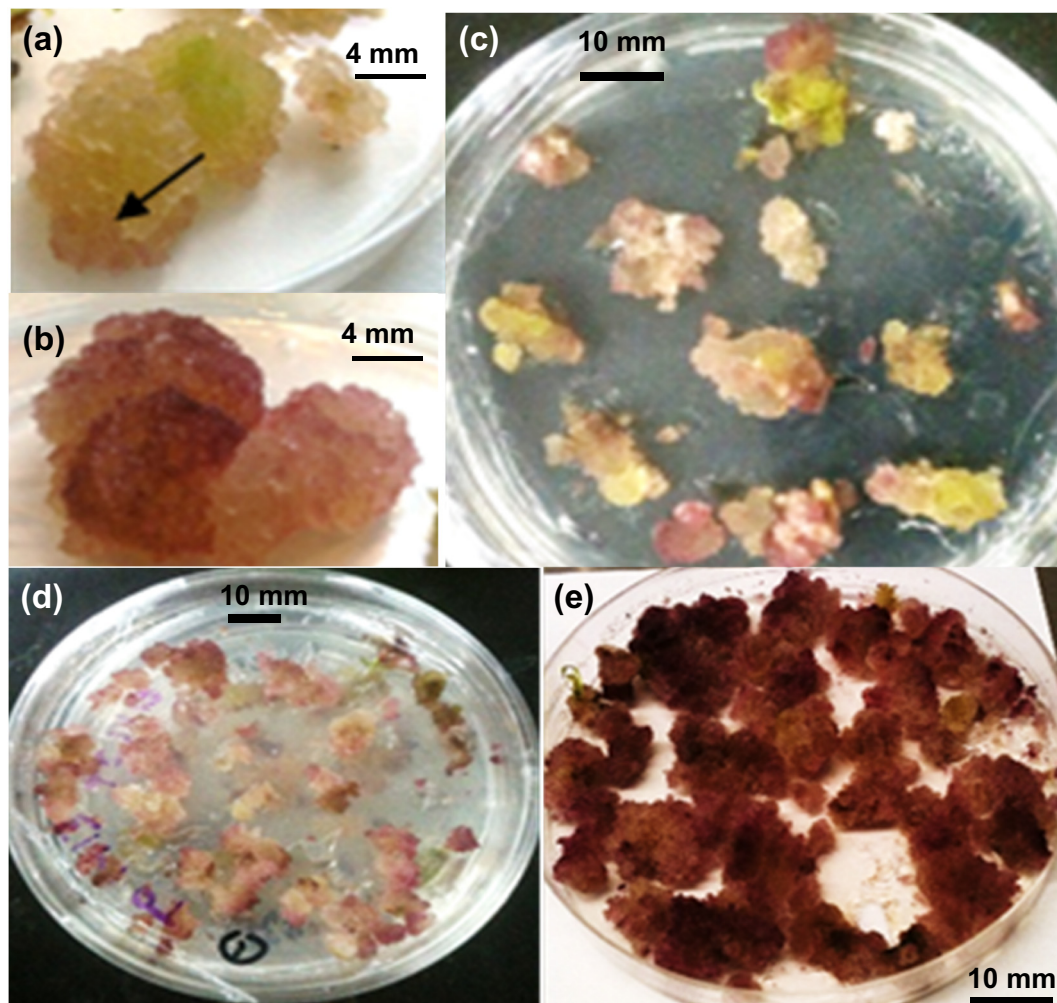


Fig. 2. Stereoscopic observation of *in vitro* anthocyanin-induced callus at week (a) 0, (b) 2, (c) 3, (d) 4, and (e) 5 after transferring to a propagation MS medium supplemented with 5.5% sucrose with 2.0 ppm BAP and 0.225 ppm NAA.

or *in vitro* species such as *Camptotheca acuminata* [29], *Arabidopsis thaliana* [30], *I. batatas* [14], and *D. carota* [27], among others. In the two latter cases, the total anthocyanin content approached the maximum with 5% sucrose concentration in the medium. In the particular case of *T. officinale*, anthocyanin was induced with a sucrose concentration of 5.5% (Table 2).

However, cells turned pink only with an application of a 10:1 ratio of hormone. This could mean that anthocyanin biosynthesis was promoted by a synergistic effect between sucrose and hormone treatments. Light could also be a key factor in promoting the synthesis of anthocyanin pigments, as suggested by some authors [28]. Chaudhary and Mukhopadhyay [11] observed that, in *S. melongena* callus, purple cells appeared when light intensity increased to 4000 lux, and Ryu et al. [16] concluded that the light was effective in anthocyanin accumulation in *T. officinale* leaves. Blando et al. [31] considered light as a triggering factor in the enhancement of pigment production in *Prunus cerasus* L. callus, thus obtaining a yield of 45 $\mu\text{g g}^{-1}$ fw (using sucrose in ranges from 3% to 7% and a 1:10 ratio of BAP/NAA).

However, the mechanism that activates anthocyanin biosynthesis in cells remains unclear. This study demonstrated that the media required for callus induction and anthocyanin production were different. This preference could be due to a shift from the growth state to the metabolite production state in cells, as suggested by Simoes et al. [9].

The statistical analysis of data considered exogenous treatments and evaluated the influence concentration, carbon source, and NAA/BAP supplementation effect on callus characteristics. Carbon type and concentration were the most important variables at the induction stage, thereby significantly influencing callus color and friability ($P < 0.001$). Tukey's test indicates that the carbon source influenced the process with similar results, between 1.0% and 2.3% and between 3.2% and 5.5%. The PGR treatment also greatly influenced ($P < 0.001$) the callus induction process, thus displaying similar results within the following groups: 1.0 to 0.125 ppm BAP-NAA, 1.0 to 0.225 ppm BAP-NAA, 2.0 to

0.125 ppm BAP-NAA, and 2.0 to 0.225 ppm BAP-NAA, considering both sucrose and glucose (Table 4).

In general, callus induction was complete by the third or fourth week after sowing under all conditions tested. Although callus size was not a variable considered for statistical analysis, it should be mentioned that the callus diameter was influenced by the carbon concentration: concentrations of 1.0%–2.3% resulted in 15–20 mm diameters, whereas 3.2%–5.5% concentrations displayed 5–10 mm diameters, for both glucose and sucrose indistinctly.

In previous experiments, we also evaluated the absence of light on the proliferation of calluses and the induction of anthocyanins considering the same experimental treatments, thereby resulting in the fact that the induction and growth of calluses were even better than those occurring in light; however, the possible induction of anthocyanins was not observed (data not included in this work).

3.2. Potential anthocyanin induction

The influence of varied glucose and sucrose levels on potential anthocyanin induction and accumulation on the *T. officinale* callus indicated that the earliest expression was observed when the MS medium was supplemented with 5.5% sucrose, 2.0 ppm BAP, and 0.225 ppm NAA. Small pink spots appeared on the explant surface within 4 weeks since it was sown. Growth of the AI callus during the 5-week period and AI callus and control cells are displayed in Fig. 2 and Fig. 3, respectively.

Small pink spots were present only on the surface of the AI callus (Fig. 2a), whereas no pigmentation was observed on the control callus (Fig. 2b). As expected, the AI callus exhibited pink coloration within the cells (Fig. 2c) according to an intracellular accumulation of these compounds, whereas control cells did not show any coloration under the microscope (60X) (Fig. 3c and d). No culture medium coloration was observed during the experimental period.

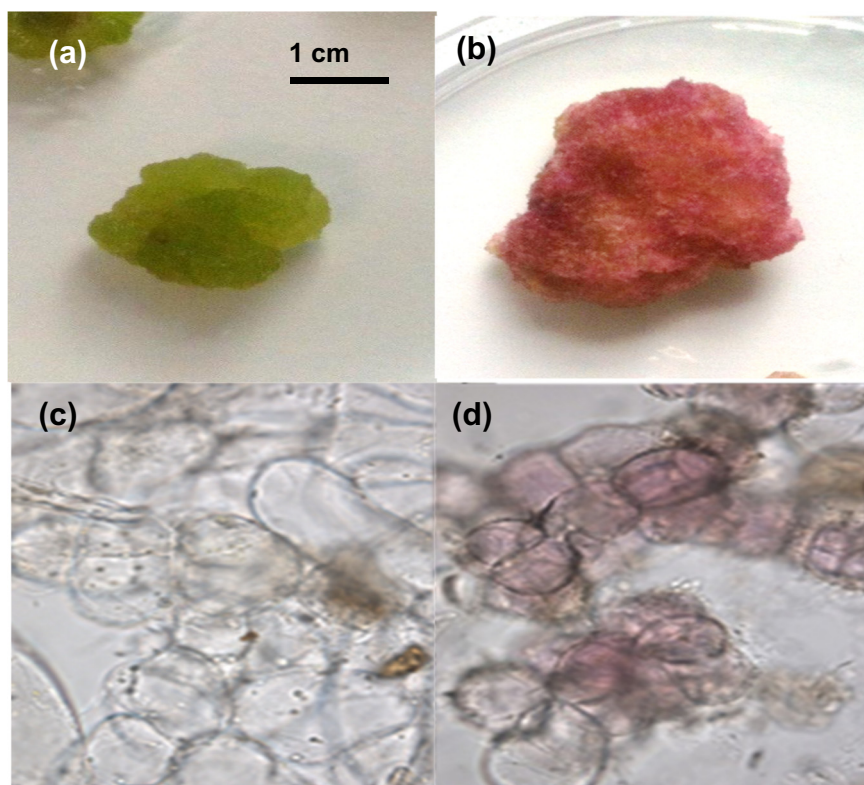


Fig. 3. Stereomicroscopic observation of *in vitro* (a) Control and (b) and anthocyanin-induced *T. officinale* callus on an MS culture medium supplemented with 5.5% sucrose with 2.0 ppm BAP and 0.225 ppm NAA (5 d), and (c) and (d) their respective cells in solution (60x).

Intense red coloration and a high quantity of anthocyanin were observed in cultures with 5.5% sucrose concentration, with the red pigmentation intensifying as the days progressed (Fig. 2e and Fig. 3b).

Isolated pink callus portions were transferred to the propagation medium and grown normally for 2 weeks, thus showing a considerable increase in biomass. Cell proliferation was primarily observed at the bottom of callus masses as an increment in the nonpigmented area, whereas the pigmented surface acquired a stronger purple coloration. As pigmentation expanded across the surface, callus growth decreased and dark, compact cells appeared. The callus weight did not increase during the ensuing third and fourth subcultures. This increased coloration was also related to a change in the aspect of the callus: the pigmented areas became dry and compact, whereas the nonpigmented areas remained soft and friable. A hard external coat was observed on the pigmented callus compared to the control, which maintained its softness, friability, and continuous growth rate.

From the sixth through the eighth week of propagation, pigmentation reversion was observed in callus masses, with the naturally pink/purple surface turning a green/white color. Callus masses lost the pink pigmentation owing to the loss of anthocyanin, and eventually, only green masses remained during continuous growth. The pigment reversion of the AI callus during the experiment and respective TLC analysis are presented in Fig. 4.

The finding that anthocyanins act as the pigment responsible for the pink pigmentation in dandelion callus is concordant with that reported in the study conducted by Akashi et al. [19]. In this study, authors also achieved callus formation and established the production of anthocyanins with a medium specifically supplemented with kinetin and 2,4-dichlorophenoxyacetic acid (1:10) resulted in a pigment content of 0.05 % of fresh weight. In the case of *S. melongena* [11],

however, the combination of NAA and BAP did not yield a positive effect on pigment induction. Instead, a combination of NAA and kinetin resulted in an anthocyanin yield of $70 \mu\text{g g}^{-1}$ fw after 8 weeks. In both cases, the values obtained in this investigation were higher than those reported in this studies.

When the callus masses lost the pink pigmentation, further experiments at lower BAP/NAA ratios (1:5 and 1:10) were conducted. Then, new, more intensely colored pink spots were observed on cell surfaces, thus indicating that anthocyanin induction capacity was not lost but unstable. Once callus masses were sown in a new, enriched culture medium, pigment production on the surface resumed.

Several studies have consistently demonstrated that high concentrations of auxin and cytokinin block or reduce anthocyanin production in callus cultures [32].

Accumulation of these compounds on the callus surface might impede cells from reaching a proper mass and gas transfer within the environment. This last point can be corroborated using a suspension culture in which cells are directly exposed to the medium and the mass and gas transfer is performed in smaller aggregates or individual cells. Anthocyanin accumulation in *Taraxacum* callus under the proposed conditions was previously indicated as a possible detriment to growth because of the hard, pigmented cell coating that developed over the callus surface, but no reports regarding this observation in a solid medium were found in the literature.

An alternative explanation for decreased AI callus growth compared to the control callus could be that anthocyanin production and growth are unrelated and occur (total or partially) through separate metabolic pathways. For example, strawberry suspension cell growth and anthocyanin production are only partially linked, but this behavior has not yet been clarified [32].

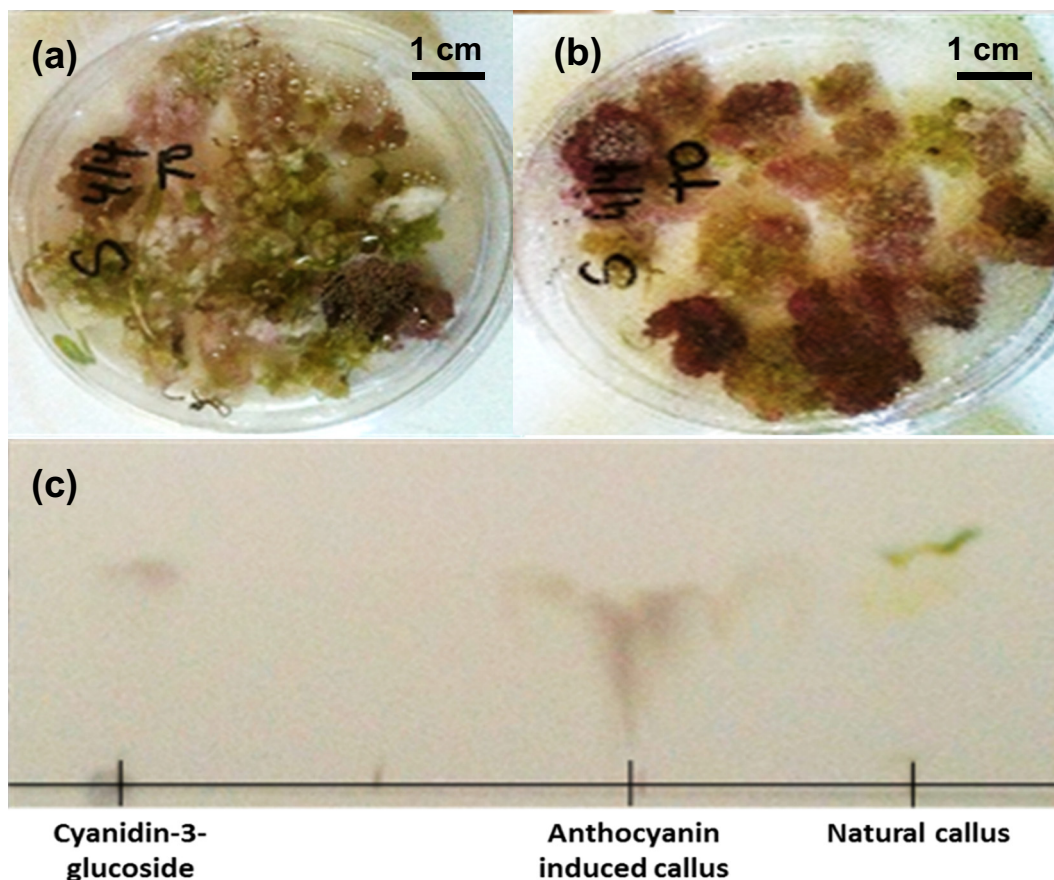


Fig. 4. Stereomicroscopic observation of *in vitro* anthocyanin-induced callus masses at week (a) 6 and (b) 8 of culture on an MS culture medium supplemented with 5.5% sucrose with 2.0 ppm BAP and 0.225 ppm NAA; (c) Thin-layer chromatography (TLC) analysis for anthocyanin-induced and natural callus.

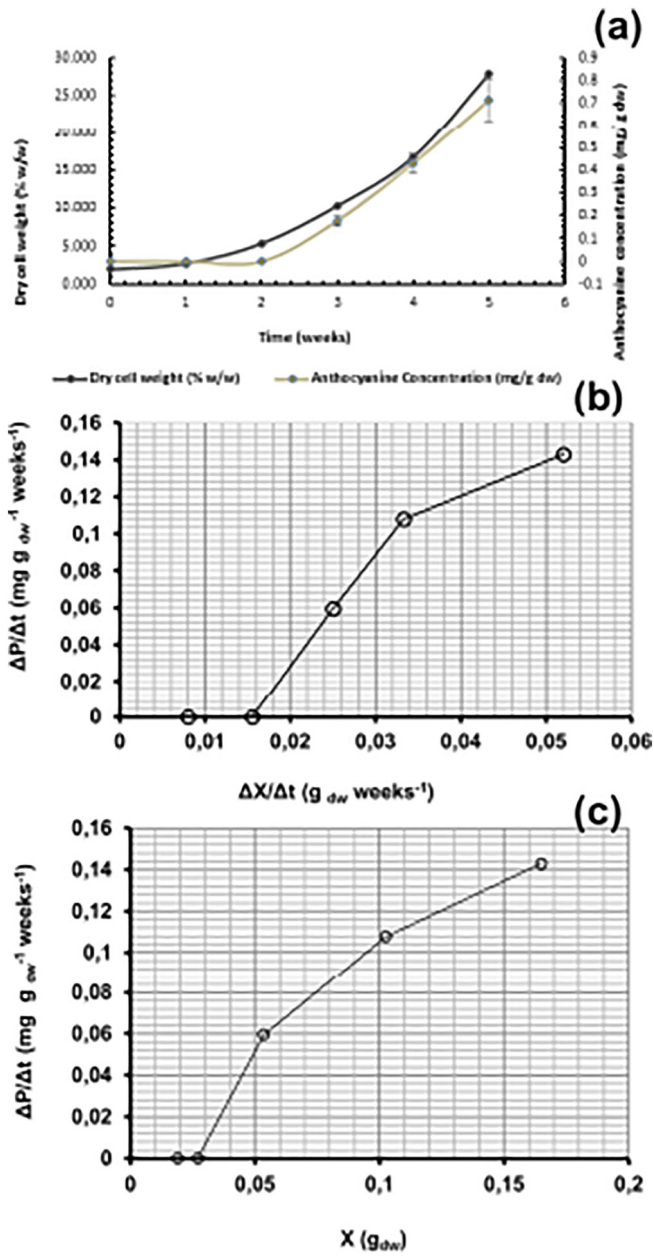


Fig. 5. (a) Growth and anthocyanin production in pigmented cultures of *T. officinale* callus at 3, 4, and 5 weeks of culture; (b) Anthocyanin- vs. biomass-specific productivities; (c) Anthocyanin-specific productivity vs. dry cell matter.

The typical growth patterns of *T. officinale* callus under optimal anthocyanin formation and cell growth conditions at different culture periods are shown in Fig. 5a and b, which indicates the specific productivities of anthocyanin and biomass. There was no detectable concentration of possible anthocyanin pigments until the third week, with an initial concentration of 0.179 mg g⁻¹ dw. A maximum concentration of 0.716 mg g⁻¹ dw was achieved on the fifth week (Fig. 5c). Total amounts of anthocyanin in cells cultured with medium optimized for anthocyanin formation increased cell growth. No further quantification could be performed from the sixth week onward owing to partial or total necrosis of the AI callus; therefore, the detection of the maximum anthocyanin content in cells during the stationary phase was not possible.

The plots shown in Fig. 5b ($\Delta P/\Delta t$ vs $\Delta X/\Delta t$) and 5c ($\Delta P/\Delta t$ vs X) indicate that a linear correlation between the respective specific productivities does not exist. This could mean that anthocyanin

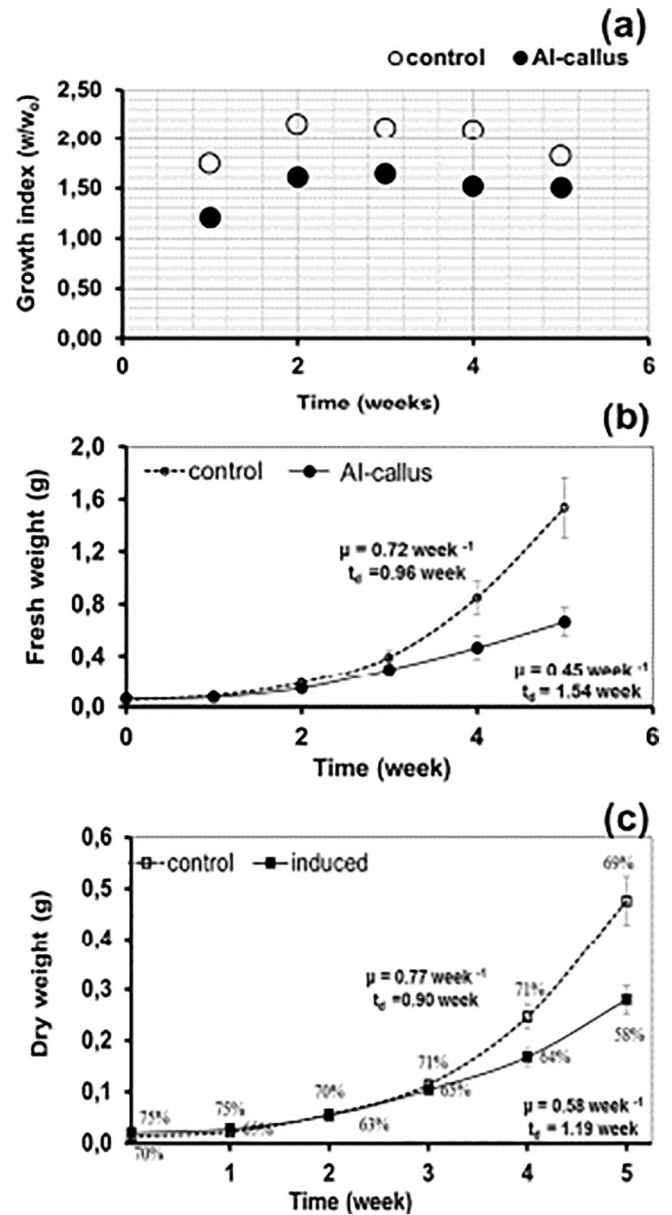


Fig. 6. (a) Variation with regard to the time of growth index for control and anthocyanin-induced calluses in fresh weight; (b) and (c) Biomass growth curves (AI-callus and control), growth rate (μ), and duplication time (t_d) in dry and fresh weights, respectively. Labels present in (c) indicate moisture percentage.

biosynthesis is not associated with cellular growth or that anthocyanin synthesis is detrimental to cellular growth on a solid medium.

Similarly, the growth indices (W/W_0) and curves for control and AI callus fresh weight (Fig. 6a) demonstrate that the AI callus grew lesser than the control callus. These results indicate that anthocyanin induction is linked to stress conditions, thus suggesting that it could have a detrimental effect on the final biomass growth (Fig. 6a). This was further corroborated in the sixth to seventh week of growth, when most AI callus masses exhibited necrosis. Compared to the growth rate (μ) and duplication time (t_d) of the control, the AI callus had the slowest growth (Fig. 6b). During the 5-week post-transfer period, both cultures displayed in their curves an exponential trend that began on the third week, with μ values of 0.77 week⁻¹ and 0.58 week⁻¹ in dw (0.72 week⁻¹ and 0.45 week⁻¹ in fw) for the control and AI calluses, respectively. Therefore, t_d was lower for the control than for the AI callus, with values of 0.90 weeks and 1.19 weeks in dw

Table 5
R² and SSE values for each growth model using experimental data from this study.

Model Type	Equation	SSE	R ²
Von Bertalanffy	$y = A * (1 - Be^{-kt})^m + d$	24.031	0.9772
Logistic	$y = A * (1 + e^{-kt})^{-m} + d$	14.056	0.9866
Logistic modified	$y = A * (1 + Be^{-kt})^{-m} + d$	8.019	0.9924
Gompertz	$y = A * e^{mBe(-kt)} + d$	14.594	0.9861
Richards	$y = A * (1 - Be^{-kt})^{-m} + d$	0.588	0.9994

(0.96 weeks and 1.54 weeks in fw), respectively. Furthermore, Fig. 6c indicates that the moisture content was also lower for the AI callus than for the control callus, with values of 58% and 69%, respectively, which agree with the dry appearance of the pigmented callus.

3.3. Modeling of Cell Growth in control and anthocyanin-induced calluses

The kinetic parameters derived from the experimental data were fitted into mathematical models such as the Von Bertalanffy, Logistic, Logistic modified, Gompertz, and Richards to compare which model best describes data relating to cell growth in a culture medium optimal for anthocyanin production. The equations for these models are detailed in Table 2.

This study is innovative in its application of different mathematical models (in order of increasing complexity) to understand cell growth in a solid medium culture of *T. officinale* to promote anthocyanin induction. Some of these models are typically used when creating a liquid culture medium for microbial cells such as vegetables, but few studies investigated optimum modeling in a solid culture medium. Parameters extracted from the fitting exercise will be employed for further secondary modeling. These mechanistic models aim to better explain the chemical, physical, and biological processes that improve callus formation and thereby enhance the production of secondary metabolites and other products of industrial interest.

Regarding the modeling of this work, the Richards model served as an apt method for describing the total cell growth process of the *T. officinale* AI callus in a solid medium; the kinetics parameters, growth velocity, and doubling times of callus masses were easily calculated using [Equation 1]. This could be because the Richard model incorporates the logistics, Gompertz, and Von Bertalanffy growth models [33,34]

The sigmoidal curve assumed in this work agrees with the curves reported for the callus cultures of *D. carota* (represented for fresh weight) [26], *Prunus cerasus* [31], and *Vitis vinifera* [28].

All models sufficiently described the cell growth of *T. officinale* *in vitro* with confidence levels more than 97.72% (t-test). However, the effect of medium composition on anthocyanin formation and the cell growth profile was successfully demonstrated using the Richards model, which proved more robust than traditional approaches. Depending on the selection criteria, the increase in the R² (99.9%) and its SSE (0.588) was much lower than the other models, although the differences in the goodness of fit equation statistics were minimal. The SSE and regression coefficients (R²) obtained from the comparison between the predicted and experimental data are displayed in Table 5. It is novel to obtain valuable growth constants for this plant using the Richards growth model.

4. Concluding remarks

To our knowledge, few publications exist regarding the establishment of *in vitro* callus lines from dandelion related to anthocyanin induction and/or production. This work reports the capacity of competent dandelion callus for anthocyanin induction and its potential accumulation under specific conditions, thereby needing a specific strategy for obtaining these compounds. The application of mathematical models to explain the growth and accumulation of

anthocyanins in *Taraxacum* callus is a powerful tool for predicting yields and responses under different environmental conditions. In conclusion, our results will aid the scale-up process by elucidating the productive potential of induced and to some extent enhanced anthocyanin from *T. officinale* callus for biotechnological purposes.

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