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# Alkaloid Production by Callous Tissue Cultures of *Cereus peruvianus* (Cactaceae)

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## Abstract

The morphologically undifferentiated cells of nonregenerant callous tissue of *Cereus peruvianus* cultured in the original medium and in medium supplemented with tyrosine were used as an alkaloid source. Comparison of alkaloid production by *C. peruvianus* plants and by callous tissues indicated that alkaloid levels were almost twice as high in callous tissues as in shoots of *C. peruvianus* plants. The ratio of alkaloid concentration between mature plant and morphologically undifferentiated cells of callous tissue was 1:1.7. A relationship between culture medium containing tyrosine and alkaloid production was also observed in the callous tissues of *C. peruvianus*. Since increased alkaloid production may be induced by additional factors such as tyrosine, increasing levels of tyrosine or other conditions of the culture medium may be considered factors for inducing higher alkaloid production by *C. peruvianus* callous tissues.

**Index Entries:** Alkaloids; cactus; callous tissue culture; columnar cactus; *mandacaru*; tyrosine precursor.

## Introduction

The ability to produce and store alkaloid substances is considered one of the most important characteristics of various cactus species *(1–6)*. Similar to other cactacea, the *Cereus peruvianus*, known in Brazil as *mandacaru*, produces alkaloid amines. Tyramine and hordenine are the main alkaloids in

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this specie *(7)*. Since alkaloids have pharmaceutical and medicinal properties, studies aiming at increasing alkaloid levels in plant tissues are highly interesting for commercial production. The use of plant cell cultures for the synthesis of natural products on an industrial scale has greatly increased over the last 20 yr *(8)* and may be a possible alternative method for increasing alkaloid production by cactus tissue cultures. The use of tissue culture for higher alkaloid production may be important since precursors of secondary metabolism could be directly introduced into the nutritive medium *(9,10)*.

Tissue culture techniques have been employed for rapid multiplication of *C. peruvianus* cactus species from callous tissues *(11)*. *C. peruvianus* plants from callous tissues have been regenerated in MS medium *(12)* supplemented with B5 vitamins *(13)* and containing different 2,4-dichlorophenoxyacetic acid (2,4-D) and *N*-(2-furanylmethyl)-1H-purine-6 amine (kinetin) combinations. However, a very small portion of callous tissue continued to grow, with no plant formation.

This nonregenerant callous tissue of *C. peruvianus* may be a possible source of alkaloids. Thus, in the present study, we investigated the production of alkaloids from callous tissue cultured in the original medium and in medium supplemented with tyrosine, using adult plants as comparative control.

## Materials and Methods

### *Plant Material*

The aerial parts (shoots) of adult plants and callous tissues of *C. peruvianus* were used for alkaloid extraction and characterization. The shoots were collected from plants maintained for 15 yr on the campus of the State University of Maringá (Maringá PR Brazil), although original callous tissue cultures were initiated in 1995.

### *Culture of Callous Tissues*

Callous tissues of *C. peruvianus* were obtained from six hypocotyls that were cut and used as explants for induction of callus in MS medium *(12)* containing B5 vitamins *(13)*, 0.8% agar, 3% sucrose, 4.0 mg/L of 2,4-D and 4.0 mg/L of kinetin or 4.0 mg/L of 2,4-D and 8.0 mg/L of kinetin and maintained at 32°C under a 16-h photoperiod (15 $m^2$/s of light intensity). Nonregenerant callous tissues were subcultured in fresh medium at 25- to 30-d intervals *(11)*. Callous tissues from long-term cultures (6 yr old) induced in medium with 4.0 mg/L of 2,4-D and 4.0 mg/L of kinetin were transferred to Petri dishes (10 pieces of callus/dish) containing fresh medium supplemented with 125 mg/L of tyrosine. The callous tissues from long-term cultures were also transferred to original fresh medium without tyrosine and used as control to verify the effect of tyrosine precursors on culture medium. Experiments were done in triplicate.

### *Extraction and Isolation of Alkaloids*

The *C. peruvianus* shoots and callous tissues were lyophilized separately. The freeze-dried material (1 g) was ground and refluxed for 1 h with a solution of 2% acetic acid prepared with 70% ethanol (50 mL [v/v]). After filtration, ethanol was evaporated under reduced pressure to produce an acid aqueous solution that was alkalinized with $NH_4OH$ to pH 8.0–9.0 and then extracted three times with the same volume of $CH_2Cl_2$. The extract was evaporated under reduced pressure, and the residue was dissolved in 10 mL of ethanol.

### *Identification of Alkaloids*

The alkaloids tyramine and hordenine were isolated and identified by analysis of their physical and spectral properties *(14)*. Alkaloids in ethanolic extracts were identified by thin-layer chromatography, silica gel G60 saturated with 0.1 *N* KOH, using $CHCl_3$:methanol (95:5 [v/v]) as eluent, while ninhydrin spray was used as the detecting reagent, by comparison with authentic standards of tyramine ($R_f$ = 0.12) and hordenine ($R_f$= 0.66).

### *Analysis of Alkaloids*

Alkaloid concentration was determined by ultraviolet (UV) spectrophotometer (Varian Carey-1E). UV wavelength detection was 274 nm, whereas tyramine (1 mg/mL) (Sigma-Aldrich, St. Louis, MO) was used as the standard (Fig. 1), since alkaloids previously identified from *C. peruvianus* were tyramine and hordenine *(7)*. The spectra in Fig. 1 were obtained before ethanol extract dilution, when required. Tyramine and hordenine had the same UV absorption spectrum; tyramine concentration is expressed as total alkaloid concentration. The absorbance of ethanolic solutions was determined without or after the necessary dilutions and converted to milligrams of alkaloids per plant or dry weight of callous tissue, using a calibration curve for tyramine between 10 and 60 μg/mL. A calibration curve equation for tyramine was obtained by linear regression of the experimental curve:

$$Y = -5.87192 + 92.22207X; \qquad r = 0.9957$$

in which $Y$ is alkaloid concentration in micrograms/milliliter, $X$ is the absorbance measured, and $r$ is the regression coefficient. Quoted data are the average of quantitative determination performed on three samples. All experiments were carried out in triplicate and the standard deviation (SD) was <4%.

## Results and Discussion

Comparison of total alkaloid production from *C. peruvianus* plants with callous tissues indicated that larger amounts of alkaloids were obtained from callous tissue cultured in original medium and from callous

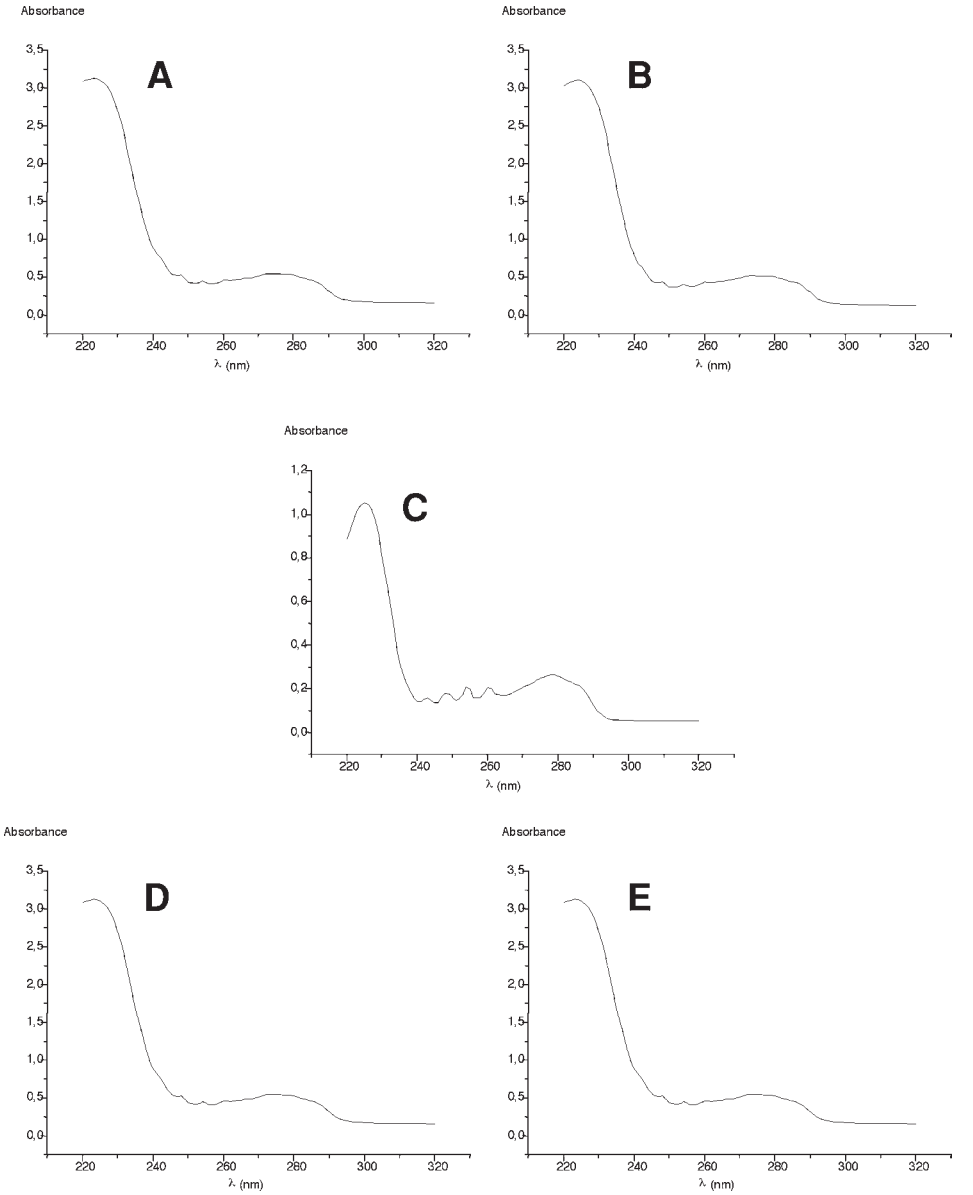


Fig. 1. UV absorption spectra of alkaloid extract of **(A)** callous C2; **(B)** shoot of *C. peruvianus* plants; **(C)** tyramine standard; **(D)** callous A: control callous tissue cultured in original medium; and **(E)** callous C1. The C1 and C2 callous tissues were cultured in medium containing 125 mg/L of tyrosine precursor.

tissue cultured in medium containing tyrosine as supplement (Table 1). Despite the greater resolving power of other techniques used in alkaloid analysis, the speed and simplicity of quantitative analysis based on UV absorption and its relative cost especially make this technique an effective tool for detecting differential alkaloid production by callous tissues and

Table 1
Alkaloid Production from Plant Shoots and Callous Tissue of *C. peruvianus* Cultured Under Original Conditions (A: MS Medium and Absence of Tyramine) and in MS Medium Containing 125 mg/L of Tyrosine (C1: Green-Yellow Callous tissues; C2: Dark-Green Callous Tissues)

| Tissue | Total alkaloid production (mg/100 g of dry wt)[a] |
|---|---|
| Plant shoot | 375.9 ± 5.232 |
| Callous tissue A | 638.3 ± 0.007 |
| Callous tissue C1 | 460.2 ± 0.017 |
| Callous tissue C2 | 718.0 ± 3.053 |

[a]± SD for triplicate experiments for each treatment.

shoots of *C. peruvianus*. Even though a large number of plant tissue cultures failed to produce the expected secondary natural products or produced only small amounts of the desired compounds *(15)*, Table 1 shows that the level of total alkaloids was almost two times higher in callous tissues than in shoots of *C. peruvianus* plants.

The development of a certain level of differentiation in callous tissue cultures of several species has been reported important for the successful production of phytochemicals by cell cultures *(16)*. Many examples exist in the literature demonstrating a relationship between differentiation and secondary metabolite accumulation. Hiraoka and Tabata *(17)* showed that alkaloid concentration in mature plant and callous tissue of *Datura innoxia* was 0.1 and 0.01% of dried weight, respectively (10:1 ratio). The current study showed that in *C. peruvianus* the ratio of alkaloid concentration between mature plants and morphologically undifferentiated cells of callous tissue was 1:1.7 (Table 1).

A relationship between culture medium containing tyrosine and total alkaloid production was also observed in the callous tissues of *C. peruvianus*. When total alkaloid content was measured about 4 wk after callous tissue transfer to culture medium containing tyrosine, it was found that the growth rate of callous tissue did not decrease; however, callous tissues with differential morphology and total alkaloid content were observed. Two cell groups supplemented with tyrosine consisting of green-yellow callous tissues (C1) and dark-green callous tissues (C2) were detected. The higher and lower alkaloid production by C2 and C1 callous tissues, respectively, cultured in medium containing tyrosine may have been caused by selection of a certain type of cells, or by an adaptation of the callous cells to different culture media. Plant cell cultures such as callous tissues of *C. peruvianus* have the ability to respond to external stimuli with changes in gene expression *(18–24)* that could result in altered secondary metabolism. Differential

alkaloid production has also been recorded in callous tissue culture of *Catharanthus roseus* in which the callus with dark yellow or green color, compact quality, and slow growth rate can synthesize more alkaloids; by contrasting, the pale yellow, more fragile, fast-growing callus usually has a low capacity for alkaloid synthesis *(25)*. In *C. peruvianus*, tyramine content decreased by 14% in C1 callous tissue and increased by 11% in C2 callous tissue, compared with control callous tissue (Table 1).

On the basis of these results, we suggest that in C2 callous tissue, tyrosine may be incorporated into the alkaloid pathway, acting directly as a precursor of tyramine synthesis, since tyrosine has been considered to be a precursor for numerous secondary metabolites *(26)*. Production and induction media for alkaloids have been well characterized in other plant species in terms of high sugar concentrations and different ammonium/nitrate ratios *(27,28)*. Concentration and carbon source, basal medium components, concentration and type of growth regulator, pH, temperature, light intensity, photoperiod, and stress conditions are all factors that may increase productivity of cultured plant cells *(16,29)*. Cytokinins have also been reported to stimulate alkaloid synthesis by different plant cell tissue cultures *(30)*.

The lower alkaloid content in cell lines of C1 callous tissues may be explained by differential mechanisms such as a shift in metabolic flow from tyrosine, decreased tyrosine synthetic activity, or suppression of enzyme activity from tyrosine to final product, which may suppress product accumulation in callous culture *(31)*.

The selection of cell lines with a higher level of alkaloids in callous tissue maintained in medium containing tyrosine indicates that some interesting possibilities to increase production have yet to be explored. Since increased alkaloid production may be induced by additional factors such as tyrosine, increasing levels of tyrosine or other conditions of the culture medium may be considered factors for inducing a higher production of alkaloids by *C. peruvianus* callous tissues.

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