

Alkaloid Production in Tissue Cultures of *Papaver somniferum* L. cv. Office-95

Esin Akçam Oluk

*Department of Biology, Faculty of Science, Ege University, 35100 Bornova-Izmir/
Turkey. E-mail : esak_ol@yahoo.co.uk*

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Abstract

The capacity of alkaloid synthesis was examined in embryogenic callus tissues of Turkish opium poppy, *Papaver somniferum* L cv. office-95. Eight months old cultures grown in hormone-free MS were examined for alkaloid content. They were found to produce codeine at a level of 1.2×10^{-2} , the baine 2.5×10^{-3} , noscapine 2.2×10^{-3} , morphine 1.6×10^{-3} and papaverine 5.2×10^{-4} % FW.

Introduction

Papaver somniferum L. opium poppy, is known for its medicinally important alkaloid content in the laticifer cells of the intact plant. In the past three decades, poppy tissue cultures have been highly recommended as an alternative source for the production of these alkaloids (Robets 1988). The presence of noscapine, narceine and papaverine as nonmorphinan alkaloids in poppy cell cultures have been reported (Khanna et al. 1978). However, in only some cases morphinan alkaloids (i.e. morphine, codeine and thebaine) have been detected and mostly in quite low levels (Staba et al. 1982). The absence of morphinan alkaloids in tissue cultures of the plant has been explained by Galewsky and Nessler (1986). The results of many studies have shown that the lack of tracheary elements and laticifer cells in the calli was one of these factors. So, differentiation has been reported essential in tissue culture of this plant for morphinan vascular production (Hsu and Pack 1989). In the present study, morphinan and nonmorphinan alkaloid productivity was investigated in tissue cultures of *Papaver somniferum* L. cv. Office-95 of differentiation ability; i.e., embryogenic callus cultures of the plant.

Materials and Methods

Tissue cultures: Eight months old tissue cultures established in our previous work (Akçam and Kaskar 2005) were employed in this study. These cultures were maintained in hormone-free MS medium subcultured at every three weeks of

intervals. They were grown in a growth chamber under 16 h light/8 h dark photoperiod and at $25 \pm 2^\circ\text{C}$ temperature.

Alkaloid analysis: About 34 g of callus tissue were harvested and extracted for alkaloid analysis. The final residue was dissolved in 5 % acetic acid and sonicated for 10 min (Staba et al. 1982). This extract was used for HPLC analysis. HPLC system (Waters Associates Inc., 1100 series) with Nova-Pak C18 column (3.9 mm \times 150 mm) was used. The mobile phase was NaAc : Acetonitril : EtOh : THF (880 : 0.75 : 0.30 : 0.15). The flow rate was 1 ml/min; the wavelength setting on the DAD detector was 280 nm. Pharmaceutical grade opium alkaloids were used as standards and included morphine sulfate, codeine sulfate, the baine, papaverine and noscapine (Sigma Chemical Corp).

Results and Discussion

The embryogenic callus tissues of the plant (Fig. 1) were found to synthesize both morphinan alkaloid as well as nonmorphinan alkaloid (Fig. 2). Total alkaloid amount was detected at a level of 1.93×10^{-2} % fresh weight (FW) (Table 1). Major

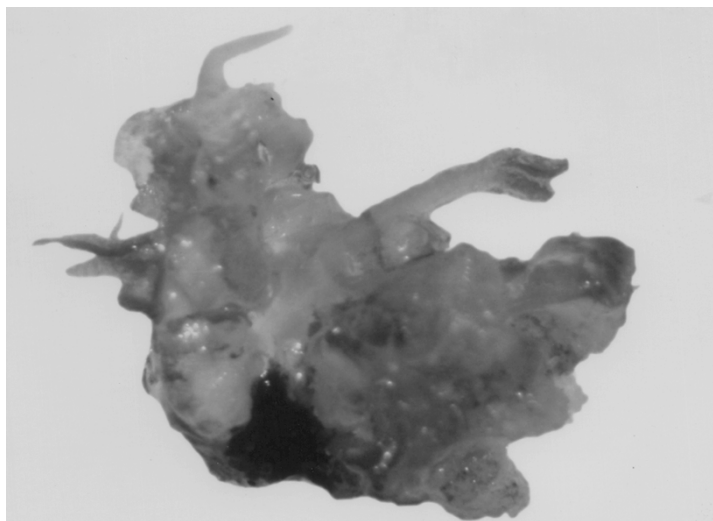


Fig. 1. Embryogenic callus cultures of *Papaver somniferum* utilized in alkaloid analysis.

alkaloid was codeine to average quantity being 1.2×10^{-2} % FW. The baine, noscapine and morphine were present at three different levels, 2.5×10^{-3} , 2.2×10^{-3} and 1.6×10^{-3} % FW, respectively. Papaverine was also produced in the tissues, but in trace amounts (5.2×10^{-4}) (Table 1).

Previous studies revealed that morphine, in particular is not produced in tissue cultures of the plant (Galewsky and Nessler 1986; Kamo et al. 1982). More recently, Laurain-Mattar et al. (1999) reported that three-month-old differentiated tissues produce morphine (0.35×10^{-3} % DW), besides codeine (8.5×10^{-3} % DW), the baine (0.7×10^{-3} % DW) and papaverine (0.35×10^{-3} % DW). They couldn't detect noscapine these cultures. Our results are in agreement with those

Table 1. Alkaloid amounts of the cultures.

Kind of alkaloid	% FW
Codeine	1.2×10^{-2}
Thebaine	2.5×10^{-3}
Noscapine	2.2×10^{-3}
Morphine	1.6×10^{-3}
Papaverine	5.2×10^{-4}
Total	1.93×10^{-2}

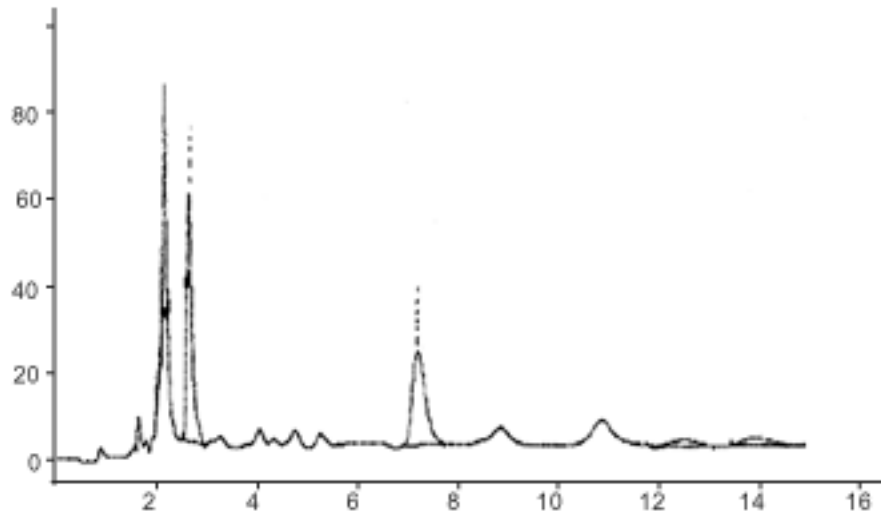


Fig. 2. HPLC chromatogram shows alkaloid profiles of the cultures.

of the above authors in that codeine was found to be the major alkaloid (8.5×10^{-3} % DW) in their cultures like ours (1.2×10^{-2} % FW). However, the difference between the two studies is, that our cultures contained noscapine (2.2×10^{-3} % FW) as against none reported by them (Table 1). Thus it can be concluded that under certain conditions, embryogenic callus tissues of opium poppy yield five

known alkaloids, namely codeine, baine, noscapine, morphine and papaverine. Our is the first to report the preserve of alkaloids in embryogenic callus tissues *Papaver somniferum* L. cv. Office-95.

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