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High-Yielding Tissues of Two Papaver species Grown *In Vitro*

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Callus cultures of *Papaver somniferum* and *P. rhoeas* were established on revised tobacco medium. The colour of the tissue varied from light grey, grey and finally to black depending on the age of the tissue. The high yielding tissues of dark grey colour were selected out of the three types of tissues maintained separately as static and suspension cultures. The tissues contained morphine, codeine, thebaine, narceine, narcotine and papaverine in *P. somniferum* and morphine, thebaine and narcotine in *P. rhoeas*.

Key Words - Morphine Codeine Thebaine Narceine Narcotine Papaverine *Papaver* Tissue cultures

Production of major opium alkaloids (morphine, codeine, thebaine, narceine, narcotine and papaverine)from the tissue culture of *Papaver somniferum* (Khanna & Khanna, 1976) and morphine, thebaine, and narcotine from *P. rhoeas* (Khanna & Sharma, 1977) has been reported. Effect of ascorbic acid, tyrosine and auxins on the alkaloid production has been studied (Khanna *et al.*, 1978; Sarin *et al.*, 1983). Recently, the presence of codeine, thebaine and cryptopine in *P. somniferum* and thebaine in *P. bracteatum* callus cultures has been reported (Staba *et al.*, 1982). In this report the sector of tissues of high yield of alkaloids has been separated from the heterogenous callus cultures of *P. somniferum* and *P. rhoeas*.

MATERIAL & METHODS The seeds of a high yielding (12-14% of morphine) variety of *P. somniferum* were obtained from Chittorgarh and inoculated aseptically on revised tobacco (RT) medium (Khanna & Staba, 1968) supplemented with 1 ppm of 2,4-dichlorophenoxyacetic acid (2,4-D) and 1% agar after surface sterilization with 0.1% mercuric chloride. The seeds germinated within 10-12 days and the seedlings thus formed resulted in an unorganised callus tissue. The tissue was a mixture of light grey, dark grey and black colour. The tissue was maintained on RT medium by subculture after every 4.6 weeks. The 7 year old tissue of *P. rhoeas* was also maintained on RT medium. The colour of the tissue varied from light grey, dark grey and finally to black depending upon the age of the tissue. Six month old tissue of *P. somniferum* and 7 year old tissue of *P. rhoeas* were harvested separately after 2,4,6 and 8 weeks, dried and extracted for opium alkaloids (Longman & De Bussy, 1972). The alkaloids were isolated and identified by co-chromatography, mp, IR and UV spectra.

The tissues of *P. somniferum* and *P. rhoeas* of different colours (light grey, dark grey and black) were maintained on RT medium by subculture after every 4-6 weeks and subsequently clumps of different colours were isolated so as to obtain high yielding tissue of one colour. The tissues of different colours (light grey, dark grey and black) were separately multiplied and maintained on RT liquid medium by subculture after every 14 days. The tissues of different colours (light grey, dark grey & black) of *P. somniferum* and *P. rhoeas* grown on RT liquid medium for 6 months were harvested separately after 14 days, dried and extracted separately for major opium alkaloids.

RESULTS & DISCUSSION Morphine, codeine, thebaine, narceine, narcotine and papaverine in *P. somniferum* and morphine, thebaine and narcotine in *P. rhoeas* callus were present in all the sample of tissues. The total alkaloid content was maximum in 6 week old tissues of *P. somniferum* and *P. rhoeas* (6.10% crude and 1.86% crude, Table 1) In the suspension culture of callus of three colours of both the species the amount of alkaloids was maximum in the dark grey tissue in *P. somniferum* and *P. rhoeas* (6. 87% crude

Table 1 Production of Opium Alkaloids from Tissue Cultures of *Papaver somniferum* and *P. rhoeas*

Age of Tissue (Week)	a GI	Total alkaloid Content (%)
P. somniferum		
2	2.6	3.3
4	4.6	5.4
6	3.8	6.1
8	3.5	4.2
P. rhoeas		
2	2.6	1.0
4	5.6	1.3
6	3.8	1.8
8	2.9	0.7

a = Growth Index = Final dry weight of the tissue − Initial dry weight of the tissue/Initial dry weight of the tissue.

Table 2 Production of Opium Alkaloids from Suspension Culture of *P. somniferum* and *P.rhoeas* (14 Day Old Tissue)

Colour of the tissue	a GI	Total alkaloids (%)
P. somniferum		
Light grey	5.25	4.9
Dark grey	3.50	6.9
Black	2.86	5.5
P. rhoeas		
Light grey	5.50	1.7
Dark grey	3.50	2.4
Black	2.18	2.3

a = Growth Index = Final dry weight of the tissue − Initial dry weight of the tissue/Initial dry weight of the tissue.

and 2.43% crude respectively, Table 2). We conclude that the dark grey tissue was the high yielding type and it was selected for the extraction of major opium alkaloids.

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