# CALLUS AND PLANTLET INDUCTION IN PAPAVER SOMNIFERUM

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## Abstract

Callus was induced in *Papaver somniferum* seedlings grown on MS medium supplemented with 0.1 of 2,4-D, 0.5 mg/l of Kn and 2% CM. However, luxurient growth was induced when MS was supplemented with BAP, AS and $GA_3$.
Shoot buds were then induced in this callus. Tissue browning was controlled by maize extract.

## 1. Introduction

*Papaver somniferum*, an annual plant of the family Papaveraceae, is the most important source of opium alkaloids codeine and morphine. Opium of different countries differ from one another not only in morphine and moisture content but in relative proportions of different alkaloids. The seed contain small amounts of papaverine and morphine, while plants contain codeine and morphine. Tissue culture of papaver may provide an alternate source for these alkaloids (Ranganathan et al., 1963; Staba et al., 1982). As reported by Ikuta et al. (1974) the plantlets produced from callus cultures have more specific alkaloidal pattern, being similar in content to the original plant.

The objective of the present study was to establish papaver callus cultures, its differentiation into shoots and to make a comparative analysis of their alkaloidal content.

## 2. Material and methods

Seeds of opium poppy were surface sterilized with 1% $HgCl_2$ solution for 3 min followed by three washings in sterilized distilled water and inoculated on plain agar medium supplemented with 5% sucrose. Germination took place within 4-5 days. These seedlings were then used for further studies i.e. callus induction and its subsequent differentiation. The basal medium used consisted of Murashige and Skoog (1962) inorganic salts at ½ strength supplemented with 5% sucrose, coconut milk (CM), maize extract and various growth hormones (Kn, BAP, AS, IAA, 2,4-D, ABA and $GA_3$). The medium was solidified with 0.9% agar and the pH adjusted to 5.4 with N HCl. Cultures were given 16 hrs of light in every 24 hr cycle with the temperature regulated at 25±1°C.

## 3. Results

### 3.1 Callus induction

Various growth hormones viz. IAA, 2,4-D, Kn, AS and $GA_3$ were first tested either individually at 0.01, 0.05, 0.1, 0.5, 1.0 and 2.0 mg/l or in various combinations. Callus initiation was very slow when

the hormones were used alone in the medium. Only a small callus was observed at 0.1 of 2,4-D, whereas no callus formation occured at any concentration of other phytohormones used. Similarly, callus initiation was very poor when hormone level was increased (1.0 and 2.0 mg/l). Eventual tissue darkening and death of the explant took place after three weeks of culture.

Most rapid callus induction and its subsequent growth took place when a combination of the auxins and cytokinins were supplied together in the BM. A good friable callus was obtained with 0.1 mg/l of 2,4-D, 0.5 of Kn and 2% CM (v/v) within four weeks of culture. Similarly, good callus and its further growth was obtained when other phytohormones e.g. $GA_3$, BAP and AS were supplied in addition to 2,4-D, Kn and CM.

Usually tissues turn brown after incubation of a few weeks. These calli are difficult to grow further and loose potentials for proliferation. Such calli were cultured on BM containing maize extract at either 0.1, 0.5 or 1.0%. This callus started rapidly turning to a whitish colour followed by active growth. The extract at 0.5% was most effective. Shoot buds have been induced in this callus after treatment with proper concentrations of the phytohormones.

### 3.2 Bud induction

In another experiment brownish hyaline callus was induced on BM supplemented with 0.1 mg/l of 2,4-D, 25 of AS and 10 of $GA_3$. This callus grew at a fast rate after subculture on the same medium. However, substitution of 2,4-D with 0.5 mg/l of BAP induced luxurient callus growth which turned green and then to a friable albino type after subcultures. Bead-like structures appeared in this callus consequently forming shoot buds (Fig. 1). Various treatments for root induction, including inoculation on 0.01 mg/l of ABA, were unsuccessful. The plantlet, however, grew to a height of 1.5 cm in 2 weeks (Fig. 2). Further treatments for root induction are being tried.

## 4. Discussion

Our studies support earlier findings (Hodges et al., 1982) for necessity of 2,4-D and Kn for an efficient callus induction and its further growth. Use of maize extract retrieved an inactive callus in which shoot buds could be induced. This effect might be due to certain adenine-like substances in maize extract. Because adenine derivatives have been isolated from immature corn kernels (Letham, 1973). Similarly, maize extract promoted culture growth in apple fruitlet, tobacco stem pitj and soybean. Moreover, the menace of tissue browning predominant in latex containing species (Ilahi, 1983) was got rid off by maize extract treatment.

Addition of 25 mg/l of AS, 0.5 of BAP and 10 of $GA_3$ to the basal medium had a stimulatory effect on bud induction. Although cytokinins and $GA_3$ always stimulated bud development, addition of 2,4-D induced callus growth consequently suppressing bud differentiation. Furthermore, bud initiation occurred on white callus having beaded appearance. The calli at various stages of differentiation are being analyzed for alkaloids.

## 5. Acknowledgements

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## 6. References

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Fig. 1. Callus & bud induction

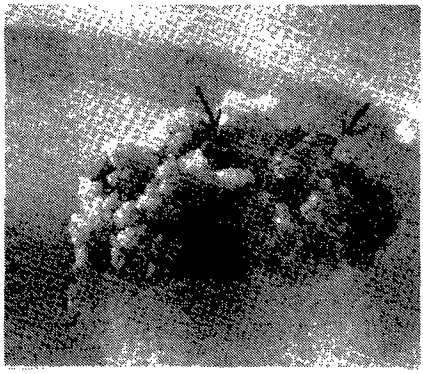

Fig. 2. Plantlet formation