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## RESEARCH ARTICLE

### STUDY OF CALLUS INDUCTION AND REGENERATION OF PAPAVER SOMNIFERUM L.

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#### ABSTRACT

Papaversomniferum (opium poppy) is an herbaceous, annual and diploid plant that is very important because of pharmacological and strategic view in the world. The limitation of this plant farming, hard and low amount extraction of this plant alkaloids cause studying about tissue culture of papaver somniferum L. for getting high level of these components. This plant has a special group of alkaloids named benzyloquinoline is due to secondary metabolites such as morphine, codeine and papaverine is a great value in pharmacy. In this research tissue culture of this plant to achieve the best hormonal levels for callus induction and regeneration examined from seed way culture. Small samples of leaves, roots and hypocotyls of 30-45 days from seedlings were transferred to different media with hormonal treatments. Best of hormone treatment for callus induction is 2mg/l 2, 4-D and 0.25mg/l BAP, 4mg/l 2, 4-D and 0.5mg/l BAP and best hormonal levels for regeneration includes 1mg/l BAP and 0.1 mg/l 2, 4-D.

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## INTRODUCTION

Opium poppy (*Papaver somniferum*) belonging to the family of papaveraceae is annuals, and diploid  $2n = 2x = 22$  plant, which have great value in terms of agriculture and pharmaceuticals. The plant is due to a specific group of alkaloids called benzyloquinoline among a diverse group of nitrogen compounds are considered as an important source for the production of drugs such as morphine and codeine, as well as housing and drug antibiotics such as sanguinarine are used. This is the reason for the tissue cultures of this plant to achieve higher quality than most of the compounds to be felt. Media after Murashige and Skoog (1962) and Gamborg *et al.* (1968) are most commonly used for *Papaver* tissue cultures and have supported successful alkaloid production. Tissue cultures of *Papaver somniferum* have been grown in modified MS media. *Papaver* callus grown *in vitro* produced morphinane alkaloids in low concentrations. Yoshikawa and Furuya (1985) gave an account of the production of codeine and the base in a green

callus with relatively high levels of kinetin. Schuchmann and Wellmann (1983) reported that high yield embryogenesis could be induced by tissue cultures of *P. somniferum* or *P. orientale* in 2, 4-dichloro-phenoxyacetic acid (2,4-D) free Gamborg's B5-medium. Daneshvar (2005) reported that the best shoot regeneration of *P. pseudo-orientale* was obtained from hypocotyl-cotyledon explant on MS medium containing 0.24 mg/l 2, 4-D and 0.19 mg/l  $\alpha$ -naphthalene acetic acid (NAA). The rooting was obtained on MS medium containing 0.25, 0.5 and 1 mg/l IBA with the best results on MS medium containing 0.5 mg/l IBA. Rostampour *et al.* (2010) reported that the optimized callus induction media for *P. bracteatum* consisted of the MS media supplemented with 1.0 mg/l 2, 4-D, and 0.1 mg/l kinetin (Kin) and 15 mg/l ascorbic acid.

## MATERIALS AND METHODS

### Plant material and seed germination

Seeds of *P. somniferum* were surface-sterilized using 70% (v/v) ethanol for 30 seconds followed by three-times washing with distilled sterile water. They were then kept in 5% (v/v) sodium

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hypochlorite solution for 7-8 min followed by three washes with distilled sterile water and germinated on MS under 16/8 h (light/dark) photoperiod at 20-22°C temperature. The roots, leafs and hypocotyls were cut into 6± 1 mm segments from 30 day-old seedlings, and used as explants.

### Media and culture conditions

The basal medium consisted of 1/2MS salts with 3% sucrose (w/v) and solidified with 7% (w/v) agar. The media were adjusted to pH 5.7-5.9 with 1.0 M NaOH or HCl before adding agar and Activated charcoal, and then sterilized by autoclaving at 121°C for 30 min.

### Callus induction

The explants from roots, leafs and shoots were placed MS (Murashige and Skoog, 1962) media supplemented with kinetin, BAP and 2,4-D at different combinations. For callus induction, roots, leafs and shoots explants were placed on 20 ml of agar-solidified medium in Petri dishes, sealed with parafilm and incubated at 20-22°C under 16 h light/8 h dark photoperiod. Well-grown callus induced from explants were transferred to the original media and sub-cultured every 40 days.

## RESULTS AND DISCUSSION

In this study, various combinations of growth regulators were used to find out their effects on callus induction. As shown in Figure 1, Results showed that among the cytokinins, the BA was more effective than Kin for callus induction when used in combination with 2, 4-D (Table 1). This study showed that the best medium for callus induction in *P. bracteatum* was MS supplemented with 0.25 mg/l BA and 4 mg/l 2,4-D with an average of 97.7 and 100% explants producing callus (Table 1).

**Table 1. Results of 4 levels of BAP and 4 levels of 2, 4-D concentration for three explants size (root, shoot and leaf), for callus induction**

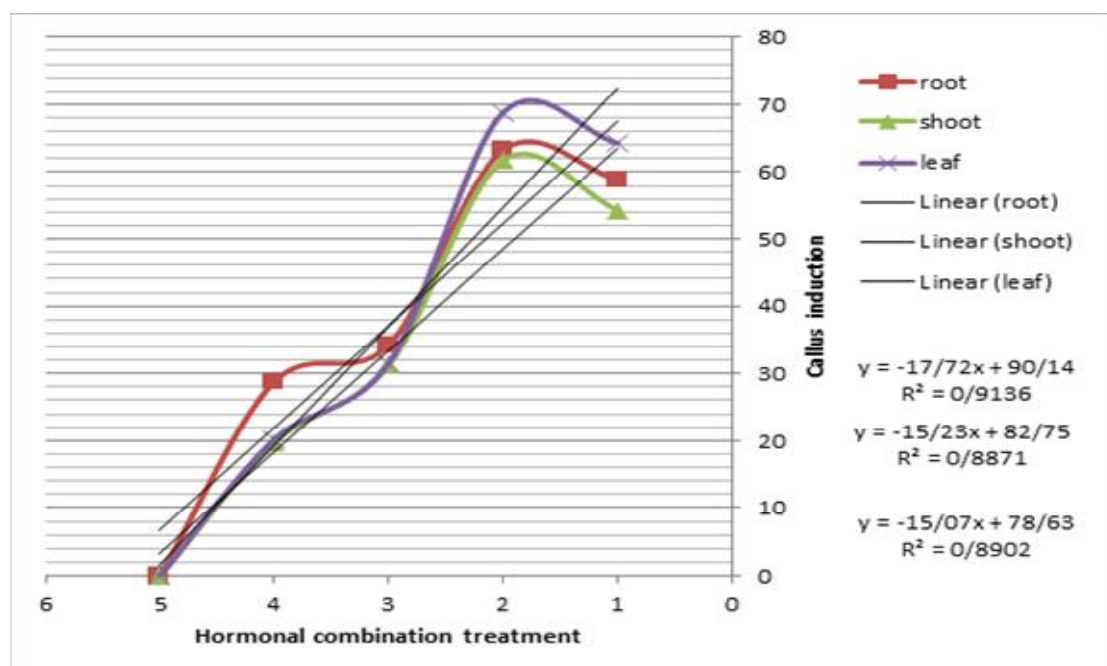
Medium	Root	shoot	leaf
0.25mg/l BAP + 2mg/l 2,4-D	58.9±3.2	54.3±5.2	64.3±3.1
0.5mg/l BAP + 4mg/l 2,4-D	63.3±2.1	61.8±2.3	68.8±2.2
0.1mg/l BAP + 1mg/l 2,4-D	34.3 ±1.4	31.3±5.8	31.6±2.5
0.5mg/l BAP + 2.5mg/l 2,4-D	28.8±2.2	19.7±4.5	20.2±4.3

**Table 2. Results of 4 levels of BAP and 2 levels of 2, 4-D concentration for three explants size (root, shoot and leaf), for regeneration**

Medium	Root	Shoot	Leaf
1 mg/l BAP + 0.1mg/l 2,4-D	45.9±2.2	57.3±3.2	67.3±3.1
0.5mg/l BAP + 0.5mg/l 2,4-D	51.3±1.3	51.8±2.7	57.8±4.2
2mg/l BAP + 0.5mg/l 2,4-D	46.3 ±1.4	53.3±3.8	52.6±1.5
2.5mg/l BAP + 0.1mg/l 2,4-D	39.8±1.2	47.7±2.5	48.2±1.3

Increasing BA concentration from 0.25 to 0.5 mg/l caused a reduction of callus production and this reduction was greater at 1 mg/l concentration of NAA. Other hormone combinations, such as 2 mg/l NAA with 0.1 mg/l Kin in MS1/2 medium and 10 mg/l IBA in MS1/2 medium were effective for callus inducing in *P. bracteatum* (Table 1).

Day *et al.* (1986) have also reported callus and meristemoid formation in *P. bracteatum* Arya II cultures on MS containing 2,4-D and Ilahi and Ghauri (1994) reported that callus was induced in *P. bracteatum* Lindl. Seedlings inoculated on MS medium supplemented with NAA (1.0 mg/l) and BA (0.5 mg/l).



**Chart 1. Results of regression Interactions between hormonal treatment and callus induction for 3 different explants**

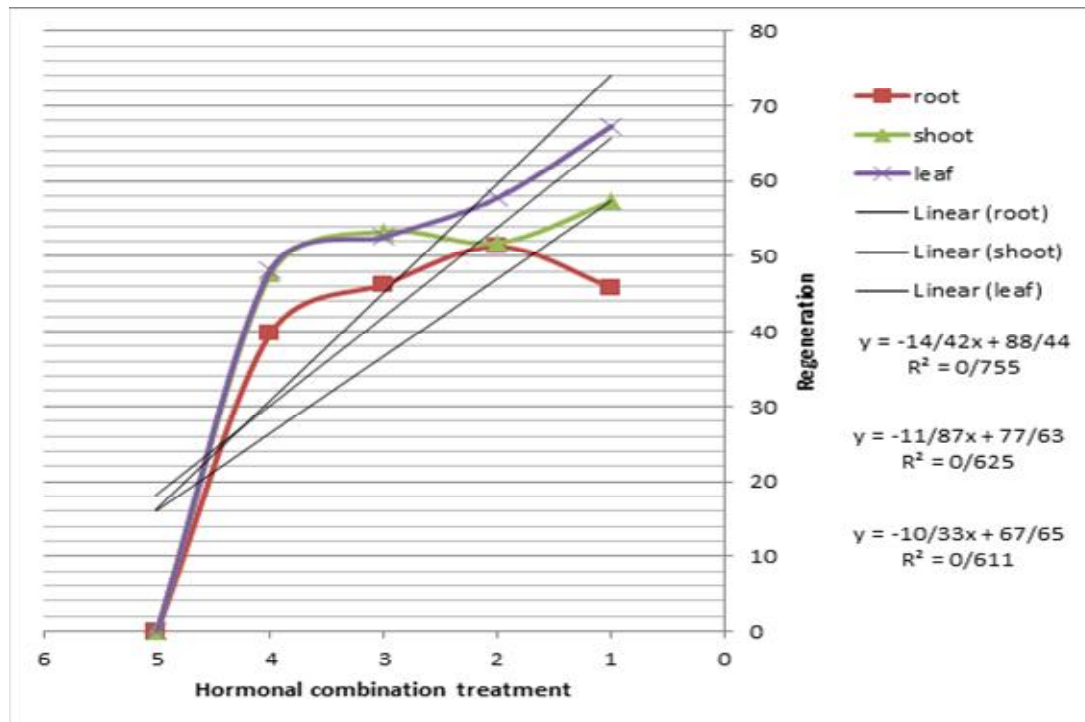


Chart 2. Results of regression Interactions between hormonal treatment and regeneration for 3 different explants

In conclusion, we reported an efficient callus induction of *P. somniferum* and the results could be helpful not only for large scale vegetative propagation, but also for genetic improvement of *P. bracteatum* through transformation studies.

In conclusion, we reported an efficient in vitro regeneration system of *P. orientale* and the results could be helpful not only for large scale vegetative propagation, but also for genetic improvement of *P. orientale* through transformation studies.

Optimized callus induction media for *P. orientale* consisted of the MS supplemented with 0.5 mg/l BA and 0.5 or 1.0 mg/l NAA. In this species, shoots were regenerated in cultures grown on MS me-dium containing 0.5 mg/l Kin and 0.5 or 1.0 mg/l NAA and were induced to root on B5 medium containing 0.5 mg/l Kin and NAA or 1.0 mg/l Kin and NAA.

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