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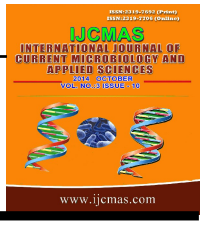


**Original Research Article**

# Callus induction and multiplication of internodal explants of *Myxopyrum smilacifolium* Blume

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**A B S T R A C T**

*Myxopyrum smilacifolium* is a large woody climbing shrub belonging to the family Oleaceae. The plant is known for many medicinal properties. For the production of secondary metabolites from callus, standardization of the protocol is very much essential. In the present study the effect of varying concentrations of 2,4-D and BAP alone as well as in combination was evaluated. The sterilized internodal explants were cultured on agar solidified MS medium supplemented with 3% sucrose and fortified with different concentrations of 2,4-D and BAP (0.05-8.0 mg/l) alone and in combination. Both 2,4-D and BAP showed callus induction. Best result was observed on MS medium supplemented with a combination of 2,4-D (0.1 mg/l) and BAP (1.0 mg/l).

**Keywords**

*Myxopyrum smilacifolium*, internode, 2,4-D, BAP, Callus

## Introduction

*Myxopyrum smilacifolium* is a large woody climbing shrub belonging to the family Oleaceae. Its root, stem, leaves are of much medicinally active and is employed in many traditional systems of medicines. The roots are used to treat various diseases like scabies, cough, rheumatism, fever, cuts and wounds (Vaidhyrathnam and Warrier, 1966). The leaves are astringent, acrid, sweet, thermogenic, anodyne, febrifuge and tonic. They are useful in vitiated conditions of kapha and vata, cough, asthma, rheumatism, cephalalgia, nostalgia, fever, otopathy, neuropathy and cuts and wounds (Orient Longman, 2006). Pharmacognostical evaluation has been made for the plant and reported for the presence of terpenoids, flavones, anthraquinones, sugars, alkaloids, phenols, tannins, and saponins (Gopalakrishnan *et al.*, 2012), antimicrobial study has been carried out in leaves (Gopalakrishnan *et al.*, 2012). Previous studies have shown the presence of triterpenoid ursolic acid in leaves (Sudharmini and Ashalatha, 2008) and the iridoid glycoside myxopyroside (Franzyk, 2001).

Various secondary metabolites present in the plant are responsible for its medicinal value. Callus culture could be employed for the production of these pharmacologically active compounds and thus it could help in

preventing exploitation of plant materials *ex-vitro*.

Callus is an undifferentiated mass of tissue which appears on explants within a few weeks of transfer onto growth medium with suitable hormones (Bhojwani and Razdan, 1996). Callus formation occurs from reversed process of cell differentiation, known as dedifferentiation or redifferentiation (Fowler *et al.,* 1993). Callus cultures are employed for shoot and root regeneration as well as micropropagation. Callus is also the starting material for suspension cultures for the mass production of secondary metabolites.

The present study was undertaken to examine the potential of internodal explants with different concentrations of hormones *viz*: 2, 4- D and BAP in MS medium (Murashige and Skoog, 1962) alone and in combination for rapid initiation of callus and percentage of biomass was also calculated. For callus cultures to be screened for standardization for secondary metabolite production many factors such as duration for callus initiation, time taken for maturity, long term maintenance, biomass content etc. need to be considered. The elite callus culture selected can be subjected to further studies.

## Materials and Methods

Internodal explants were collected from Botanical garden, Dept. of Botany, University of Kerala, Kariavattom. The explants were thoroughly washed for few minutes under running tap water and subsequently they were rinsed with 0.2% tween 20 (mild detergent). They were then rinsed several times using sterilized distilled water. Further sterilization was done under aseptic conditions in laminar air flow cabinet. The explants were dipped in 70% alcohol for 3 minutes and again washed with sterilized distilled water. For surface sterilization, the explants were first treated with 10% Sodium hypochlorite and then dipped in 0.1% aqueous solution (w/v) of $HgCl_2$ for 3 minutes. Then they were washed in sterilized distilled water for 4-5 times.

Sterilized explants were then inoculated in MS medium supplemented with 2, 4-D (0.05-8 mg/l) and BAP (0.05-8 mg/l). Callus obtained were then inoculated in combinations of 2, 4-D (0.1 mg/l) and BAP (0.05-5 mg/l). The inoculated explants were maintained in culture room with standard temperature (26±2ºC) and light conditions (16/8 hours photoperiod). Data was recorded after 4-8 weeks. Data collected includes the duration in days for callus initiation, percentage moisture content and morphology of callus.

## Results and Discussion

Surface sterilized internodal explants inoculated in MS medium supplemented with varying concentration of 2,4-D and BAP ranging from 0.05-8 mg/l. All of them produced callus with different frequencies and morphology. Present results shows that on increasing concentration callusing was found to be reducing gradually. Best results was observed on MS medium with 0.1 mg/l 2,4-D and 1 mg/l BAP. Callus obtained from 0.1 mg/l 2,4-D was selected for sub-culturing on varying concentration of BAP in combination with 0.1 mg/l 2,4-D which was kept constant. Results are as shown in Table 1. For determining the biomass content of the callus moisture content was measured. Though 2,4-D at a concentration of 3 mg/l showed good biomass content, explants inoculated in 0.1 mg/l 2,4-D was selected as the most favourable concentration with respect to its response

period, fresh weight as well as nature of callus. Friable creamy white with deep purple colouration was obtained with 0.1 mg/l 2,4-D (Fig.1). Sticky brown callus obtained at 3 mg/l concentration was very difficult to be dried and often increased chance of contamination. Complete drying of callus was impossible. Fresh weight of the callus in 0.1 mg/l 2,4-D was four times that of in 3 mg/l 2,4-D. The response started deteriorating on increasing the concentration and at 8.0 mg/l 2,4-D there was no response.

At very low concentration of 0.05 mg/l BAP no response was observed. Compact green chlorogenetic callus was characteristic of callus obtained from all concentrations of BAP. At 8.0 mg/l BAP low response was observed. Although the biomass content of callus was slightly higher for explants cultured in 3.0 mg/l BAP, the best result was obtained for 1.0 mg/l (Fig.2) with respect to duration of response, fresh weight and nature of callus.

2,4-D and BAP in combination for proper maintenance and proliferation of callus culture was optimized keeping 2,4-D concentration constant (0.1 mg/l) and varying the concentration of BAP (0.05-5 mg/l). Four week old callus was selected for sub-culturing. Best result was observed at combination of 0.1 mg/l 2,4-D and BAP concentration of 1 mg/l (Fig.3) in terms of biomass , proliferation and nature of callus. The moisture content was strikingly reduced to 65% which is a good indication of its high biomass content. Owing to the high biomass content it was selected for suspension culture and further studies.

For subculturing, callus obtained from fresh cultures of internodal explants in 0.1 mg/l 2,4-D was selected due to the fact that approximately 0.5 mg of callus could yield an average of 4.8 gms of callus on subculturing in a combination of 0.1mg/l 2,4-D and 1 mg/l BAP within short period of time. Friability of the callus was also retained with low moisture content hence high biomass.

**Table.1** Effect of 2,4-D and BAP on callus induction and moisture content

| Hormones | Concentration (mg/l) | Duration In days for callus initiation | Duration in days for data collection | Morphology of callus | Fresh weight (gm) ±SD | Dry weight (gm) ±SD | Percentage moisture |
|---|---|---|---|---|---|---|---|
| 2,4- D | 0.05 | 9 | 33 | Friable, pale white | 3.04±0.33 | 0.17±0.004 | 94.30 |
| | 0.1 | 5 | 29 | Friable, creamy white with deep purple coloration | 4.16±0.05 | 0.31±0.03 | 92.54 |
| | 0.5 | 7 | 41 | Friable, light brown | 3.10±0.07 | 0.19±0.004 | 93.87 |
| | 1.0 | 13 | 47 | Friable, light brown | 2.87±0.16 | 0.18±0.002 | 93.72 |
| | 3.0 | 17 | 53 | Sticky brown | 1.21±0.27 | 0.13±0.23 | 89.25 |
| | 5.0 | 37 | 62 | Deep sticky brown | 0.24±0.11 | 0.02±0.01 | 91.67 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | response | | | |
| | 8.0 | - | - | No response | - | - | - |
| BAP | 0.05 | - | - | No response | - | - | - |
| | 0.1 | 8 | 39 | Compact, light green | 1.61±0.04 | 0.21±0.004 | 86.90 |
| | 0.5 | 12 | 34 | Compact, light green with yellow patches | 1.58±0.31 | 0.17±0.03 | 89.20 |
| | 1.0 | 12 | 32 | Compact, slightly friable greenish yellow | 1.72±0.11 | 0.19±0.07 | 88.90 |
| | 3.0 | 19 | 42 | Green crispy compact callus | 1.45±0.12 | 0.22±0.11 | 84.82 |
| | 5.0 | 26 | 52 | Green compact callus | 1.43±0.12 | 0.19±0.04 | 86.71 |
| | 8.0 | 36 | 67 | Very slow growth with green compact callus | 0.95±0.04 | 0.11±0.02 | 88.42 |
| 2,4D-BAP | 0.05(BAP)+0.1 (2,4 D) | 11 | 31 | Compact, white | 3.216±0.26 | 0.189±0.03 | 94.10 |
| | 0.1(BAP)+0.1 (2,4, D) | 9 | 32 | Compact, green colour | 3.91±0.32 | 0.198±0.04 | 94.90 |
| | 0.5(BAP)+0.1 (2,4, D) | 8 | 30 | Compact, light green | 1.827±0.39 | 0.179±0.28 | 90.20 |
| | 1.0(BAP)+0.1 (2,4, D) | 7 | 27 | Compact, pale white greenish tint with purple colour | 4.89±0.23 | 1.68±0.24 | 65.64 |
| | 3.0(BAP)+0.1 (2,4, D) | 17 | 43 | Compact green hard callus | 3.16±0.31 | 0.43±0.09 | 86.39 |
| | 5.0(BAP)+0.1 (2,4, D) | 23 | 69 | Low response callus | 1.31±0.07 | 0.29±0.11 | 77.86 |

**Figure.1** Callus induction in 0.1 mg/l 2,4-D

**Figure**.**2** Callus induction in 1.0 mg/l BAP

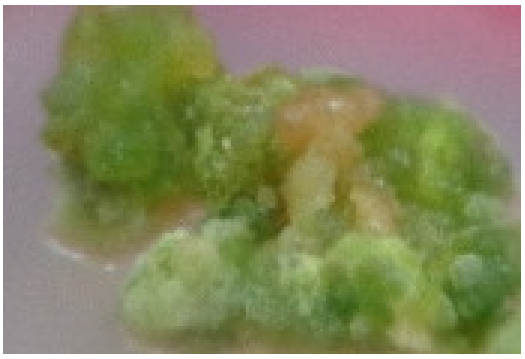

**Figure.3** Callus proliferation in 0.1 mg/l 2,4-D and 1 mg/l BAP

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