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Effect of different plant growth regulators on callus induction in leaf and stem explants of *Oxystelma esculentum* (L.F) R.Br. ex Schltes

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Abstract

Oxystelma esculentum is an important medicinal plant belonging to the family Asclepiadaceae used in the traditional systems of medicine for various ailments. The leaf and stem segments were cultured on Murashige and Skoog (MS) medium media containing various combination of auxins such as Naphthalene acetic acid (NAA) and 2,4- dichlorophenoxy acetic acid (2, 4-D) and cytokinins of 6 benzyl amino purine (BAP) for callus induction. The highest efficiency of callus formation (83.52%) was observed in concentration of 2.00 mg/l and 0.25 mg/l BAP. The leaf explant was responded high callus formation compared to stem segments. *In vitro* generated callus can be used as a source for the isolation of secondary metabolites from *O. esculentum.*

Keywords: *Oxystelma esculentum,* Asclepiadaceae, *invitro* callus, MS medium

Introduction

Oxystelma esculentum R. Br. (Asclepiadaceae) is a twiner growing in water-logged areas of the plains and lower hills of the Indian subcontinent and Java. It is used traditionally as diuretic, laxative, anti-ulcer, hepatoprotective, galactogogue and anthelmintic. It is used ethnomedicinally in throat infections, skin diseases and also in the treatment of jaundice(Kirtikar and Basu, 1975 and Pandya *et al*., 2011). It has been found to contain calogenin (Pandya and Anand,2012), lupeol (Pandya and Anand,2011a), stigmasterol (Pandya and Anand,2011b), kaempferol (Pandya and Anand,2011c) and aesculin (Pandya and Anand,2011d).

Medicinal plants are highly precious source of secondary metabolites in wide range, which are used as pharmaceuticals, agrochemicals, flavours, fragrances, colours, biopesticides and food additives (Bourgaud *et al*., 2001). In the end of 1960s, plant tissue culture technologies were introduced as a tool for both studying and producing plant bioactive compounds. A highly potent secondary metabolites that is used in pharmaceuticals and food additives have been produced through plant cell cultures, shoot cultures, root cultures and transgenic roots obtained through biotechnological means (Ramachandra Rao and Ravishankar, 2002). Callus or cell suspension culture culture also could be used for the large-scale plant cell culture where the bioactive compounds could be extracted (Taha *et al*., 2008).

So, this paper reports on the influence of plant growth regulators on induction and growth of *Oxystelma esculentum* callus culture as a starting point to produce bioactive compounds in plant cell culture.

Materials and Methods

Young leaf and stem were collected from mature field grown healthy plant of *Oxystelma esculentum* maintained in green house at Kamban College of Arts and Science, Tiruvannamalai, Tamil Nadu, India and washed thoroughly under running tap water and then treated with a few drops of Tween-80 and 1% Savlon for 10 minutes with constant shaking. This followed by successive three washing with distilled water to make the material free from savlon. Again the explants were washed with 70% ethyl alcohol for few seconds and washed with distilled water for 3-4 times. After that, the explants were transferred to laminar air flow chamber and disinfected with 0.1% $HgCl_2$ for 2 minutes and washed with sterile distilled water for 5-7 times. Then, the explants were placed in sterile Petri plates before inoculation. The sterilized explants were injured all over the surface and used for callus induction.

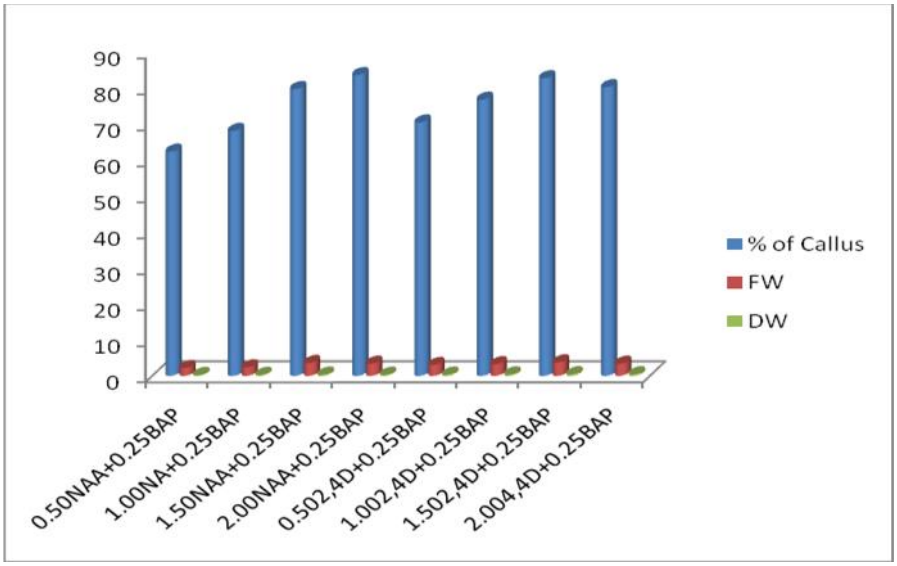
The excised explants were cultured on MS (Murashige and Skoog, 1962) medium augmented with different concentrations of auxins like NAA and 2, 4-D (0.5, 1.0, 1.5 and 2.0 mg/l) along with cytokinins i.e., BAP (0.25 mg/l) , 3% sucrose and 0.8 to 1% agar with pH adjusted to 5.8 before the addition of agar. Culture tubes containing medium were autoclaved at 121°C for 15 lbs/inch2 for 15 min. All the inoculated cultures were incubated in growth room in controlled conditions at a temperature of 25 ± 2°C, 16 h light/8 h dark photoperiod and continuous illumination was provided by cool white fluorescent tubes at 2000 lux.

Each experiment was repeated thrice. Analysis of variance was carried out and the differences between the treatments were determined by DMRT at 5% level of significance using SPSS (SPSS ver. 16.0).

Results and Discussion

The young leaf explants of *O. esculentum* on transfer to MS medium containing different concentrations of NAA, 2,4-D with BAP resulted in proliferation of callus. The explants started to form callus within five days after inoculation from the cut end and all over the areas injured. Among the attempted concentrations of NAA (0.5, 1.0, 1.5 and 2.0 mg/l) and 2,4-D (0.5, 1.0, 1.5 and 2.0 mg/l) in conjunction with BAP (0.25 mg/l), maximum callus formation (83.52 ± 0.25%) was observed with NAA (2.0 mg/l) and BAP (0.25 mg/l) combination. The minimum callusing (62.26 ± 0.84^{a} %) was observed in the MS media fortified with 0.5 mg/l NAA with BAP (0.25 mg/l). The colour of the callus was pale green and friable. The maximum growth rate in terms of fresh weight (3.73 ± 0.06 g) and dry weight (0.44 ± 0.02 g) was observed in the combination of 2, 4-D 1.5 mg/l and BAP 0.25 mg/l. Minimum growth rate 2.28 ± 0.07 g fresh weight and 0.24 ± 0.03 g dry weight was obtained in 0.5 mg/l NAA and 0.25 mg/l BAP combination (Fig 1).

Fig 1: Effect of NAA, 2,4-D with BAP on callus induction, callus growth of young leaf explants of *Oxystelma esculentum*



Values with the same superscript are not significantly different at P> 0.05level according to Duncan's multiple range test.

When the excised explants of stem were placed on MS medium containing different concentrations and combinations of NAA + BAP and 2,4-D + BAP for callus induction, the callus initiations started within 5 - 7 days of incubation. Depending upon the concentration and combinations of harmones the calli were induced of varying frequencies. The highest percentage of callus induction (74.34 ± 0.67^{e} %) was observed in MS Medium containing 1.5 mg/l of NAA+0.25 mg/l BAP. The maximum growth rate in terms of fresh weight (3.90 ± 0.05 g) was observed in the combination of 1.5 mg/l of NAA+0.25 mg/l BAP. Minimum growth rate 2.48 ± 0.09 g fresh weight and 0.25 ± 0.02 g dry weight was obtained in 0.5 mg/l NAA and 0.25 mg/l BAP combination (Table 2 and Fig 2).. The results obtained were agreement with the previous reports by other investigators, *Gymnema sylvestre* (Roy *et al*., 2008) and *Ceropegia juncea* (Nikam and Savant, 2009), *Dregea volubilis* (Yogananth *et al*.,2012) and *Sarcostemma brevistigma* (Palanivel *et al*., 2013). George, (1996) reported that the NAA, 2,4-D shows effect on the RNA metabolism by inducing the transcription of messenger RNA capable of coding proteins required for the growth and hence, promoting a chaotic cell proliferation, i.e., callus formation. These findings provide some basic information for the production of bioactive compounds from *Oxystelma esculentum* cell culture.

Table 1: Effect of NAA, 2,4-D with BAP on callus induction, callus growth of young stem explants of *Oxystelma esculentum*

Hormones mg/l	PERCENTAGE OF RESPONSE	FRESH WEIGHT	DRY WEIGHT
NAA+BAP			
0.50 + 0.25	55.02 ± 1.21	2.48 ± 0.09	0.25 ± 0.02
1.00 + 0.25	65.35 ± 1.34	3.23 ± 0.03	0.32 ± 0.04
1.50 + 0.25	74.34 ± 0.67	3.90 ± 0.05	0.39 ± 0.01
2.00 + 0.25	69.99 ± 0.78	3.60 ± 0.08	0.36 ± 0.02
2,4-D+ BAP			
0.50 + 0.25	60.20 ± 0.05	2.68 ± 0.07	0.28 ± 0.04
1.00 + 0.25	69.06 ± 0.67	3.03 ± 0.03	0.32 ± 0.01
1.50 + 0.25	72.53 ± 0.48	3.29 ± 0.00	0.40 ± 0.03
2.00 + 0.25	65.10 ± 0.55	3.10± 0.03	0.46 ± 0.02

Values with the same superscript are not significantly different at P> 0.05 level according to Duncan's multiple range test.

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