ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 9, Issue, 1(D), pp. 23103-23105, January, 2018

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

EFFECT OF DIFFERENT CONCENTRATIONS OF 2,4-D IN NUTRIENT MEDIUM ON GROWTH AND TOTAL PHENOLIC, FLAVONOID AND TANNIN CONTENT OF CALLUS OBTAINED FROM O. SANCTUM LEAF EXPLANTS

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DOI: http://dx.doi.org/10.24327/ijrsr.2018.0901.1397

ARTICLE INFO

Article History:

Received 17th October, 2017
Received in revised form 21st November, 2017
Accepted 05th December, 2017
Published online 28th January, 2018

Key Words:

2,4-D, Ocimum sanctum, callus, phenolic, flavonoid, tannin

ABSTRACT

Effect of increasing concentrations of 2,4-D was evaluated on growth and total phenolic, flavonoid and tannin content of callus obtained from leaf explants of *Ocimum sanctum*. Fresh and dry weight were highest for callus cultured on nutrient medium supplemented with 0.2 µg ml−1 2,4-D. Contents of total flavonoid and tannin increased with increasing concentrations of 2,4-D whereas total phenolic content was highest in callus cultured on nutrient medium enriched with 0.4 µg ml−1 2,4-D. The results of the present study revealed that 2,4-D is required for callus formation from O. sanctum leaf explants. Higher concentrations of it inhibit callus growth but significantly increase the production of flavonoids and tannins.

INTRODUCTION

Ocimum sanctum, a medicinal herb which belongs to the family of Lamiaceae is regarded as 'elixir of life' and an adaptogen in Ayurveda (Maheshwari, 2013; Cohen, 2014). *O. sanctum* extracts are used to cure common colds, inflammation, stomach disorders, headaches, malaria, heart disease and several forms of poisoning (Maheshwari, 2013). The holy basil stem and leaves have several bioactive constituents including secondary metabolites such as phenolic, flavonoid, tannin, and saponin that are responsible for its pharmacological and medicinal properties (Pietta *et al*., 1998; Jaggi *et al*., 2003; Chew *et al*., 2009). The amount of secondary metabolites is significantly affected by different growing, harvesting, processing and storage conditions of plant tissues thus limiting their production.

Plant tissue culture which is a technique to culture plant cells and tissue *in-vitro* on nutrient medium under aseptic controlled condition can be used to preserve endangered medicinal plants and enhance secondary metabolites production (Elangomathavan, 2017; Wang *et al*., 2017). Auxins are generally used in plant tissue culture as an integral constituent of nutrient medium (Collin, 2001). 2,4-D is generally used to induce callus growth as it regulates division and takes part in dedifferentiation of explants to produce callus (Lim *et al*., 2009). Although it is known that auxin is crucial for callus induction, different concentrations of it may be required for different plant species and different explants of the same species. Therefore, aim of this study was to determine the effect of different concentrations of 2,4-D on growth and total phenolic, flavonoid and tannin content of callus obtained from *O. sanctum* leaf explants**.**

RESULTS AND DISCUSSION

O. sanctum contains several secondary metabolites which are responsible for their antitumor, antiviral, cholesterol-lowering, antiprotozoal, antihelminth, immunosuppressant and anti-ageing activities (Maheshwari, 2013; Cohen, 2014). Callus induction is an important step for large scale production of secondary metabolites and bioactive compounds. The growth hormone auxin alone or in combination with kinetin has been shown to be responsible for induction and development of callus from leaf explants in most plant species in plant tissue culture. However, optimum concentration of it needs to be

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defined for different species and explants. No callus induction form *O*. sanctum leaf explant was observed in nutrient medium without auxin. Callus was obtained from *O. sanctum* leaf explants in all media supplemented with 0.2 to 0.6 µg/ml 2,4-D (Fig. 1).

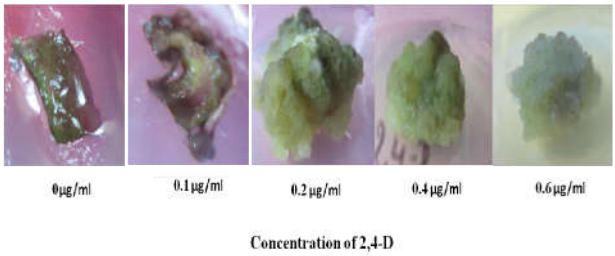

Figure 1 Callus formation from *O. sanctum* leaf explants cultured on nutrient medium enriched with different concentrations of 2,4-D. The young leaves of *O. sanctum*, collected from campus of Central University of Jharkhand, Brambe, Ranchi were cut into small pieces (1 cm x 1 cm), surface sterilized and placed on MS medium (pH 5.8) containing 0.2, 0.4 and 0.6 µg/ml 2,4-D at 25 ± 2°C under constant illumination of 125 µmol m^{-2} s^{-1} white light for 20 days.

Among the various concentrations of 2,4-D, fresh and dry weight of callus was highest in callus cultured on nutrient medium containing 0.2 µg/ml 2,4-D (Table 1). In comparison to 0.2 µg/ml 2,4-D supplementation, 0.4 and 0.6 µg/ml 2,4-D concentration led to 42 % and 47 % reduction in fresh weight and 25% and 32% reduction in dry weight, respectively (Table 1). The calluses were green and friable in nature. Similar to our results, Elangomathavan (2017) also observed soft, friable callus of green white color in *Orthosiphon stamineus* at 1.0 -6.0 ug/ml 2, 4–D concentration. Growth of callus at 0.2 µg/ml 2,4-D was found to be the optimum. Increasing concentrations of 2,4-D led to less growth of callus (Fig. 1). This result corroborates the findings in *Achyranthes aspera* (Chew *et al*., 2009) and *Brucea mollis* (Das *et al*., 2017). At higher 2,4-D concentrations lower growth of callus could be due to enrichment of 2,4-D in the tissues, as was demonstrated in Arabidopsis (Meijer *et al*., 1999).

Table 1: Fresh weight and dry weight of callus obtained from *Ocimum sanctum* leaf explants cultured on nutrient medium enriched with different concentrations of 2,4-D.

2,4-D(µg/ml)	Fresh weight[a](gm)	Dry weight[b] (gm)
0	No callus	No callus
0.1	No callus	No callus
0.2	4.028±0.110	0.2885±0.112
0.4	2.377±0.186	0.2164±0.121
0.6	2.136±0.025	0.1976±0.034

[a]Fresh weight of callus was measured using weighing balance.
[b]For dry weight determination, samples were dried in an oven for 72 hr or until constant weight was achieved

Total phenolic content was highest in callus grown in nutrient medium containing 0.4 µg/ml 2,4-D whereas flavonoid and tannin content was highest in callus grown in nutrient medium supplemented with 0.6 µg/ml 2,4-D (Fig. 2). Nutrient medium supplemented with 0.4 µg/ml and 0.6 µg/ml of 2,4-D led to 38.46 % and 64.10 % increase in tannin content of callus, respectively compared to nutrient medium supplemented with 0.2 µg/ml of 2,4-D (Fig. 2).

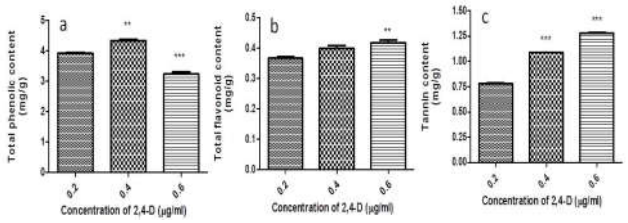

Figure 2 Effect of 2,4-D (0.2, 0.4, 0.6 µg/ml) in nutrient medium on (a|) total phenolic content (b) total flavonoid content and (c) tannin content of callus from *O. sanctum* leaf explant. All data presented are means of three replicates along with standard deviations. *, **, and *** represent significant differences compared to 0.2 µg/ml 2,4-D at probabilities of 0.05, 0.01, and 0.001, respectively. Total phenolic content was measured following the method of Kim *et al*. (2003) and was expressed as mg gallic acid equivalent /g f wt. The flavonoid content was measured following the method of Chang et al. (2002) and expressed as mg quercetin equivalent/g f wt. Total tannin concentration in the methanolic extract of callus was estimated by modified method of Price and Butler (1977) and expressed as mg tannic acid equivalent /g f wt.

Similar to our studies, in callus of *Byrsonima verbascifolia* also, auxin both promoted and increased the production of phenolic compounds, especially tannin (Castro *et al*., 2016). The variation in the total phenolic, flavonoid and tannin content in presence of different concentrations of 2,4-D may be attributed to the activation of key enzyme phenylammonia lyase which participates in the biosynthesis of these compounds (Davies, 1972). Overall, our results show that 2,4-D is required for *O. sanctum* callus formation. Higher concentrations of it inhibit callus growth but significantly increase the production of flavonoids and tannins.

Acknowledgements

The authors are grateful to Department of Biotechnology (DBT) for providing funding in the form of Builder project No. BT/PR-9028/INF/22/193/2013. KK is thankful to University Grant Commission (UGC), New Delhi for providing Rajiv Gandhi National Fellowship. PS is thankful to UGC-FRP.

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How to cite this article:

Kiran Kachhap *et al.*2018, Effect of different concentrations of 2,4-D in nutrient medium on growth and total phenolic, flavonoid and tannin content of callus obtained from *O. sanctum* leaf explants. *Int J Recent Sci Res.* 9(1), pp. 23103-23105.
DOI: http://dx.doi.org/10.24327/ijrsr.2018.0901.1397

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