Influence of different combinations of 2,4-D and Kin on callus induction, texture and colour of callus in *Ocimum sanctum* leaf explants.

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ABSTRACT

Our experiment was focused on the enhancement of callus growth in combination of different concentration of auxin (2,4-D) and cytokinin (Kinetin) in invitro culture of *Ocimum sanctum* leaf explants. Various concentration of combination of 2,4-D (0.2,0.4 µg/ml) and Kinetin (0.1-0.5 µg/ml) are used in Murashige and skoog (MS) media. We find callus was obtained from *O. sanctum* leaf explants in all media supplemented with combination of 2,4-D and Kin were friable and whitish green in colour and maximum callus was obtained in the combination of 0.4 mg/ml 2,4-D and 0.2 µg/ml Kin concentration.

Key words: *Ocimum sanctum*, 2,4-D, Kinetin, Callus.

I. INTRODUCTION

Ocimum sanctum belonging to the family Lamiaceae and widely occur throughout in India. *Ocimum sanctum* have been spiritual sanctity, religious importance and use as traditional medicine for the treatment of many diseases from ayurvedic era. (Warrier PK.1995). It contains huge number of phytochemicals which has medicinal properties and cure many diseases such as malaria, bronchitis, skin disease, dysentery etc. (Luthra 2010). Due to the harmful side effects and toxicity of drugs, modern society prefer drugs of natural origin. Tissue culture is an alternative technique to growth of cell in short duration of time. Although, callus induction was recommended to investigate and invitro studies to improve phytochemicals. (Rajinikanth et al. 2013). Plant growth hormones are important role to regulate growth of callus. Auxin as well as cytokinin are the most active compound to callus induction. (Kakani et al. 2009). We are trying to develop and callus growths from manipulation of concentration of auxin (2,4-D) and cytokinin (Kinetin). Callus extract may be further use in pharmaceutical, dye, food and flavor industries.

I. METHODOLOGY

In-vitro callus formation:

Young leaf explants was collected and cut into small pieces (1 cm approximately). It was sterilized by three treatments: 1.25% sodium hypochloride, 0.1% mercuric chloride, 70% ethanol and gradually washed with distilled water 4-5 times. These sterilized leaf was inoculated into different combination of concentration of 2,4-D (0.2, 0.4 µg/ml) and Kinetin (0.1-0.5 µg/ml) contiaining Murashige and Skoog(MS) media. After that these culture was kept into light for 24 hr or 20 days.

II. RESULTS AND DISCUSSION

Effect of different combinations of 2,4-D and Kin on callus fresh weight

2,4-D is essential auxin to growth of callus. Lower 2,4-D concentration gave better callus growth. Callus was obtained from *O. sanctum* leaf explants in all media supplemented with combination of 2,4-D and Kin and callus obtained were friable and whitish green in colour (Table 1; Fig. 1). *O. sanctum* is source of many useful phytochemicals which are used in treatment and/or prevention of many diseases (Murashige and Skoog 1962) (Aqil et al. 2006). Due to increased demand, the production of these phytochemicals needs to be ramped up. Auxins and cytokinins have been used alone and in combination to stimulate callus proliferation and enhance phytochemical production *in vitro*. (Abbasi et al. 2007). Therefore, the effects of fortification of combinations of 2,4-D (0.2,0.4) µg/ml and Kin (0.1-0.5) µg/ml in MS medium on callus formation and biomass were studied. Among the various 2,4-D concentrations, maximum callus fresh weight (10.29$\pm$0.54 g) was achieved in medium containing 0.4 mg/ml 2,4-D and 0.2 µg/ml Kin (Fig. 2). Leaf explants of *Coleus blumei* also showed maximum callus induction in MS medium fortified with 1 µg/ml 2,4-D with 0.1 µg/ml Kin (Meghana 2015). Castro et al. 2016 reported highest callus biomass of *Byrsonima verbascifolia* in MS medium fortified with 4.52 µM 2,4-D and 4.44 µM BAP. The use of auxin in combination with cytokinins for induction of callus has been suggested to be due to their role in DNA replication and mitosis (Skoog and Miller 1957). Overall, our results suggest that highest callus growth can be achieved using MS medium containing combination of 2,4-D and Kin.

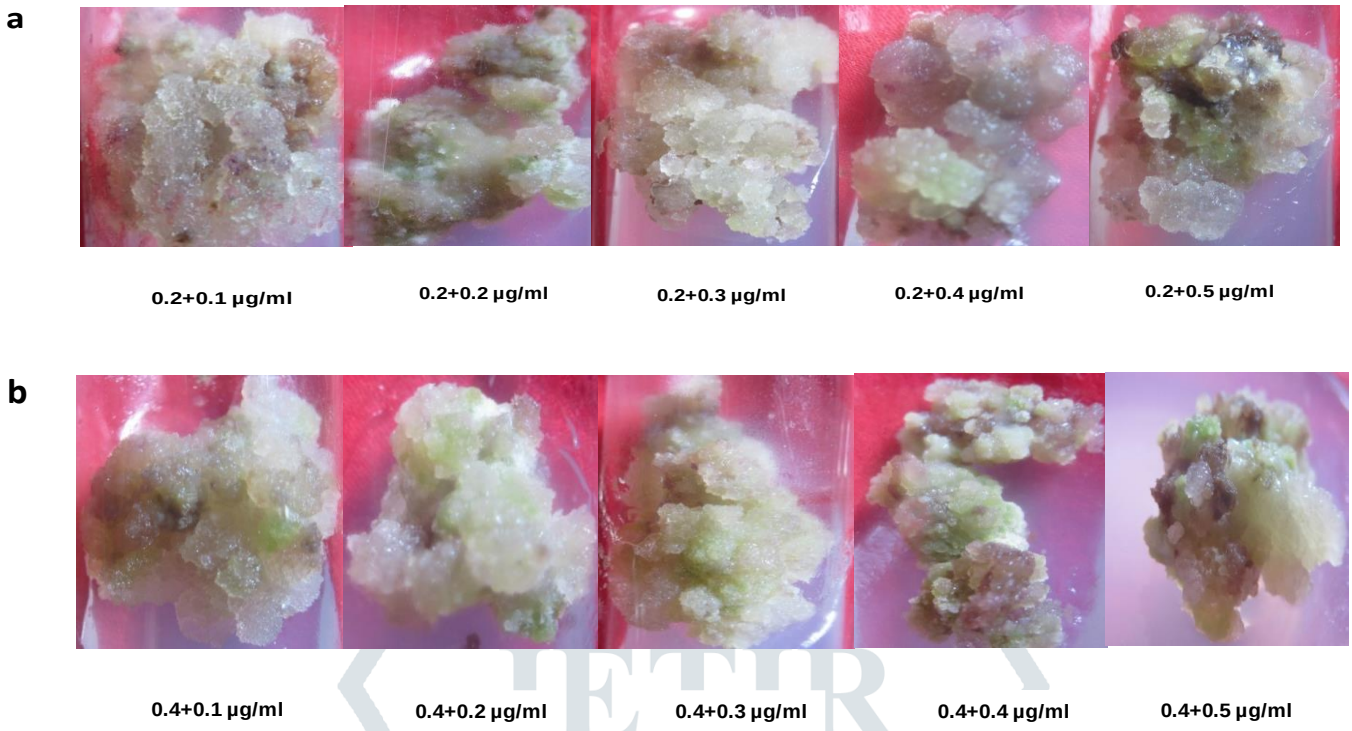

Fig. 1. Effect of combination of 0.2 µg/ml 2,4-D and 0.1-0.5 µg/ml Kin (a) and 0.4 µg/ml 2,4-D and 0.1-0.5 µg/ml Kin (b) on formation and growth of *O. sanctum* callus.

Table 1: Effect of plant growth hormone in combination of auxin (2,4-D) and cytokinin (Kin) on texture and colour of *O. sanctum* callus obtained from leaf explants.

Plant growth hormone			
2,4-D (mg/l)	**Kinetin (mg/l)**	**Texture of callus**	**Callus colour**
0	0	-	No callus
0.2	0.1	Friable	Light green
0.2	0.2	Friable	Light green
0.2	0.3	Friable	Whitish green
0.2	0.4	Friable	Whitish green
0.2	0.5	Friable	Whitish green
0.4	0.1	Friable	Whitish green
0.4	0.2	Friable	Whitish green
0.4	0.3	Friable	Whitish green
0.4	0.4	Friable	Whitish green
0.4	0.5	Friable	Whitish green

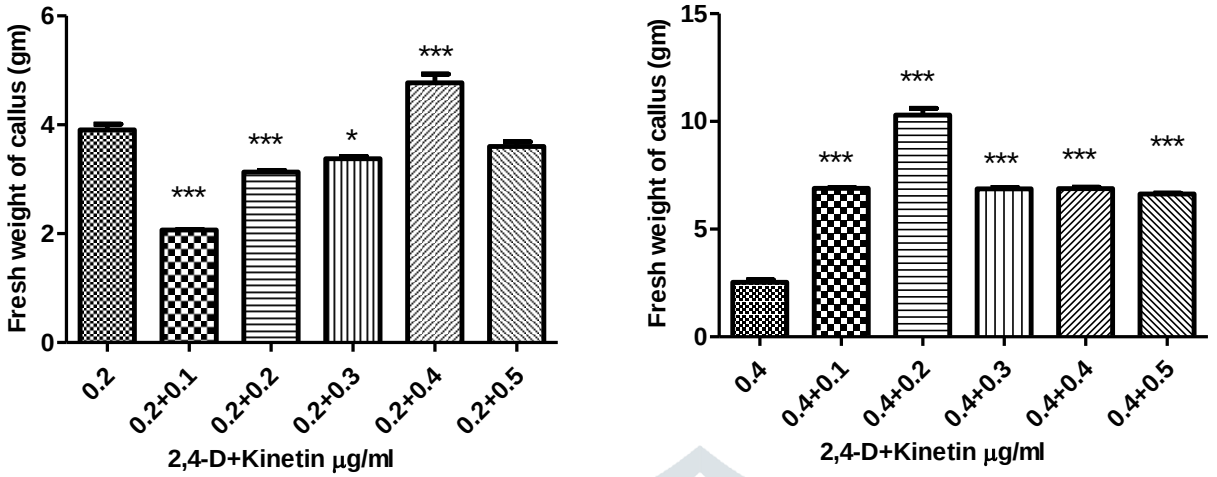

Fig. 2. Effect of combination of 0.2 µg/ml 2,4-D and 0.1-0.5 µg/ml Kin (a) and 0.4 µg/ml 2,4-D and 0.1-0.5 µg/ml Kin (b) on *O. sanctum* callus fresh weight. All data presented are means of three replicates along with standard deviations. *, **, and *** represent significant differences compared to 0.2 µg/ml 2,4-D (a) and 0.4 µg/ml 2,4-D (c) at probabilities of 0.05, 0.01, and 0.001, respectively.

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