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Modulation of callus induction and growth by 2, 4-d from leaf explants of *Ocimum Sanctum* (L.)

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Abstract
Ocimum sanctum (L.) plant parts, especially the leaves contain secondary metabolites which are economically important due to its medicinal, culinary and aromatic properties. Callus culture is an alternative source to whole plants for the production of secondary metabolites. The objective of this study is to increase secondary metabolite production in *Ocimum sanctum* (L.) callus culture. Callus cultures were initiated on Murashige and Skoog (MS) medium supplemented with different concentrations (0.1-1.4 mg/l) of 2, 4-dichlorophenoxy acetic acid (2,4-D). The cultures were maintained under continuous illumination of white light (125 uμmole. $m^{-2}s^{-1}$). Maximum callusing was observed in MS medium supplemented with 0.1 mg/l 2, 4-D and decreased at higher concentrations (0.2, 0.4, 0.6, 0.8, 1 mg/l). Initiation of callus was totally arrested when explants were grown in MS media supplemented with 1.2 or 1.4 mg/l of 2, 4-D.

Keywords: Ocimum sanctum, 2, 4-D, Murashige and Skoog, Callus.

Introduction
Ocimum sanctum L. is one of the most widely used and commercially exploited herb of India (Misra 1998). It contains essential oil and a wide array of other natural products including polyphenols such as flavonoids and anthocyanins (Misra 1998). Jharkhand is reach in the distribution, abundance and variation in *O. sanctum.* In order to avoid its gradual depletion and natural exctinction due to anthropogenic interference, mass propagation through tissue culture of elite and local genotypes and chemotypes will be an asset (Misra *et al*. 1998). Tissue culture produces clones, in which all product cells have the same genotype. The objective of this study is to standardize a protocol for initiation of a callus culture which will be utilized for multiple shoot formation and mass propagation of Ocimum *Sanctum* (L.) from Jharkhand through callus culture.

Material and method:
Leaf explants of *ocimum sanctum* L. were collected from the field grown Medicinal & Aromatic Plants garden of CUJ. Leaves were cut into small pieces (around 1cm), sterilized sequentially 3x (i) with 1.25% sodium hypochloride for 20 minutes and washed thoroughly in distilled water (DW), (ii) 0.1% mercuric chloride for 4-5 minutes and washed with DW, and (iii) then sterilized with ethyl alcohol for 15 minutes and washed with DW. The sterilized leaf explants were inoculated in MS basal medium containing 3% (w/v) sucrose with supplement of different concentration of 2, 4-D (0.1-1.4 mg/l) for callus initiation. The pH of the medium was adjusted to 5.7 with 1 N NaOH or 1 N HCl before gelling with 0.8% (w/v) agar. Vertically implanted explants in test tubes were maintained at 25 ± 2 °C temperature, continuous illumination of white light (125 µmole. $m^{-2}s^{-1}$).

Result and Discussion
The inoculation of leaf explants for one week in MS medium showed swelling of the explants at the cut end of the leaf adjoin the medium. Callus initiation was observed in the second week after inoculation. Four weeks later, callus initiated at the cut edge of the explants, developed into a full grown callus. The cultures produced different amounts of fresh mass depending on the different concentration of 2, 4-D. The manipulation of plant growth regulators is essential to optimize the induction of callus and callus growth (Misra *et al*. 1998). Maximum callusing was observed in MS medium supplemented with 0.1 mg/l 2, 4-D and decreased gradually at higher concentrations (0.2, 0.4, 0.6, 0.8, 1 mg/l) of 2, 4-D (Fig. 1 and Fig. 2). Initiation of callus was totally arrested when explants were grown in MS media supplemented with 2, 4-D

concentration more than 1.2 mg/l (Fig. 1). The callus formed was friable, light green with some white callus in color distributed on the top of the light green callus.

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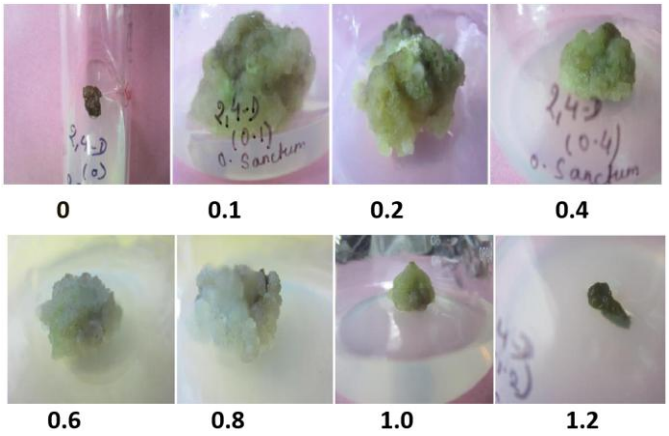

Fig 1: Callus formation in *Ocimum sanctum* L. leaf explants cultured for 4 weeks in MS ± 2, 4-D Different concentration in mg/l of 2, 4-D is shown on each Figure.

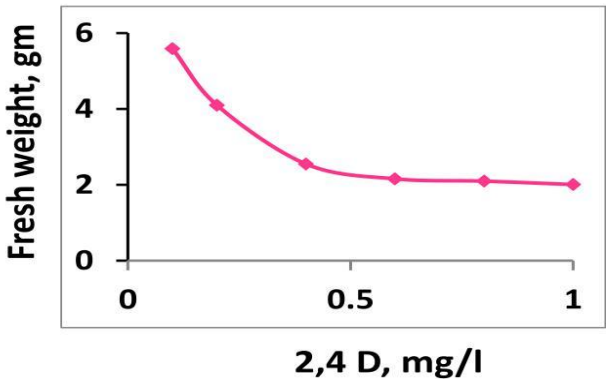

Fig 2: Effect of different concentrations of 2, 4-D on callus growth of *O. sanctum* L

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