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Protocol Establishment for Multiplication and Regeneration of 'Holy Basil' (*Ocimum sanctum* Linn). An Important Medicinal plant with High Religious Value in India

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Abstract
Ocimum sanctum Linn, also known as "Holy Basil" is one of the most common medicinal plants used by diverse cultures and tribal groups. An efficient protocol for rapid propagation of *O. sanctum*, of the family Lamiaceae, was developed using leaf explants culture. The leaf explants cultured on Murashige and Skoog (MS) basal medium were supplemented with various concentrations and combinations of auxins and cytokinins. Callus induction was obtained within 7-10 days, Picloram at 3mg/l formed profuse callus and the degree was found to be the highest (+++++) among all the treatments. The best response to shoot induction, with maximum shoot elongation 6.8±0.14 was obtained using 1.0mg/l 6-benzyl aminopurine (BA) in combination with 0.5 mg/l Indole Acetic Acid (IAA). This medium showed 82% shoot bud proliferation with 23.8±0.23 mean number of shoots. *In vitro* shoots were rooted on 1.5mg/l of Indole Butyric Acid (IBA) supplemented medium. The rooted shoots were successfully acclimatized and established under natural conditions, with 90% survival rate.

Keywords: *Ocimum sanctum*, medicinal plant, micropropagation

1. Introduction
O. sanctum has been known from as early as the Vedic period, commonly known as "Tulsi", belonging to the family of Lamiaceae. The plant is held sacred by Indians all over the world as it is an herb that is used for religious purposes, in addition to its great medicinal values [1]. The plant is an aromatic bushy shrub. It grows all over India up to 2000 meters in the hills. It is grown in houses, temples and gardens. Leaves have a sweet, clove-like fragrance. There are several varieties of this species. It shows a range of leaf colour (purple and green), leaf shape and texture, flower colour, height and fragrance. The flowers are tiny, purple or white and inflorescence is a long spike. Of all the *Ocimum* species, *O. sanctum* is most widely studied for its pharmacological properties. Traditionally leaves are used in bronchitis, gastric, skin and hepatic disorders [2]. Diverse pharmacological properties of *O. sanctum* for example antidiabetic, immunostimulant [3], antioxidant [4], cardioprotection [5], antifungal [6], have attracted entrepreneurs to set eyes on this plant. Leaf extract of *O. sanctum* had showed significant ability to scavenge free radicals [7]. The industry sector perceives *O. sanctum* not just as a spice used in household kitchen. They also see it as a potential lead to the production of drugs that are important to answer the insatiable needs of the population. These drugs would be cost effective and temperamentally quite suiting to millions of our masses, as this herb and its remedies are in use from ancient times. One outstanding obstacle in production and extraction of compounds from this plant is the standardization of quality and quantity of the chemicals. As discovered by various researches, even close members of the same genus of *Ocimum* do not possess the same chemical constituents [8]. Razdan (2003) revealed that as *O. sanctum* is able to cross-pollinate with other plants of the similar genus, certain plants would not be true-to-type, and if there are genetic variations in the plant, the chemical constituents would be different [9]. Under such a situation it is important to develop techniques for rapid mass propagation of this species to meet up the commercial need and also for protecting the genetic erosion. *In vitro* culture is an effective mean for rapid multiplication of species, in which it is necessary to obtain a high progeny uniformity.

2. Material and Methods
Young leaves of *O. sanctum* were collected from the medicinal plant garden of P.G Dept. Of Botany, Utkal University, Bhubaneshwar, India. Leaves (explant) were collected from two

years old field grown mature plants (Fig a). Young leaves were rinsed under running tap water for 20 minutes and were surface sterilized separately in an aqueous solution of 0.1% (w/v) mercuric chloride for 3-4 minutes. The sterilized young leaves were cut into 5mm x 5mm squares and these explants were inoculated on Murashige and Skoog's (1962) basal medium (MS medium) supplemented with various concentrations of auxins 2,4 Dichlorophenoxyacetic acid (2,4-D), picloram and IBA (Table 1). The medium was solidified with 0.8 % agar. The pH of the medium was adjusted to 5.8 before autoclaved at 121^{0}C for 20 minutes. The cultures were maintained in a culture room at 25±20C with 16 hrs photoperiod. For each treatment, 10 replicates were taken and each experiment was repeated thrice. Later different concentrations and combinations of BA, IAA and NAA were added on MS basal medium for differentiation of the callus (Table 2). The excised shoots were transferred to liquid medium for rooting. The *in vitro* raised shootlets were subcultured on ½ strength MS medium supplemented with various concentrations of NAA and IBA (Table 3). *In vitro* rooted plantlets were transplanted to small earthen pots containing a mixture of soil and compost (2:1) and covered with transparent polyethylene bags to maintain high humidity. They were hardened in plastic cups containing vermiculite soil and kept in the green house for further acclimatization and finally transferred to the field.

3. Result and Discussion

3.1 Induction of Callus and Shoot bud proliferation

The manipulation of plant growth regulators is essential to optimize the induction of callus. After 4 weeks of observation, all the plant growth regulators tested on leaf explants showed 100% of callus formation (Table 1). However the degree of the callus induced from the leaf explants varied from each plant growth regulator. Picloram was the best auxin used for callus induction. The callus formed was compact, light green in colour with some white callus distributed on the top of the light green callus (Fig b). Picloram at 3mg/l concentration showed best response in the degree of callus formation. Luczkiewcz and Cisowski (2001) reported that plant growth regulators (IAA and NAA) could stimulate the callus to synthesize anthocyanins and further increase the anthocyanin contents in the callus up to 44% [10]. During redifferentiation, the callus cells were no longer prepared for cell division, and rather ready for organogenesis [11]. The leaf explants took 8±1 days for induction of callus and later it showed profuse callus formation on this medium. The phase of cell growth in callus also influences the yield of flavonoid [12]. During early exponential phases of tobacco callus culture, anthraquinones were supposed to be the predominant secondary metabolites [13]. Among the various treatments to study shoot induction in *O. sanctum,* the best response with maximum shoot elongation (6.8±0.14) was obtained after four weeks of subculture, using 1.0 mg/l BA in combination with 0.5 mg/1 IAA (Fig c). This medium also showed a high response to shoot bud proliferation (82%) with 23.8±0.23 mean no. of shoots (Fig d). This was followed by 71% response and 12.5±0.11 mean no. of shoots, in a medium supplemented with 0.5 mg/l each of IAA and BA. NAA supplemented medium showed a lower response (52%) to axillary bud break. Response of BA and IAA was found to be the best combination for bud break response as well as shoot bud regeneration in *O. sanctum.* Similar findings have been reported by various authors [14, 15]. Establishment and multiplication of shoot buds in MS nutrient media was achieved with high cytokinin BA (1.0-3.0 mg/l) and lower concentration of auxin IAA or NAA (0.2- 0.5 mg/l). This result is in accordance with Harris and Stevenson, (1982) and Chee and Pool, (1985) [16, 17]. In contrast to this, Pattanaik and Chand, (1996) observed that at higher concentrations of BA (2-3 mg/l), the number and length of shoots drastically reduced [18].

Table 1: Effects of auxins on callus induction from leaf explant of *O. sanctum* after 4 weeks of culture in MS medium

Auxins	Concentration (mg/L)	Callus formation (%)	Morphology and colour of callus	Degree
Control 2, 4-D	0	0	---	---
	1	100	White, light greenish compact callus	+++
	3	100	White, light greenish compact callus	++++
	5	100	White, light greenish compact callus	++++
Picloram	1	100	White, light greenish compact callus	++++
	3	100	White, light greenish compact callus	+++++
	5	100	White, light greenish compact callus	+++
IBA	1	100	Purplish, light greenish compact callus	+
	3	100	Purplish, light greenish compact callus	+++
	5	100	Purplish, light greenish compact callus	++++

- = no callus formed, += poor callus, formation, ++=minor callus, formation, +++ average callus formation, ++++=moderate callus formation, +++++= profuse callus formation.

Table 2: Effects of growth regulators on MS medium for shoot regeneration of *O. sanctum*

Growth regulators mg/l			Shooting response %	Mean No. of shoots (Mean±SE)	Mean length of shoots cm (Mean±SE)
BA	IAA	NAA			
0.5	0.0	0.0	30	8.6±0.11	1.8±0.21
1.0	0.0	0.0	42	7.9±0.07	1.5±0.14
2.0	0.0	0.0	28	6.2±0.08	6.6±0.22
3.0	0.0	0.0	24	7.3±0.24	5.9±0.24
0.5	0.5	0.0	71	12.5±0.11	5.3±0.10
1.0	0.5	0.0	82	23.8±0.23	6.8±0.14
2.0	0.0	0.0	48	12.9±0.09	2.4±0.17
3.0	0.5	0.0	62	14.6±0.16	1.6±0.11
0.5	0.0	0.5	52	21.6±0.24	4.9±0.21
1.0	0.0	0.5	49	13.5±0.06	2.6±0.18
2.0	0.0	0.5	40	10.7±0.12	2.5±0.29
3.0	0.0	0.5	36	7.3±0.16	1.9±0.18

The mean values given in the table are the average of 10 replicates with the standard errors

3.2 Induction of Root, Hardening and Acclimatization of plantlets

After four weeks, the well developed shoots (2-3 cm) were excised and transferred to half strength MS medium supplemented with IBA singly and in combination with NAA (Table 3). In different concentration of IBA tested, 1.5 mg/l of IBA in half strength MS was found to be most suitable with 89% rooting response (Fig e). Use of half strength MS media for root induction was also observed by Das *et al.* (1997) [19]. The supplementation of auxin either singly or in combination was also reported in many plant species [20, 21]. However, the addition of IBA also favored rooting in other medicinal plants like *Picrorhia kurrooa* [22] and *Sida cordifolia* [23]. Approximately 90 percent of the regenerated plants survived under the field condition (Fig f). There were no morphological differences observed, among the *in vivo* and *in vitro* grown plants. These results exhibited that the protocol described for clonal propagation of *O. sanctum* was very efficient in regeneration of this species.

Table 3: Effect of auxins on root induction of *O. sanctum* in half strength MS medium

Growth regulators mg/l		Rooting response %
IBA	NAA	
0.5	0.0	56.25±1.41
0.0	0.5	45.71±1.72
0.1	0.0	47.14±2.56
0.5	0.0	62.50±3.10
1.0	0.0	81.25±0.83
1.5	0.0	89.00±0.73
2.0	0.0	60.00±1.22
1.0	0.5	51.25±2.03
1.5	0.5	38.88±2.02
2.0	0.5	00.00±0.00

Fig a: Complete plant *Ocimum sanctum*

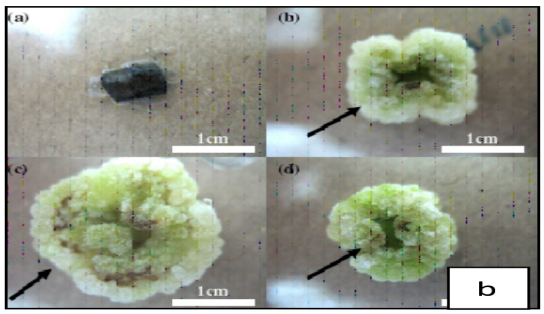

Fig b: Callus induction from the leaf explants of *O. sanctum* after 4 weeks of culture in MS medium supplemented with different concentrations of picloram. (a) 0 mg/L; (b) 1 mg/L; (c) 3 mg/L; (d) 5 mg/L

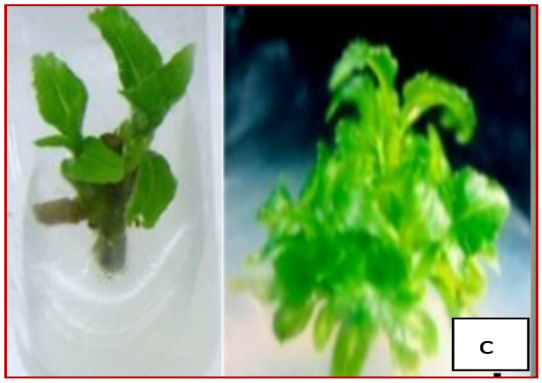

Fig c: Shoot induction and bud break response of *O. sanctum* on MS medium supplemented with BA (0.5 mg/l) and IAA (0.5 mg/l) after two weeks of culture.

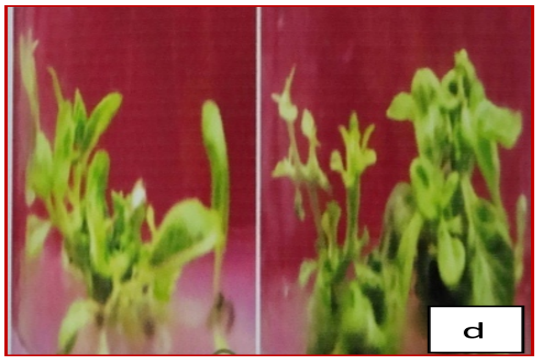

Fig d: Shoot elongation and multiplication of *O. sanctum* on MS medium supplemented with BA (1.0 mg/l) and IAA (0.5 mg/l) after first subculture

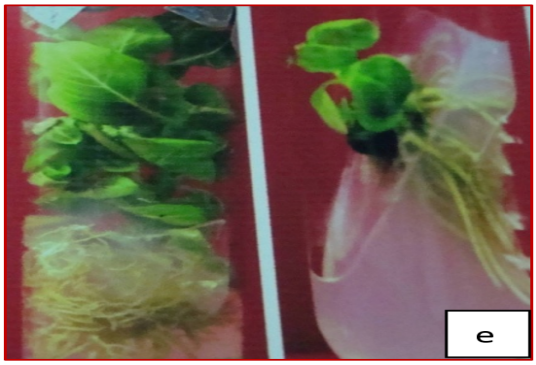

Fig e: Root induction from *in vitro* regenerated shoots of *O. sanctum* on half strength MS medium supplemented with IBA (1.5 mg/l) after five weeks of transfer.

Fig f: Regenerated plants of *O. sanctum* maintained in green house on a potting mixture of 2:1 of compost and soil.

4. Acknowledgements
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5. References

1. Banu LA, Bari MA, Haque E. *In vitro* propagation of *Ocimum sanctum* L. through nodal explants. Bangladesh J. Genet. Biotechnol 2001; 2:143-146
2. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants, Council of Scientific and Industrial Research, (CSIR), New Delhi, 1986, 39-40.
3. Mukherjee PK, Maitib K, Houghton, PJ. Leads from Indian medicinal plants with hypoglycemic potential. J. Ethnopharmacol 2006. 106(28):1-28.
4. Khosla MK. Sacred Tulsi (*Ocimum sanctum* L.). Traditional Medicine and Pharmacology 1995; 15:53-61.
5. Sood S, Sethi J, Seth S, Talwar A. Evaluation of hypoglycemic and antioxidant effect of *Ocimum sanctum*. Indian. J Clin Biochem. 2006; 19(2):152-155.
6. Awuah RT, Ellis WO. Effects of some groundnut packing methods and protection with *Ocimum* and *Syzygium* powders on kemel infection by fungi. Mycopathologia 2002; 154(5):264-269.
7. Geetha, Kedlaya R, Vasudevan DM. Inhibition of *Ocimum sanctum*: *In vivo* and *In vitro* studies. Life Sci 2004; 76:21-28.
8. Singh, NK, Sehgal CB. Micropropagation of Holy Basil (*Ocimum sanctum* L) from young infloerescence of mature plants. Plant Growth Regul 1999; 29:161-166.
9. Razdan RZ. Introduction to plant Tissue Culture. Science publishers, U.S.A, 2003.
10. Luczkiewcz M, Cisowski W. Optimisation of the second phase of a two phase growth system for anthocyanin accumulation in callus cultures of *Rudbeckia hirta*. Plant cell, Tissue and organ culture. 2001; 65(11):57-68.
11. Mohr HP, Schopfer P, Lawlor G, Lawlor DW. *Plant physiology*. Springer Kerlog, Berlin, Heidelberg 1995; 6:198-230.
12. Rahman A. Studies in Natural Products Chemistry. Elsevier, Netherlands, 1988, 155-156.
13. Bohm B, Stuessy TF. Flavonoids of Sunflower family (Asteraceae). Springer. U.S.A, 2001, 240-242.
14. Sahoo Y, Pattnaik SK, Chand PK. *In vitro* clonal propagation of an aromatic medicinal herb *Ocimum basilicum* L. (Sweet basil) by axillary shoot proliferation. In vitro Cell Dev. Biol. Plant 1997; 33:293-296
15. Begum F, Amin N, Azad MAK. *In vitro* rapid clonal propagation of *Ocimum basilicum* L. Plant Tissue Culture 2002; 12:27-35.
16. Harris RE, Stevenson JH. *In vitro* propagation of *Vitis*. *Vitis* 1982; 21:22-32
17. Chee R, Pool RM. The effects of growth substances and photoperiod on the development of shoot apices of *Vitis* cultured *in vitro*. *Scientia Horticulturae* 1985; 16:17-27
18. Pattnaik SK, Chand PK. *In vitro* propagation of the medicinal herbs *Ocimum americanum* L. Syn. *O. canum* Sims. (hoary basil) and *Ocimum sanctum* L. (holy basil). Plant Cell Rep 1996; 15:846-850.
19. Das P, Samantaray S, Roberts AV, Rout GR. *In vitro* somatic embryogenesis of *Dalbergia sissoo* Roxb.-A multipurpose timber yielding tree. Plant Cell Report. 1997; 16:578-582.
20. Gopi C, Sekhar N, Ponmurugan P. *In vitro* multiplication of *Ocimum gratissimum* L. through direct regeneration.African Journal of Biotechnology 2006; 5(9):723-726.
21. Baksha R, Miskat AJ, Khatun R, Munshi JL. *In vitro* Rapid Clonal Propagation of *Rauvolfia serpentina* (Linn.) Benth Bangladesh J. Sci. Ind Res 2007; 42(1):37-44.
22. Chandra B, Palni LMS, Nandi SK. Propagation and conservation of *Picrorhia kurrooa* Royle ex Benth: An endangered Himalayan medicinal herb of high commercial value. Biodivers. Conserv. 2006; 15:2325-2338.
23. Sivanesan I, Jeong BR. Direct shootregeneration from nodal explants of *Sida cordifolia* Linn. *In vitro* Cell. Dev. Biol 2007; 43:436-441.