International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 11-12-2021, Accepted: 27-12-2021, Published: 12-01-2022
Volume 7, Issue 1, 2022, Page No. 225-229

In vitro micropropagation of *Ocimum sanctum* L.

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Abstract
Tulsi or holy basil (*Ocimum sanctum* L) is a common plant seen in most of the Indian household. It is treated as a spiritual plant but scientifically it is one of the immediate antibiotics in hand. It is also one of the plants used extensively in Ayurvedic medicines where of all the herbs used, Tulsi is the most important herb in Ayurveda, and modern study is now verifying its health benefits.It has a unique combination of pharmacological activities that can relieve physical, physiological, metabolic, and psychological stress. Tulsi has also been proven to help with metabolic stress by lowering blood sugar, blood pressure, and cholesterol levels, as well as mental stress by improving memory and cognitive performance and acting as an antipanic and anti-depressive agent. Its broad-spectrum antibacterial action, which includes activity against a variety of human and livestock infections, implies that it could be utilized as a hand sanitizer, mouthwash, and water purifier, as well as in animal husbandry, wound repair, food preservation, etc. The study gives an insight about the preferable media with given composition which can be used for the micropropagation of Tulsi.The selected explants of the shoot were inoculated *in vitro* in the growth media i.e., Murashige and Skoog media with varying concentrations of auxin and cytokinin, (2,4-D, BAP, IBA). The explants showed results after 22 days (about 3 weeks) of inoculation, where callusing was observed in nodes, internodes, and young inflorescence.

Keywords: *Ocimum sanctum*, *in vitro*, explants, holy basil, murashige and skoog media, tulsi

Introduction
Holy basil (*Ocimum sanctum*), often known as Tulsi or Tulasi, is a mint family (Lamiaceae) phanerogam cultivated for its fragrant foliage. Holy basil is a plant that thrives across Southeast Asia and is indigenous to India [1]. The plant is profusely used in Ayurvedic and traditional ailment, and is revered in Hinduism [2]. It is commonly used as an herbal tea for a range of disorders. It's also used as a flavor enhancer, with a strong flavor that gets stronger as it cooks. It has a zesty spice and is reminiscent of clove, Italian basil (*Ocimumbasilicum*), and mint. In certain regions beyond its usual habitat, it is considered an agricultural weed and an invasive plant [3].Tulsi is a prominent emblem of tradition in Hindu faith. Due to its extensive medical characteristics, it has made a significant contribution to science since olden days and continues to do so in modern studies. It has long been used as an anti-inflammatory, antimicrobial, and antipyretic medication to treat common cold, coughs, and flu [4, 2, 22]. Scientists from all around the globe have conducted extensive research on this plant, establishing that it has features such as tissue repairing, anti-oxidant, anti-carcinogenic, anti-inflammatory, and anti-ulcerogenic effects [5].

The advent of micropropagation methods using *in vitro* propagation techniques is a crucial step toward gaining independence from natural reservoirs.

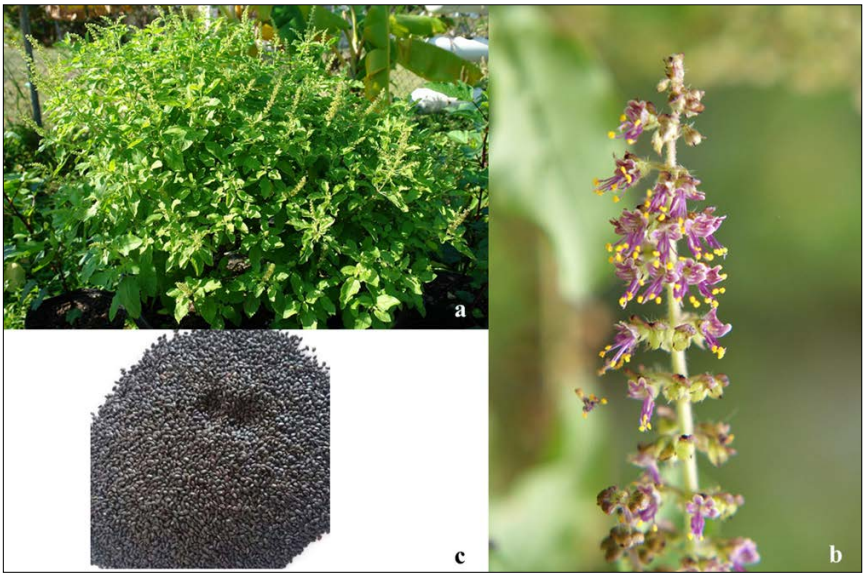

Fig 1: Ocimum sanctum: a) Habit b) inflorescence c) seed

Tissue culture is the *in vitro* sterile technique cultivation of cells, tissues, organs, or entire plants under regulated nutritional and environmental conditions, which is commonly used to make plant clones (6). The regulated circumstances provide favorable conditions for the culture's growth and reproduction (6). These parameters include adequate nutrition supply, a pH medium, an appropriate temperature, and a suitable gaseous and liquid environment. For bulk plant propagation, the technology is frequently used. Plant tissue culture techniques have gained significant industrial importance in the areas of plant regeneration, disease removal, plant enhancement, and secondary metabolite production, in addition to their use as a research too. (3)(7)(8) Small plant parts, explain it is called is enough to produce several plantlets from it hence the efficiency of plant propagation is increased by this method. The method is widely used in propagation of endangered and endemic plant species, economically important species, and in plant specific drug extraction. (9)(10) In present paper *in vitro* propagation of Tulsi explants were done using MS media and the results obtained are presented and discussed.

Materials and methods

A healthy and mature plant with many inflorescences was selected from Nagarabavi locality in Bengaluru. Shoots with leaves, axillary buds and young inflorescence was cut from the mother plant. The plant parts were washed in running tap water for several times to remove dirt particles from it. Following which they were washed with 10% Teepol solution along with 4% Sodium hypochlorite (NaOCl) for 10 minutes by shaking them inside a closed jar. Again, they were washed with tap water to remove the traces of detergent from them and was kept inside a jar containing autoclaved distilled water until further sterilization process. Later further chemical sterilization was carried out in the inoculation chamber inside the Laminar Air Flow (LAF), where the plant parts were then sterilized with 0.1 % mercuric chloride ($HgCl_2$) solution for 15 minutes, 20% Hydrogen peroxide (H_2O_2) solution for 10 mins and with three changes in autoclaved distilled water for 2 minutes each to remove the traces of mercuric chloride and hydrogen peroxide which usually are potent toxins for plants.

Table 1

Murashige - Skoog (MS) media							
Stock Solutions							
Stock	Components	Amount per liter of original media (mg/L)	Concentration of stock solution	Amount of stock solution prepared (mL)	Amount of component used in stock solution (mg)		Amount of stock used for 1 liter of media (mg/L)
					mg	g	
Stock 1	$MgSO_4.7H_2O$	370	10x	500	3700	3.7	50
	KNO_3	190			1900	1.9	
	NH_4NO_3	1650			16500	16.5	
	KH_2PO_4	170			1700	1.7	
Stock 2	$CaCl_2.2H_2O$	440	10x	500	4400	4.4	50
Stock 3	$MnSO_4.4H_2O$	22.3	100x	100	2230	2.23	1
	$ZnSO_4.7H_2O$	8.6			860	0.86	
	H_3BO_4	6.2			620	0.62	
	$NaMoO_4.2H_2O$	0.25			25	0.025	
Stock 4	$CuSO_4.5H_2O$	0.025	200x	200	5.0	0.005	1
	$CoCl_2.6H_2O$						
Stock 5	KI	0.83	100x	100	83	0.083	1
Stock 6	$FeSO_4.7H_2O$	27.85	20x	200	557	0.557	10
	EDTA(Na)	37.25			745	0.745	
Vitamins							
Component			MS media 1 (mg/L)		MS media 2 (mg/)		
Myoinositol			100		100		
Glycine			2		5		
Pyridoxin			1		1		
Thiamine			1		1		
Hormones							
6-benzylaminopurine (BAP)			2		-		
Indole 3-butyric acid(IBA)			2		2		
2,4-dichlorophenoxyacetic acid (2,4-D)			-		1		
Other Components							
Coconut Water			100		100		
Sucrose			30		30		
Agar agar			11		11		

The sterile plant parts were then removed on to a sterile plate and then the explants were excised from it using a sterile blade. The explants included the leaves (both dorsal and ventral surface), internodes, nodes having axillary buds and young inflorescence. The instruments employed here were sterilized inside hot air oven for 1-2 hours at 200°C. and the process of explants removal and inoculation was done inside the LAF unit in the inoculation room. The explants were then inoculated using sterile forceps into the culture bottles containing sterile growth media. The growth media employed here was Murashige and Skoog media with concentrations of different constituents mentioned in the *Table 1*. Media was prepared according to the standard procedure using the stock solutions; pH was maintained at 6.5 – 7.0 before autoclaving following which the media was poured into the culture bottles capped, wrapped, and

autoclaved at 121°C at 15 psi pressure for 20 minutes. Later the media was left for solidifying by altering the concentrations of the hormones. Two different media sets were prepared namely, M.S (Murashige and Skoog) media 1 and 2. Varying concentrations of hormones were considered based on the literature survey carried out. The concentrations of hormones were as mentioned in *Table 1*. Explants were inoculated in 20 culture bottles of each type of the media. After inoculation of the explants, the culture bottles were capped and sealed with cling wrap to avoid any contamination and were transferred into the culture room with temperature maintained at 25 (±2) °C and providing 2000 lux of light. Observations were recorded at weekly intervals and changes if any were noted.

Results

Table 2: Growth seen in media with varying concentrations of hormones over the period of 4 weeks. (2, 4-D- 2,4-dichlorophenoxyacetic acid, IBA– Indole 3-butyric acid, BAP- 6-benzylaminopurine)

Media	Conc. Of 2,4-D	Conc. Of IBA	Conc. of BAP	After 2 weeks	After 3 weeks	After 4 weeks	After 5 weeks
M.S media 1	-	2 mg/L	2 mg/L	No growth observed	No growth observed	No growth observed	No growth observed
M.S media 2	1 mg/L	-	2 mg/L	No growth observed	Callus formation observed	Callus turns green, leaves formed	Callus formation in leaf explant

Out of the best two media selected with varying concentrations of cytokinin and auxins, M.S media 2 with Indole 3-butyric acid (IBA) as cytokinin and 2,4-dichlorophenoxyacetic acid (2,4-D) as auxin with concentration 2mg/L and 1mg/L respectively showed positive results. The explants from lateral buds of nodal region, internode showed the formation of callus after 3 weeks of inoculation.

Cultures were observed every week to track the growth in explants. After 4 weeks, callus turned green and formation of leaves were seen from the callus. Out of all the explants inoculated.

Fig 3: a) Callus formation seen in node explant after 3 weeks – Media: M.S Media 2 (a_1& a_2). b) Formation of leaves from nodes and internodes after 4 weeks – Media: M.S Media 2 (b_1-b_3).

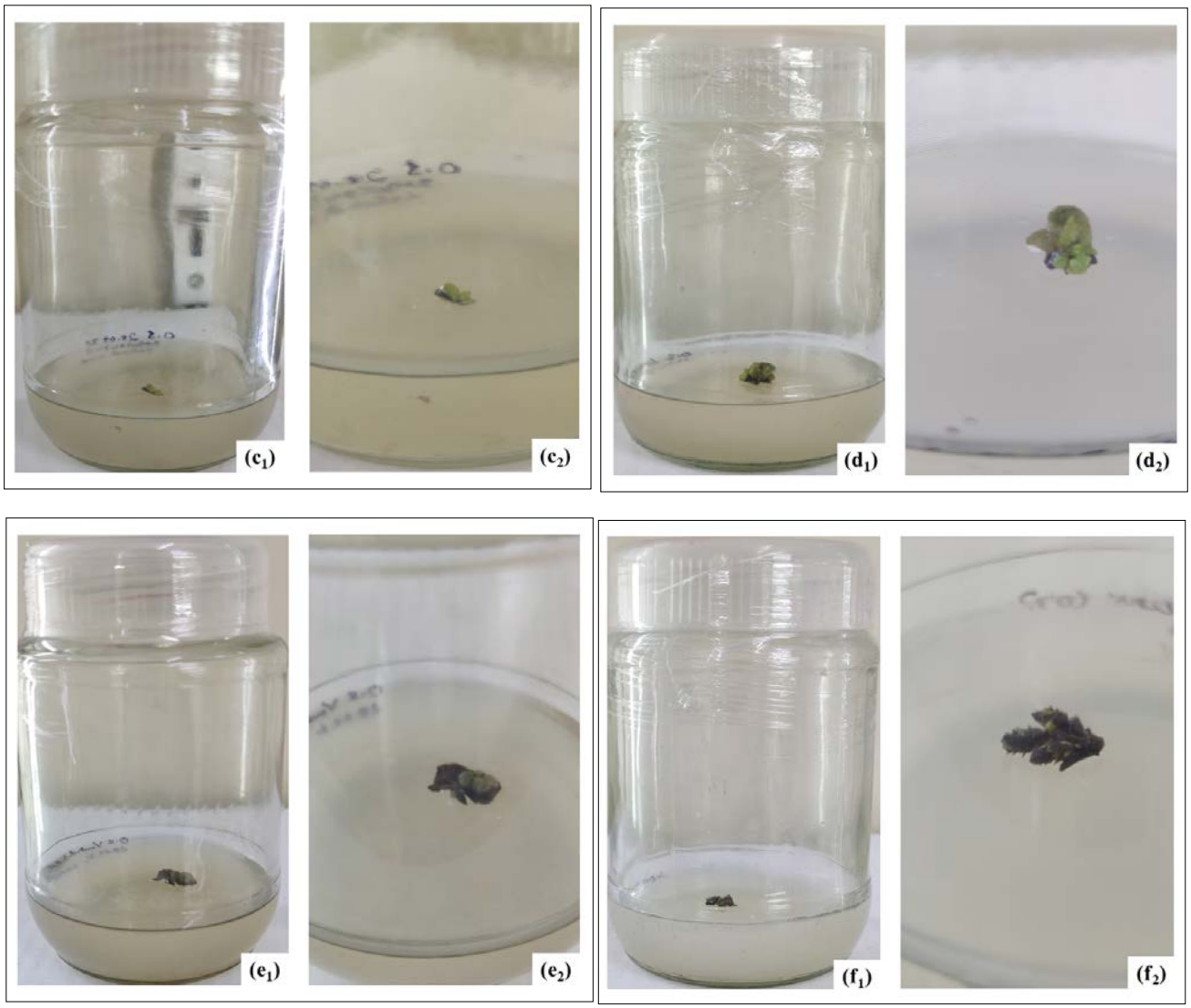

Fig 4: 3) Callus growth on explants after 5 weeks– Media: M.S Media 2: node (c_1& c_2), internode (d_1& d_2), leaf (e_1& e_2), inflorescence (f_1& f_2).

Discussion

Media with BAP and 2,4-D showed good results with the formation of callus after 3 weeks and formation of leaves after 4 weeks. Similar results were seen [11] with the similar hormone combination. Whereas, media with BAP and IBA did not show any results after 4 weeks of observations. Adventitious shoots can be obtained either by direct method or indirectly by the forming of callus. Indirect growth is generally not preferred, as chances of somaclonal variation are high by the formation of callus [4].Somaclonal variations results in variation of desired characters in the cultures. With higher concentrations of cytokinin than auxin, M.S media 2 showed callus formation with formation of small leaf appendages. In order to obtain direct shoot or root formations, cytokinin and auxin ratio is to be increased respective to obtain shoot and root cultures. Other than IBA, NAA can also be used as synthetic auxin [4].

The present work is a differential study conducted to find the mediasuitable among the two media compositions prepared for obtaining good cultures of *O. sanctum* in the given media sets. Therefore, the process of hardening or sub culturing were not taken up.It was found during the study that media with concentrations BAP – 2mg/L and 2,4 D – 1mg/L showed better results against BAP and IBA – 2mg/L concentrations respectively. The explants are closely monitored further to study the rate of growth.

Tulasi, *Ocimum sanctum* is an important plant with umpty medicinal and cosmetic benefits. It is an integral part of many Ayurvedic, Siddha and Unani medication. Micro propagation of *O. sanctum* is helpful in harnessing the full potential of the plant. In order to extract essential secondary metabolites, micro propagation techniques are useful. Through suspension cultures, the secondary metabolites can be extracted in large quantities and meet the growing commercial demands for Tulsi based medications.

Acknowledgment

The authors gratefully acknowledge the support and encouragement by the management of St. Joseph's College Autonomous, Bengaluru and the faculty of Departure of Botany to conduct and publish this work.

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