IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Tissue Culture Of Tulasi (*Ocimum Sanctum*) From Stem Nodes

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Abstract:

Tulasi, also known as Holy Basil (*Ocimum sanctum*), is revered in Hindu culture and prized for its medicinal properties. It aids in respiratory health, digestion, and stress relief due to its antimicrobial and adaptogenic qualities. Tulasi is used in traditional medicine for treating asthma, indigestion, and anxiety. It's also valued for its role in promoting heart health and maintaining oral hygiene. Culturally significant, Tulasi is worshipped for its spiritual significance and widely used in cooking for its aromatic flavour. Tulasi cultivation faces challenges such as climate sensitivity, pest susceptibility, and the need for precise watering and soil management. These factors can affect growth, yield, and overall plant health, requiring careful attention and management practices for successful cultivation. Tissue culture of Tulasi offers rapid multiplication of disease-free plants, ensuring genetic purity and uniformity. It enables year-round cultivation regardless of climatic conditions, contributing to consistent supply and preservation of this culturally significant plant. Additionally, tissue culture techniques facilitate research and development for enhancing Tulasi's medicinal properties and agricultural traits. in this present study evaluate the tissue culture of Tulasi plant from stem nodes in Murashige and Skoog. According to this study stem node turn green colour with in 4 weeks of incubation and started to form callus. On fifth week ending a small leaves developments were observed in stem nodal callus area from explants. In this state, we haven't noticed any root formation emerging from the callus.

Key words: Tissue culture, Tulasi (*Ocimum Sanctum*), Stem nodes.

Introduction:

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 (Smitha et al. 2014). The plant is profusely used in Ayurvedic and traditional ailment, and is revered in Hinduism (Singh and Chaudhuri, 2018). It is commonly used as an herbal tea for a range of disorders. It's also used as a flavor enhancer, with a strong flavour that gets stronger as it cooks. It has a zesty spice and is reminiscent of clove, Italian basil (*Ocimum basilicum*), and mint. In certain regions beyond its usual habitat, it is considered an agricultural weed and an invasive plant (Cohen, 2014). 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 (Gulati et al. 2015; Singh and Chaudhuri, 2018, Rameshv et al. 2021). 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 (Hussain et al. 2012).

Tulsi, an annual herb ranging from 35 to 70 cm in height, features branched stems, typically adorned with purple hues and soft, spreading hairs. Its leaves measure 2.5-5 cm in length and 1.7-3.3 cm in width, exhibiting various shapes and margins, with both sides bearing pubescent surfaces and dotted glands. Flowers occur in racemes 15-20 cm long, forming verticillate inflorescences, accompanied by broad bracts and slender, pubescent calyxes. The corolla, 4 mm long and purple, displays a bilabiate upper lip with pubescence on its back. Exserted stamens feature slender filaments, with the upper pair possessing small appendages at their bases. The nutlets, 1.26 mm long, are broadly ellipsoid, smooth, and marked with yellow and black hues. Seeds are brownish and globose or subglobose, with a distinct aromatic odor and sharp taste. Additionally, it bears fruit of a cerulean hue. In Fig 1. Tulasi plant images have given.

Fig 1: Tulasi (*Ocimum sanctum*) plant from House hold of Guntur District, Andra Pradesh, India

The chemical composition of Tulsi is highly complex, containing many nutrients and biological active compounds. Standardization of the active components of Tulsi is very complicated due to its inherent biochemical complexity and botanical nature. However, best known of many active components of the Tulsi, leaves are the source of an essential oil i.e eugenol and ursolic acid (Lawrence et al. 1972, Skaltsa et al. 1987). Ursolic acid, that have been identified and extracted are one of the major constituents. It has been suggested to possess antifertility effects in male mice and rats of both sexes. Ursolic acid possess anti-estrogenic effects, causes a decrease in sperm counts (Godhwani et al. 1988) and reduces spermatogenesis. Eugenol is a phenolic compound and also a major constituent of essential oils extracted from different parts of Tulsi plant. The therapeutic potential of the extracted essential oils from the fresh leaves of *Ocimum sanctum* L. has been found to be largely due to major constituent i.e eugenol which itself is a phenolic compound (l-hydroxy-2-methoxy-4- allylbenzene) (Mukherjee et al. 2005).

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 (Thorpe, 2007). The regulated circumstances provide favourable conditions for the culture's growth and reproduction (Thorpe, 2007). 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 research too (Cohen 2014; Pandey et al 2013; Chandran et al. 2020). 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. (Coelho et al. 2020; Espinosa-Leal et al. 2018)

Tissue culture is an age-old practice for in vitro regeneration of plants, especially the medicinally valuable plants that are difficult to propagate in natural environment (Atal and kapoor 1989). This method is also used for studying the molecular studies of plant development thereby artificially increasing the plant output molecules (Dhar et al. 1968). For the clonal propagation of plants with high medicinal value, the use of controlled environment systems is ideal due to the highly regulated growth conditions of water, light, temperature and soil. The use of greenhouses and in vitro techniques of growing plants. Both presents the controlled environment systems. Propagation of plants under in vitro conditions is the use of a sterile, controlled environment in which plants are provided all the necessary nutrients through a pathogen free medium (George et al. 2008) In vitro propagation has also been used in different medicinal plants. Being a unique tool, tissue culture provides more maintenance of numerous plant species or germplasm lines in a effective manner at very young growth stage which can be later on transferred into greenhouse environments (George et al. 2008). Changes of media components and external inputs can increase production of medicinal constitutes (Rout et al. 1999).

Different studies have been done on the micropropagation of Tulsi. Culturing of shoot tip culture is widely used for rapid propagation of many species due to its great advantages with respect of traditional methods. Young shoot cultured in Murashige and Skoog, (MS) medium containing sucrose and different growth regulators like as indole-3 acetic acid (IAA), α-naphtholene acetic acid (NAA), 6 benzyl aminopurine (BAP) forms callus which further elongated by transferring to fresh media after a fixed time interval.

In present paper in vitro propagation of Tulsi explants were done using MS (Murashige and Skoog) media and the results obtained are presented and discussed.

Materials and methods:

Plant collection and cleaning process:

In vitro regeneration of *Ocimum sanctum* (holy basil) using nodal segments as explants. This protocol aims to efficiently propagate *Ocimum sanctum* in a controlled laboratory environment: Fig 2. Has given detailed information about work flow of *Ocimum sanctum* tissue culture.

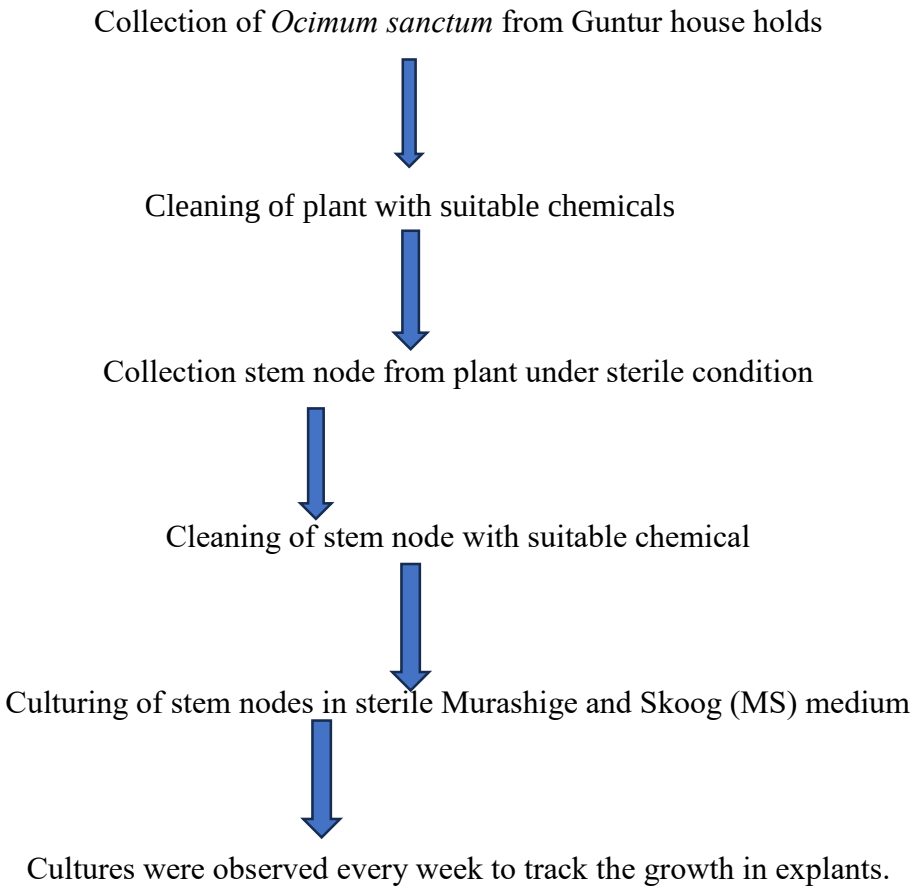

Fig 2. Work flow chat of *Ocimum sanctum* tissue culture

A healthy and mature plant with many inflorescences was selected from Guntur locality in Andhra Pradesh. 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 (HgCl2) 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.

Explants Preparation:

Use nodal segments (small sections of stem with one or more nodes) as the starting material. Sterilize the nodal segments using appropriate disinfectants to prevent contamination.

Medium Preparation:

Prepare Murashige and Skoog (MS) medium. The basal MS medium should be supplemented with specific growth regulators.

Organogenesis and Multiplication:

Culture the nodal segments on MS medium supplemented with: 6-Benzyl amino purine (BAP) at a concentration of 0.1 mg/L. Indole-3-acetic acid (IAA) at a concentration of 0.025 mg/L. Observe the development of leave from the explants.

Result and Discussion:

Cultures were observed every week to track the growth in explants. After 4 weeks of inoculation, the explants from stem nodal area of culture converted to green hue and started to form callus. On fifth week ending, callus turned green and formation of leaves were seen from the callus (Table 1). Development of leave on stem nodes of Tulasi in tissue culture has given Fig. 3.

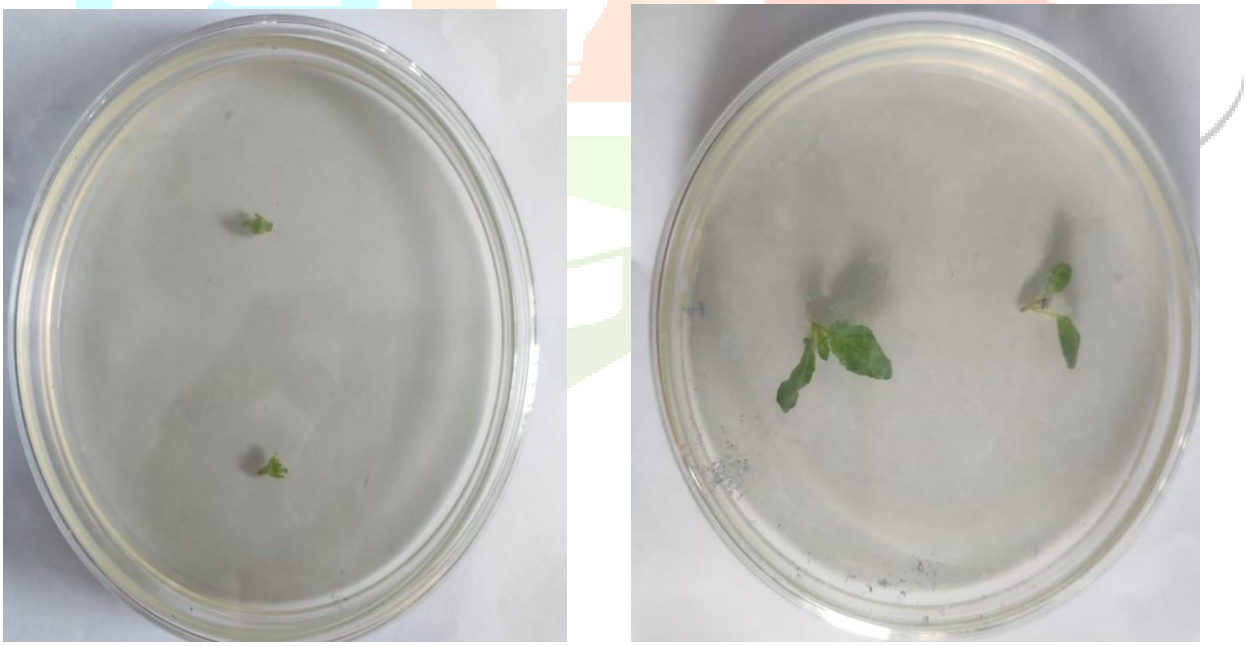

Fig. 3. Stem node tissue culture of Tulasi (*Ocimum sanctum*) in MS Medium

Table 1: Cultures were observed every week to track the growth in explants

Incubation period in weeks	Response of callus
2 weeks	No growth
3 weeks	No growth
4 weeks	Explant convert to green hue and formation of callus
5 weeks	Callus start to form small leaves from the node area.
6 weeks	No root formation were observed

Various explants of holy basil such as inflorescence, nodal, leaf, stem, shoot tip, axillary buds and cotyledons have been used to initiate callus cultures using BA or TDZ (Singh and Sehgal, 1999) or 2,4-D (1.0 mg/L) in combination with kinetin (Hakkim et al. 2007) Nodal segments were induced to form callus and shoots with the combination of auxin and cytokinins, NAA (naphthalene acetic acid) (5 mg/L) and BA (0.5 mg/L) or 2,4D at 0.2 mg/L25 similar to multiplication from shoot tips with NAA 0.1 mg/L and BA at 0.2 mg/L16 and axillary buds with NAA and kinetin (Gogoi and Kumaria 2011).

Direct root formation on micropropagated shoots of holy basil also requires a higher auxin to cytokinin ratio and is accomplished by the addition of NAA. Shoots derived from axillary buds were found to root optimally in media containing 1.0 mg/L NAA, with 98% root formation after 10-12 days (Pattnaik and Chand,1997; Girija et al. 2006).

Conclusion:

Plant tissue culture involves growing plant cells, tissues, or organs in a controlled, sterile environment. Its applications include micropropagation for rapid multiplication of plants, genetic modification to introduce desired traits, germplasm preservation for genetic diversity conservation, and somatic embryogenesis for mass plant production. It's also used for haploid and doubled haploid production, secondary metabolite synthesis, disease eradication, and research/education in plant biology and biotechnology. Tissue culture offers a powerful tool for plant propagation, genetic improvement, conservation, and scientific investigation.

Tulasi plant tissue culture finds various applications in research, conservation, and commercial propagation: Micropropagation: Tissue culture enables rapid multiplication of Tulasi plants, producing large numbers of genetically identical individuals for commercial cultivation or research purposes. Genetic modification: Tissue culture techniques facilitate the introduction of desired traits into Tulasi plants, such as enhanced medicinal properties or resistance to pests and diseases, through genetic engineering. Secondary metabolite production: Tissue culture can be optimized to enhance the production of bioactive compounds in Tulasi, such as essential oils and antioxidants, for pharmaceutical, cosmetic, or nutraceutical applications. Disease eradication: Tissue culture allows for the production of disease-free Tulasi plants from healthy explants, helping to conserve disease-resistant genotypes and ensure the availability of high-quality planting material. Germplasm preservation: Tulasi germplasm can be cryopreserved using tissue culture techniques, preserving genetic diversity and ensuring the availability of plant material for future breeding and research efforts. Research and education: Tissue culture serves as a valuable tool for studying Tulasi growth and development, as well as investigating its biochemical and molecular properties. It is used in both academic and industrial settings for research and educational purposes. Overall, Tulasi plant tissue culture plays a crucial role in the propagation, genetic improvement, conservation, and utilization of this important medicinal herb.

Acknowledgement:

The author expresses gratitude to the PEARL LAB for their meticulous review of this article and their invaluable support during the successful completion of the internship program in food technology.

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