IN VITRO MICROPROPAGATION OF *Ocimum tenuiflorum* (Tulsi)

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Abstract

Ocimum tenuiflorum is an aromatic perennial medicinal plant in the family Lamiaceae. Direct regeneration of nodal explants and their multiplication have been optimized using cytokinin BAP (0.5-4.0 mg/l) and combination of BAP (0.5 mg/l) + NAA (0.5-3.0 mg/l) respectively. MS media with nodal explants supplemented with BAP (2.0 mg/l) produced maximum average number of shoots (2±0.37) and average shoot length was found to be 2.8±0.15 cm. Best initiated shoots then sub-cultured for shoot multiplication, an improved shoot multiplication in terms of average number of shoots (5.3±0.41) and average shoot length 6.5±0.12 cm was observed on MS media in combination with BAP (0.5 mg/l) + NAA (1.5 mg/l). Maximum average number (12±0.20) and average length 9.8±0.26cm of roots were observed on MS media supplemented with IBA (2.0 mg/l) out of different IBA (1-5 mg/l) concentrations were taken in to consideration during the study. Regenerated plantlets were successfully transferred to greenhouse condition.

Keywords: *Ocimum tenuiflorum, In vitro, Murashige and Skoog medium, BAP, NAA.*

Introduction-

Tulsi is cultivated for religious and traditional medicine purposes, and for its essential oil. It is widely used as a herbal tea, commonly used in Ayurveda, and has a place within the Vaishnava tradition of Hinduism, in which devotees perform worship involving holy basil plants or leaves. Holy basil is an erect, many-branched subshrub, 30-60cm tall with hairy stems. The discovery might suggest the evolution of tulsi is related with the cultural migratory patterns in the Indian subcontinent. Some of the phytochemical constituents of tulsi are oleanolic acid, ursolic acid, rosmarinic acid, eugenol, carvacrol, linalool, B-caryophyllene (about 8%). Tulsi essential oil consists mostly of eugenol (-70%) B-elemene (-11.0%), B-caryophyllene (-8%) and germacrene (-2%), with the balance being made up of various trace compounds, mostly terpenes.



The three main morphotypes cultivated in India and Nepal is Ram tulsi, the less common purplish green-leaved (Krishna tulasi) and the rare wild "vanatulsi". Tulasi (Sanskrit:-Surasa) has been used in Ayurveda and Siddha practices for its supposed treatment of diseases. Traditionally, tulasi is taken as herbal tea, dried powder, fresh leaf or mixed with ghee. *Ocimum tenuiflorum* is reported to have Antioxidant activity and Anti-microbial activity:

2. Objective

1) To study the effect of growth hormones on shoots initiation, multiplication and rooting of *Ocimum tenuiflorum*.

3. Material and Methods:-

Material:-

Nodal segments were used as explants.

3.1 Collection of Plant material:-

The plant of *Ocimum tenuiflorum* was collected from Dhanwantari Medicinal and Aromatic plants project, Mahatma Phule Krishi Vidyapeeth, Rahuri.

3.3 Methods:-

3.3.1 Sterilization of glassware's, equipments, media and working environment:-

The glassware's such as culture bottles, measuring cylinders and petriplates etc. were washed in running tap water using detergent followed by rinsing with double distilled water and then kept for dry heat sterilization for 30 min at 45°C in hot air oven. After cooling they were wrapped in aluminium foil and autoclaved subsequently at 121°C at 15 lbs. pressure for 20 minutes. Ready to use Murashige & Skoog medium of Himedia, specially formulated for plant cell, tissue and organ culture was used. Murashige and Skoog is a defined medium which consists of inorganic salts, vitamins and Carbohydrate.

3.3.2 Inoculation and Incubation of explants:

Surface sterilized explants were washed with doubled distilled autoclaved water for 2-3 times under Laminar Air Flow sterile condition. Finally with the help of forcep the explants were inoculated on the surface of the MS media in a such a way that ¾ part of the nodal explants would be in contact with MS media. The culture bottles containing explants inoculated on MS media supplemented with respective different conc. of BAP) and were incubated at 25±3°C under white fluorescent light (2000 lux) for 16/8 hours light and dark conditions. In total 6 weeks of initiation period one subculture was done in 3rdweek and data for average shoot number per explants and average shoot length was recorded at the end.

3.3.3 Shoot multiplication:

The best initiated grown shoots were then transplanted on MS media supplemented with different concentrations of BAP and NAA as listed

below. Again the data for average shoot number per explants and average shoot length was recorded after 6 weeks of incubation period.

3.3.4 Rooting:

For complete plant development regenerated shoots were excised and transferred to MS medium supplemented with different concentrations of IBA.

Data was recorded after 6weeks of inoculation in growth chamber

Average number of roots.

Average length of roots (cm).

Statistical analysis was done by calculating the standard error (SE).

3.3.5 Hardening:

The rooted plants were removed from culture vessels and washed in running tap water to remove agar. The number of roots that developed was counted and the plants were transferred to plastic pots containing sterile soil: sand: vermiculite (1:2:1, v/v/v).

4. Result and Discussion:-

4.1 Results :

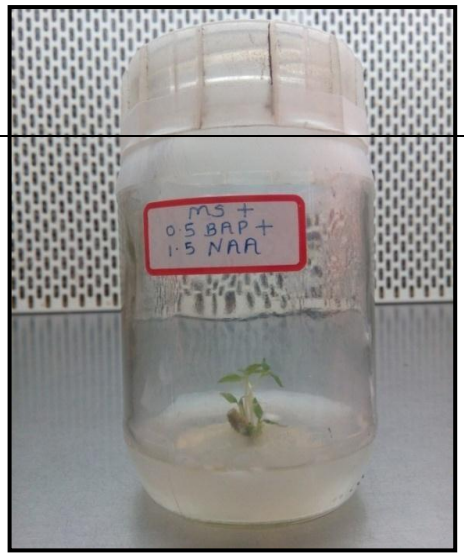
4.1.1 Initiation:

Table 4.1 Effect of different conc. of BAP on *invitro* shoot induction from Nodal explants of *Ocimum tenuiflorum* after 6 weeks of inoculation-

Sr. No.	MS medium + BAP(mg/l)	Averae Shoot Numbe r (mean ± SE)	Average Shoot Length (cm) (mean ± SE)	Plate 4.1 Result of Initiation
1.	MS medium+0.0(control)	-	-	
2.	MS medium + 0.5	1 ± 0.28	1 ± 0.26	
3.	MS medium + 1.0	1.3 ± 0.05	1.6 ± 0.43	
4.	MS medium + 1.5	1 ± 0.20	1.4 ± 0.25	
5.	**MS medium+ 2.0**	**2 ± 0.37**	**2.8 ± 0.15**	
6.	MS medium + 2.5	1.3 ± 0.11	1 ± 0.20	
7.	MS medium + 3.0	1.3 ± 0.41	1.2 ± 0.11	
8	MS medium + 3.5	1 ± 0.20	1.6 ± 0.15	
9.	MS medium + 4.0	1.6 ± 0.41	2.3 ± 0.34	

4.1.2 Multiplication:

Table 4.2 Effect of different conc. of BAP and NAA on *invitro* shoot multiplication *Ocimum tenuiflorum* after 6 weeks-

Sr. No.	MS Medium + BAP(mg/l)+ NAA (mg/l)	Average Shoot Number (mean ± SE)	Average Shoot Length (cm) (mean ± SE)	Plate 4.2 Result of Shoot multiplication
1.	MS Medium+0.5+0.5	2.6 ± 0.25	3.4 ± 0.28	
2.	MS Medium+0.5 +1.0	1.6 ± 0.30	2 ± 0.32	
3.	**MS Medium+0.5+ 1.5**	**5.3 ± 0.41**	**6.5 ± 0.12**	
4.	MS Medium + 0.5+2.0	4 ± 0.43	4.7 ± 0.23	
5.	MS Medium+0.5+2.5	2.6 ± 0.36	2.3 ± 0.23	
6.	MS Medium +0.5+3.0	2.5 ± 0.32	2.1 ± 0.11	

4.1.3 Rooting:

Table 4.3 Effect of different conc. of IBA on *invitro* rooting of *Ocimum tenuiflorum* after 6 weeks-

Sr. No.	MS Medium + IBA (mg/l)	Average Root Number (mean ± SE)	Average Root Length(cm) (mean ± SE)
1.	MS Medium +1.0	6.6 ± 0.37	5.3 ± 0.20
2.	**MS Medium+ 2.0**	**12 ±0.20**	**9.8 ± 0.26**
3.	MS Medium +3.0	5.3 ± 0.15	4.7 ± 0.43
4.	MS Medium + 4.0	3.6 ± 0.25	4 ± 0.32
5.	MS Medium +5.0	3.3 ± 0.36	3.7 ± 0.28

Here,	**Plate 4.3 Result of Rooting**
r1-MSmedium +1.0IBA (mg/l)	 
r2-MSmedium+ 2.0 IBA(mg/l)	
r3 - MS medium + 3.0 IBA (mg/l)	
r4-MSmedium+4.0IBA(mg/l)	
r5 - MS medium + 5.0 IBA (mg/l)	

Discussion:

During initiation of explants it has been observed that nodal explants cultured on MS media devoid of growth hormone (Cytokines) failed to induce the initiation of explants. Out of all concentrations of BAP (0.5-4.0 mg/l) used for shoot initiation BAP (2 mg/l) promoted the shoot initiation i.e. average number of shoot (2±0.37) and average length of shoot (2.8±0.15cm). Axillary and apical buds gave maximum response with respective to the initiation of explants when inoculation on MS media fortified with 1mg/l BAP. They found 75% response with 1-3 no. of shoot/explants and shoot length was 2 cm. Good *in vitro* response (average shoot number 5.3±0.41 and average shoot length 6.5±0.12cm) was observed of *Ocimum tenuiflorum* initiated shoots were cultured on MS media augmented with BAP (0.5 mg/l) + NAA (1.5 mg/l) increasing concentration of BAP and NAA resulted in gradually increased in *in vitro* response of shoots, while further increased concentration was found to be directly proportional to poor response of the shoot multiplication.

Finally initiated elongated shoots were excised and implanted on MS media augmented with IBA (1-5 mg/l). The optimum root induction i.e. average number of roots (12±0.20) and average root length (9.8±0.26cm) was obtained on MS media supplemented with 2 mg/l IBA.

Summary and Conclusion:-

This medicinally significant plant species has been depleted from its natural habitat and is now included in the list of endangered species by the International Union for Conservation of Nature and Natural Resources (Kavidra *et al.*, 2000; Supe *et al.*, 2006). In vitro micropropagation of *Ocimum tenuiflorum* has been achieved by using nodal segment as a explants. The explants was initiated on MS media supplemented with different concentration of BAP (0.5 to 4.0 mg/l) resulted in best response on BAP (2.0mg/l) produced in terms of average number of shoots 2±0.37 and average shoot length was 2.8±0.15cm. The best initiated shoots then transferred of multiplication on MS medium supplemented different concentration of BAP in combination with NAA. After six weeks of incubation BAP and NAA (0.5mg/l+ 1.5mg/l) produced maximum average number of shoot 5.3±0.41 and average shoot length 6.5±0.12cm. MS medium supplemented with IBA 2.0mg/l produced maximum average of number roots 12±0.20 and average root length was 9.8±0.26cm. After rooting, rooted plantlets were successfully established in primary and secondary hardening. After 30 days of inoculation on rooting medium, the rooted plantlets were removed from the culture tube and washed with distilled water. These *in vitro* derived plantlets were transferred to plastic pots containing mixture of sand and FYM in 1:1 ratio for 18 days for hardening. It was concluded from this study that plant regeneration from nodal explants of *Ocimum tenuiflorum* offered a great Potential in agriculture and this in genetic transformation of this important species. The protocol can be exploited for *in vitro* generating new genetic variability and production of bioactive constituents from the plant.

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