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TISSUE CULTURE STUDY OF *Ocimum sanctum* FOR INITIATION OF MICROSHOOT ON NODAL EXPLANTS

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ABSTRACT

Tissue culture study for initiation of microshoots from the nodal explants of *Ocimum sanctum* L, an important medicinal herb has been carried out. Nodal explants were cultured on MS medium supplemented with different concentrations of 6- Benzyl amino purine (BAP) alone or with NAA, and Kinetin at (0.5-3.5 mg/l) concentrations. Maximum percentage of response for the induction of microshoots was obtained in MS + 1.5 mg/l BAP + 0.5 mg/l NAA (96.28), where as the number of microshoots at this concentration was 5.78 with average length of shoots 4.5 cm. BAP alone at 1.5 mg/l in MS medium could induce microshoots in 90.86 % of the explants where the mean number of microshoots was 4.58 and mean length 3.42 cm, while at the same concentration kinetin in MS medium induced microshoots in 80% of the explants with mean number of microshoots 4.16 and the mean length 3.14 respectively. Here the highest response was 82.36 % the mean number of shoots 4.42 and mean length of shoots 3.24 cm in MS + 2.0 mg/l KN. Shoots with fully expanded leaves were used for rooting in MS medium supplemented with (0.5-2.5 mg/l) concentrations of NAA and IBA separately. Maximum response for rooting was found in MS + 1.5 mg/l IBA (78.36%) and the mean number of roots was 3.48. At the similar concentration of NAA the response was poor that is 26.24% only.

KEYWORDS : Microshoots, Medicinal Plant, Nodal Explants, *Ocimum sanctum*, Tissue Culture

Ocimum sanctum L. of Lamiaceae is an important medicinal plant, which is commonly planted in the kitchen garden in general and of the Hindu in particular. Due to its medicinal importance the plant is worshiped by Hindus. Common applications are in cough and cold. Because it has anti-inflammatory properties so it is used externally to reduce swelling and pain. Leaf extract is used to cure different skin diseases. Drop of leaf extract if put in the nostrils, it help to relieve headache. It also helps to sharpen memory. It helps in expectoration of excess mucus secretion. It acts as cardiac tonic and blood purifiers. Seeds of tulsi help to increase the sperm density and reduce premature ejaculation. Reduces blood sugar & cholesterol.

Plant tissue culture techniques are being used for induction of calli and multiple shoots in case of different medicinal herbs and trees. Micropropagation may help us to produce clones of a particular plant species that is true to its parents for its genotype, and is faster than the conventional methods. We get different literatures with respect to tissue culture of medicinal plants such as Ahuza et al*.,* 1982 in (*Ocimum* spp. Vincent et al*.,* 1992) Kaemferia galangal L., George et al., 1993. *Gardenia jaminoides*, Krishna and Seenis*,* 1994. *Woodfordia fruticosa*, Balachandran et al*.,* 1990); Bhat et al., 1995; Pipper spp; Das and Pal, 2005; Ajit Kumar and Seeni, 1998; Aegle marmelos; Andrade et al., 1999; Faisal and Anis, 2003) *Tylophora indica*, Gopi et al., 2006; Saha et al., 2010; in *Ocimum basilicum*, Ganraj et al., 2012 in *Achyranthes aspera* and *A. bidentata*. Shahzad et al*.,* 2012.

Even today mass scale cultivation of *Ocimum* is not found. The natural means of propagation is through seeds, but it is not available for commercial cultivation at one hand while on the hand the viability of seeds is very poor. Therefore, micropropagation technique is the best alternative for the production of large number of genuine planting materials. Keeping all these ideas in mind present work was done.

MATERIALS AND METHODS

Ocimum sanctum growing in the campus of University Department was maintained for healthy plants. As they were growing in open air so before culture the nodal explants were sterilized by washing under running tap water for 45 min, followed by treatment with 70% alcohol for 1 min. Above nodal explants were treated with 0.1% aqueous solution of $HgCl_2$, for 2-3 min. During this treatment the flask was shaken manually for uniform contact of the chemical with the explants. Explants were taken out and rinsed with sterile distilled water three to four times, each time having 3-4 min durations to remove even a trace of the chemical. Above explants were stored in pre-sterilized and moist clother at low temperature.

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MS basal medium was supplemented with 3% sucrose and different concentrations of growth regulators separately. The pH was adjusted to 5.8 with 1N, HCl, and gelled with 0.8% agar (m/v) (Hi-Media). One liter of medium was dispensed in 125 cc culture tubes 20 ml each and after plugging with cotton plugs, were autoclaved at 15 lb pressure. Inoculation of explants was done under aseptic conditions of Laminar flow chamber. Inoculated cultures were incubated in culture room maintained artificially at 26±1^{0}C and 65-70% humidity. The light period was maintained at 16 h with cool white fluorescent tube light, Philips, India.

Axillary buds were initiated at different concentrations and combinations. Best concentrations and combination was selected. Sub-culture of the initiated shoots was done in that medium. For root initiation, plantlets with fully expanded leaves were cultured in MS medium supplemented with different concentrations of two auxins.

Observation was made on alternate day and culture showing contamination was discarded. During observations, the percentage response of explants for shoot initiation, number of shoot per explants, length of the shoots all were noted. All the experiments were done in triplicate and the mean of the data was taken for calculation of standard error by applying the standard formula for it.

RESULTS AND DISCUSSION

For initiation of microshoots, tissue culture study of *Ocimum sanctum* was done. During the experiments, different data were collected and the mean was taken for each, parameters viz., percentage response, number of

Table 1 : Impact of Different Growth Regulators on Nodal Explants of *Ocimum* in MS Medium

Culture Growth Regulator Concentration in mg/l in MS Medium			% Response of internodes	No. of shoots + SE	Shoot length after 18th days of Initiation
BAP	**KN**	**NAA**			
0.5	--	--	42.66	1.42 ± 0.15	1.32 ± 0.14
1.0	--	--	68.24	2.68 ± 0.36	1.86 ± 0.26
1.5	--	--	90.86	4.58 ± 0.26	3.42 ± 0.32
2.0	--	--	72.38	3.72 ± 0.32	2.74 ± 0.26
2.5	--	--	68.78	3.48 ± 0.28	2.28 ± 0.24
3.0	--	--	62.54	2.84 ± 0.38	1.82 ± 0.28
3.5	--	--	44.18	1.46 ± 0.28	1.24 ± 0.26
--	0.5	--	42.26	1.22 ± 0.11	1.18 ± 0.12
--	1.0	--	56.74	2.32 ± 0.28	1.64 ± 0.28
--	1.5	--	80.64	4.16 ± 0.32	3.14 ± 0.26
--	2.0	--	82.36	4.42 ± 0.36	3.24 ± 0.28
--	2.5	--	64.42	2.84 ± 0.38	2.66 ± 0.32
--	3.0	--	60.84	2.26 ± 0.24	1.58 ± 0.24
--	3.5	--	41.32	1.32 ± 0.18	1.18 ± 0.14
0.5	--	0.5	52.34	1.84 ± 0.22	1.42 ± 0.22
1.0	--	0.5	74.52	2.92 ± 0.36	2.14 ± 0.26
1.5	--	0.5	96.18	5.78 ± 0.38	4.52 ± 0.28
2.0	--	0.5	83.88	4.72 ± 0.32	3.72 ± 0.30
2.5	--	0.5	72.22	4.22 ± 0.36	2.88 ± 0.26
3.0	--	0.5	68.80	3.18 ± 0.24	1.64 ± 0.28
3.5	--	0.5	59.92	2.46 ± 0.28	1.32 ± 0.24
Control MS medium alone			**No response**		

Data indicates Mean + SE.

Experiments in triplicate, Each time 15 cultures were used.

Final data after 30 days.

Table 2 : Table Showing % Response, Number Roots Per Explants and Average Root Length in ½ MS Basal Medium Supplemented With Different Concentrations of Auxins

MS + Growth Regulators		% Response for Rooting	Mean No. of Roots / Shoots	Mean Length
IBA (mg/l)	**NAA (mg/l)**			
--	0.5	14.24	0.58 ± 0.18	0.18 ± 0.06
--	1.0	16.88	0.63 ± 0.20	0.24 ± 0.08
--	1.5	26.24	0.94 ± 0.22	0.38 ± 0.13
--	2.0	22.30	0.80 ± 0.22	0.27 ± 0.11
--	2.5	18.64	0.62 ± 0.16	0.24 ± 0.08
0.5	--	18.68	0.86 ± 0.22	0.36 ± 0.12
1.0	--	42.52	1.18 ± 0.24	0.44 ± 0.14
1.5	--	78.36	3.48 ± 0.32	1.28 ± 0.16
2.0	--	58.26	2.16 ± 0.34	1.08 ± 0.12
2.5	--	26.38	1.28 ± 0.32	0.56 ± 0.08
Control		---------		

Data indicate mean value + SE.

Triplicate experiment each time 15 cultures were taken.

microshoots, the shoot length etc. The data have been presented in the Table 1. After perusal of the table it is found that nodal explants responded to all the concentrations of growth regulators (0.5-3.5 mg/l). However, there were variations in the percentage of response.

Highest percentage of response was found in MS + 1.5 mg/l BAP + 0.5 mg/l NAA that was followed by MS + 1.5 mg/l BAP alone. At these two conditions the percentage of response was 96.28 and 90.86 respectively. Here the mean number of microshoots was 5.78 and 4.58, while the mean length of shoots was 4.52 cm and 3.42 cm. It was further observed that kinetin at 2.0 mg/l when supplemented in MS medium could induce multiple shoots in 82% of the explants and the mean number of the shoots was 4.16 and mean length 3.24 cm. A gradual increase in the percentage response, number of microshoots, mean length may be noted from the table up to 1.5 mg/l from 0.5 mg/l. However, above this concentration there was gradual reduction in all the parameters considered. Further it may be noted that when MS + 1.5 mg/l BAP was supplemented with 0.5 mg/l NAA, again there was increase in all the parameters that is percentage of response, number of shoots, per explants and mean length of the shoots at all the concentrations of BAP alone.

The cytokinin type and concentration greatly influence axillary shoot generation from nodal explants.

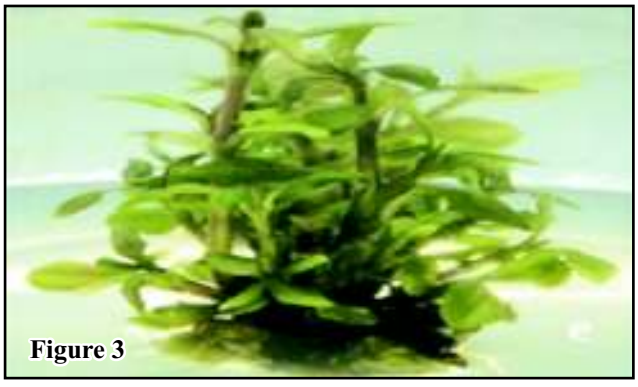

Figure 1 and 2 : Showing 45 Days Old Subculture of *Ocimum* Showing Multiple Shoots

Figure 3 : Showing Root Initiation in 40 Days Old Plantlet of *Ocimum*

Saha et al., 2010; Present findings corroborate with the findings of the above workers. Here BAP was found more suitable than that of the kinetin. It may be noted that at higher concentrations of BAP there was gradual reduction in the percentage of response as well as in the number of shoots per explants. Similar results have been observed by Kukreja et al., 1990, Vincent et al., 1992). George, 1993, reported that BAP overcomes apical dominance and promotes lateral buds. That BAP promotes axillary shoot formation has also been reported in different medicinal plant- *Ocimum* by (Ahuja et al., 1982; Begam et al., 2002; Shaha et al., 2010), Balachandran et al*.,* 1990 reported in Curcuma and Zingiber, Faisal and Anis*,* 2003 in *Tylophora indica*.

The effect of sub-culture on the multiplication rates achieved in culture is known to differ from one species to another. Siddique and Anis, 2007; reported increase in the rate during subculture in *Cassia angustifolia*. Debergh and Maena, 1981 reported that two media are required for propagation and elongation, but in the present experiments the same culture conditions were found suitable for the above two.

Shoots having well expanded leaves were inoculated in half strength MS basal medium gelled with 0.6% agar and supplemented with (0.5-2.5 mg/l) IBA and NAA separately. The mean of the percentage response, root number and mean length have been presented in the table 2. Here 1.5 mg/l IBA was more suitable as the percentage of response for rooting was 78 mean number of roots 3.48 and mean length 1.28 cm. This was followed by ½ MS + 2.0 mg/l IBA. It may be noted from the table that similar concentration of NAA gave very poor response which was 26.24 and the mean number of roots was 0.94 cm and the length 0.38 only. Similar findings were reported by Saha et al; (2010). Frequency of rooting in Dendroclamus longispathus (Saxena and Bhojwani, 1993); Chlorophytum borivilianum (Purohit et al; 1994); Lavandula vera (Anrade et al.; 1999) was higher at lower strength of MS medium. Further IBA at its all concentration was more promising for root initiation than at the similar concentration of NAA. Findings of the present research work are in agreement of the findings of Bhujan et al., 1997 in Murraya Spp; Banerjee et al; 1999) in *Centella asiatica* and Faisal and Anis*,* 2003; in Tolyphora. Here both the lower and higher concentrations of IBA and NAA had very poor response with respect to root initiation.

The findings of the present work may be exploited for the production of large number of planting materials at the commercial scale.

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