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In Vitro Shoot Regeneration of *Elaeocarpus sphaericus* (Rudraksha) – An Important Medicinal Plant

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Abstract

Elaeocarpus sphaericus (syn: *Elaeocarpus ganitrus*; Elaeocarpaceae) is a large evergreen big-leaved tree. *Elaeocarpus sphaericus commonly* known as "Rudraksha". *Elaeocarpus sphaericus* is a medium sized tree occurring in Nepal, Bihar, Bengal, Assam, Madhya Pradesh and Bombay, and cultivated as an ornamental tree in various parts of India. The *Elaeocarpus sphaericus* is an inhabitant shrub that has a good rich history of traditional uses in medicine. The experiment was conducted to optimize growth medium and growth conditions for *in vitro* propagation of *Elaeocarpus sphaericus* through nodular stem sections. Varying concentrations of different growth regulators BAP in combination with Kn were applied through growth medium to access their effects on shoot development. The sterilized explants were inoculated on MS media supplemented with concentration of BAP (1.5 mg/l) in combination with kinetin (1.5 mg/l) showed a good degree of shoot induction. The bud breaking and shoot induction frequency was 80% after 3 weeks of inoculation. The multiple shoots per explants was recorded highest in MS media supplemented with BAP+Kn (1.5 mg/l+1.5 mg/l) & Casein hydrolysate (100mg/l).

Key words : *Elaeocarpus sphaericus*, *In-vitro* shoot regeneration, nodal explant.

Introduction

Elaeocarpus sphaericus commonly known as 'Rudraksha' belongs to family *Elaeocarpaceae*. It is a large evergreen broad-leaved tree found in tropical ever green forests and preferably grows in suitable climatic regions. These fruits are also known as Amritphala (Fruits of nectar). Rudraksha is used for the treatment of various diseases like stress, palpitation, nerve, pain, epilepsy, migraine, lack of concentration, asthma, hypertension, arthritis and liver diseases. Nearly 360 species of Elaeocarpus trees are found in different parts of the world like Australia, East Asia, Malaysia and the Pacific Islands. About 120 Elaeocarpus species belonging to this genus from different parts of Asia and out of this, 35 species occur in India alone. The Rudraksha seeds are primarily used in India, Indonesia and Nepal as beads for organic jewellery and malas and valued similarly to semi-precious stones. An HPTLC densitometry method has been developed to estimate *E. sphaericus* beads in order that plant may be standardized on the premise of its bioactive marker (1).

The *Elaeocarpus sphaericus* seeds exhibit pharmacological properties that include anti-inflammatory, analgesic, antidepressant, antiasthmatic, hypoglycaemic, antihypertensive and anticonvulsant. According to Ayurveda, the bead, bark and leaves of the rudraksha tree, which have antibacterial effects, are used for treating various ailments such as mental disorders, headaches, fever, skin diseases, etc. The leaves of the species are considered medicinally important due to the presence of secondary metabolites like alkaloids, flavonoids, tannins, terpenoids and cardiac glycosides. Flavonoids are used in the management of anxiety (2, 3, 4). The Kani tribes of Kerala use the plant Elaeocarpus for curing arthritis in ancient Ayurvedic medicative system. In situ and ex situ conservation techniques are endorsed in view of their ethnic medicinal importance (5) and to derive economic benefits due to increasing demand of Rudraksha beads at various Rudraksha centers in Asia. Nut collection for beads has caused the shrinkage of the seed bank in the soil. Besides, poor seed has adverse effects on the regeneration of species, pushing it to threatened category. The use of biotechnology on trees has now opened up new possibilities for rapid mass multiplication of existing stocks of germplasm as well as conservation medicinally important plants/plant parts (6, 7, 8). The present research was, thus, undertaken to expand a fast and efficient in vitro propagation technique with the usage of nodal explants of *E. sphaericus* brought from its natural habitat in India for large scale production at regions harbouring subtropical climatic conditions suitable for the growth of this plant species.

Materials and Methods

Plant material and sterilization : Plant parts were collected from nursery of Forest Research of institute (FRI) Dehradun. Nodal segments were cut and washed in running tap water to remove the dust particles followed by several washings. Surface sterilization procedure of nodal explants included treatments in 0.1 % Tween-20 (v/v; 10min), 0.1 % mercuric chloride (w/v; 8-10 min) 70 % ethanol (30 s) and followed by repeated rinses in double

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distilled water. The nodal segments of explant were washed with surfactant Tween-20 (3 drops/100 ml solution) for 10 minutes and after washing under running tap water. Nodal explants were surface sterilized by soaking in Mercuric Chloride (0.1%) for 10 minutes followed by repeated washings with sterilized distilled water in laminar flow cabinet to remove the traces of sterilant.

Culture media and incubation conditions : For induction and shoot multiplication MS medium was used. For preparation of working media 100ml of 10x macronutrient stock solution was used and 10 ml 100x micronutrient stock was used for preparation of 1 litre working media. The required amount of disodium EDTA salt and additives (agar, sucrose, myoinositol) were added fresh to medium. B5 vitamins were added from stocks (1 mg/ml). The constituents were mixed properly and distilled water was added slightly less than final volume. Final volume was made 1litre after adjusting the pH to 5.8 by drop wise addition of 1N NaOH or 1N HCL. Finally, required amount of agar was added and after heating the solution till it become transparent, the medium was poured in conical flask, tubes etc. and autoclaved at 15 psi, 121°C for 20 min.

For bud breaking and shoot initiation MS media supplemented with BAP (1.5 mg/l) in combination with Kn (1.5 mg/l) were used. The best concentration was further sub-cultured with BAP (1.5mg/l), Kn (1.5mg/l) and Casein hydrolysate at a concentration of 50mg/l - 100mg/l was used to study the effect of Casein hydrolysate on the rate of shoot multiplication. Data pertaining to average number of shoots was recorded after a period of 6 weeks. Sub-culturing was carried out at periodic interval of 3 weeks using MS medium supplemented with BAP (1.0 mg/l) and CH (50mg/l). All the cultures were incubated in a culture room at 250C ±20 C for 16 hours in light (illuminated by 40-watt cool white fluorescent tubes, 1200 lux) and 8 hours in dark.

Results and Discussion

The results indicated that a very simple and efficient clonal propagation can be successfully achieved for *E. sphaericus* through tissue culture technique.

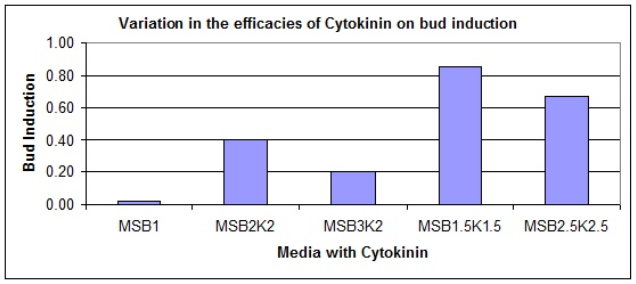
Sterilization procedure : The sterilization procedure adopted resulted in 80% aseptic and responsive cultures. The sterilization of explant was carried out at concentration of $HgCl_2$ for different time and was allowed to culture on basal MS medium. The explants were treated with 0.1% for 10 minute showed survivability.

Bud breaking and Shoot initiation for proliferation of *Elaeocarpus sphaericus* : Bud break occurred in nodal explants only after 3 weeks of inoculation. 80% explants have shown bud break in MS + BAP (1.5 mg/l) + KN (1.5 mg/l). Whereas MS + BAP (2 mg/l) + KN (2 mg/l) gave 40% bud break (Table 1, Fig1.a). After 3 weeks, the bud breaking from nodal explants were transferred to fresh medium of the same composition to shoot initiation for proliferation (Fig 1.b). The shoots were subsequently used for further *in vitro* shoot multiplication.

Bud Induction :

Table-1: Response to growth regulators and mean shoot induction in *E. sphaericus.*

Regulators	Percentage of response (%)	Shoot induction Mean±SE	Signifi-cance	CD
BAP3	00	0.02± 0.015		
BAP1.5K1.5	83%	0.85 ± 0.029	***	0.060
BAP2+KN2	57%	0.40 ± 0.058	(P<0.001)	
BAP2.5+Kn2.5	60%	0.67 ± 0.035		
BAP3+Kn2	40%	0.20 ± 0.058		



Interpretation : The analysis revealed that the variation among the regulators was highly significant (at p< 0.001). The regulator BP3 maximum shoots induction closely followed by (BP1 +KN1). BP1 shows the minimum shoot induction.

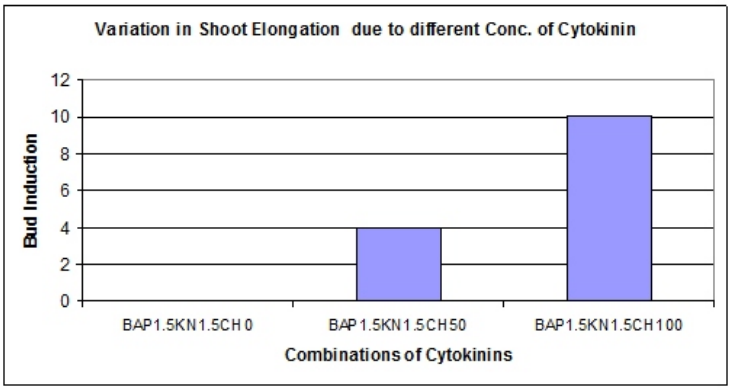
Multiplication of shoots : For shoot multiplication, Casein hydrolysate used. Casein hydrolysate can be a source of calcium, phosphate, several microelements, vitamins and most importantly, a mixture of up 18 amino acids. Several Casein hydrolysates are available commercially but their value for plant tissue culture can vary considerably. The data recorded on number of shoots per explants obtained after shoot regeneration on BM supplemented with different concentrations of growth regulators BAP+KN combination with Casein hydrolysate (Table-2, Fig.-1.c). The number of shoots per explants inoculated on $MSB_{1.5}K_{1.5}$ & CH (100 mg/l). Maximum number of shoot per explants was observed in $MSB_{1.5}K_{1.5.}$

This concentration of plant growth regulators is suitable for multiplication of shoots $MSB_{1.5}K_{1.5}$ with Casein hydrolysate.

Interpretation : The analysis revealed that the variation among the regulators was highly significant (at p< 0.001). The combination of regulators (BP1.5KN1.5CH100)

Table-2 : Response to casein hydrolysate and mean number of shoots in *E. sphaericus*.

S. No.	MS medium and plant growth regulator Concentration			Observations after 3 weeks		Significance	CD
	BAP	KN	CH	No. of Flask cultured (1explant/ flask)	No of Shoots	(p<0.001)	0.189
Control	1.5	1.5	0.0	02	0 ± 0		
1	1.5	1.5	50	03	3.983 ± 0.101		
2	1.5	1.5	100	03	10.00 ± 0.208		



shows maximum shoot elongation whereas the combination of regulators (BP1.5KN1.5CH100) shows no elongation in shoots.

The outcome indicated that a very simple and efficient clonal propagation can be successfully carried out for *E. sphaericus*. through tissue culture technique. The sterilization procedure adopted resulted in 80% aseptic and responsive cultures. The sterilization of explant was carried out at concentration of $HgCl_2$ for different time and was allowed to culture on basal MS medium. The explants were treated with 0.1% for 10 minute showed survivability. Similarly, (9) studied effects of concentration surface disinfectants ($HgCl_2$) along with variation in treatment time duration. This finding also related with the work done by (10). The effectiveness of 0.1% $HgCl_2$ in surface disinfection of explants of tree species has earlier been reported in Quercus robur (11), Citrus lemon, Eucalyptus F1 hybrid (*E. citriodora* x *E. torelliana*) (12) and *Eucalyptus tereticornis* (13).

Bud break occurred in nodal explants only after 3 weeks. The importance of cytokinin for shoot proliferation is well established and is an essential step to successful micropropagation. In the present study, both BA and Kn were ineffective when used alone for shoot proliferation and growth in the initial stages but showed good results when used in combination and better results were obtained along with growth additive, casein hydrolysate. Similar investigations have been reported in the same species (9) and other species such as *E. robustus* by (14) wherein equal concentrations of BA and Kn along with casein hydrolysate were used for optimal shoot multiplication. Further, for routine sub culturing of multiple shoots of *E. sphaericus*, BAP and kinetins were used. The maximum bud breaking and shoot initiation frequency was obtained in MS medium supplemented with BAP 1.5 mg/l and kinetin 1.5mg/l. This is in accordance of (9) multiple shoots were produced on the medium containing BAP, Kn & casein hydrolysate. However, in *E. tuberculatus* (15) axillary bud proliferation was achieved in MS medium supplemented with BA alone but in vitro maintenance was carried out by sub culturing on the same medium containing same concentrations of BA and Kn. The shoot initiation also observed in MS medium supplemented with BAP 2.5 mg/l with 2.5 mg/l Kn. This is accordance with the findings of (10). As, they reported with BAP, Kn & Casein hydrolysate, an increase in number of multiple shoots was observed. By comparing all parameters of all treatments, 1.5 mg/l BAP & 1.5 mg/l Kn for bud breaking and casein hydrolysate for shoot multiplication gave best performance. This result partially supported (9).

The present findings also indicate that a permissive balance of cytokinin may be required by a plant which can be species specific as is evident from various *in vitro* shoot multiplication protocols of *Elaeocarpus* species. The synergistic effect of growth additive Casein hydrolysate with PGRs on shoot multiplication and elongation of other plant species and enhanced multiplication on sub-culturing of proliferated shoot culture at regular intervals (16) have also been reported. Therefore, this method is a useful alternative to the conventional methods of propagation and will help in conservation of this threatened tree species under suitable habitat conditions in India.

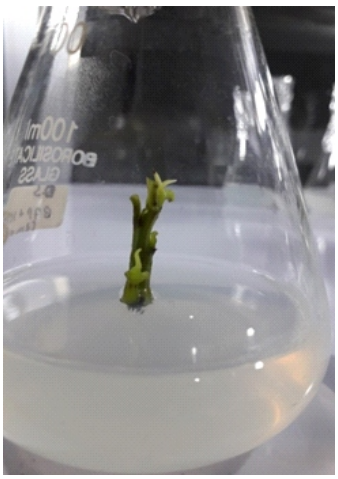


Fig.-1.a : Bud Breaking

Fig.-1.b : Shoot Initiation for Proliferation

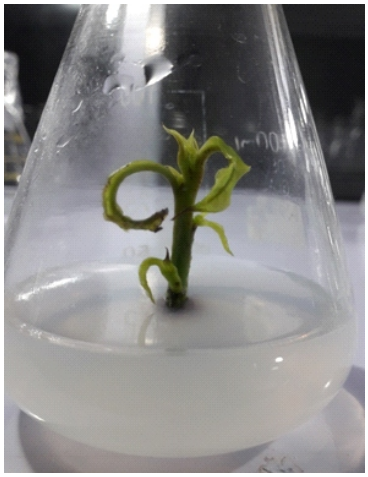


Fig.-1.c : Shoot Multiplication

Fig.-1(a-c) : *In vitro* Shoot Regeneration of *Elaeocarpus sphaericus.*

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