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Somatic Embryogenesis in *Elaeocarpus ganitrus* seen for the first time

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ABSTRACT

Somatic embryogenesis is the process of formation of non-zygotic embryos of plant material from somatic cells under in-vitro conditions. It is an essential biotechnology technique for clonal propagation and genetic transformation in plants. This study focuses on the somatic embryogenesis of Elaeocarpus ganitrus, commonly known as Rudraksh, a tree species with great religious and therapeutic significance. The research involves the somatic embryos were induced from in vitro cultured leaf tissue of Elaeocarpus ganitrus. The explants were grown in Murashige and Skoog (MS) media after surface sterilization. The medium was supplemented with various concentrations and combinations of PGRs, especially Indole acetic acid (IAA) and 2, 4-Dichlorophenoxyacetic acid (2, 4 -D). The induced somatic embryos were subsequently cultured to enhance development and maturation. The development from explants was critical, but has led to advancement in the conservation of Elaeocarpus species. The current study aims to develop somatic embryos from explants of the species to conserve Elaeocarpus ganitrus spp. and even other endangered tree species. The in-vitro micropropagation and plant tissue culture of Elaeocarpus ganitrus have been reported in various studies, whereas globally, we found the somatic embryogenesis in Elaeocarpus ganitrus for the first time.

Keywords: *Somatic embryo, Elaeocarpus ganitrus, MS media, in vitro.*

Introduction

Rudraksha plant, scientifically known as *Elaeocarpus ganitrus*, belongs to the family Elaeocarpaceae. It is an important plant which has broad medicinal as well as spiritual applications [1]. Currently, more than 38 different species of *Elaeocarpus* are known to exist and are grown in Nepal, Indonesia, Java, Sri Lanka, India, and a few other Southeast Asian nations, including Japan, Australia, Malaysia, Southern China, New Zealand, Fiji, and Hawaii. When we talk about the Indian geographical origin of *Elaeocarpus Spp*, the following states and regions come into frame. These are the Himalayan Strips, the Garhwal area, Dehradun, Madhya Pradesh, Maharashtra, Bengal, Bihar, Assam [2]. It is reported that Elaeocarpus has its origin in the mountain range of the Himalayas. It is also considered sacred and has a number of therapeutic and spiritual positive aspects [3]. The phytoconstituents of *Elaeocarpus* are some alkaloids, phenolics, and flavonoids. Alkaloids that are present in the species include Elaeokanine C, elaeocarpenine, isoelaeocarpiline, isoelaeocarpicine, elaeocarpidine, isoelaeocarpine, grandisine A, B, C, D, E, F, and G, habbemine A & B. Phenolics and Flavonoids are found in the species involves Gallic acid, ellagic acid, quercetin, rutin, nargenin, kaempferol, rutinoside, luteolin, etc. [4]. Rudraksha seeds are associated with ethereal and supernatural qualities, and they are assigned a special location. Used as an agent to counter stress, anxiety, sadness, palpitations, nerve pain, epilepsy, lack of concentration, asthma, hypertension, arthritis, and liver illnesses, it is used in traditional medicine. The *Elaeocarpus parvifolius* bark is specifically used to treat malaria. *Elaeocarpus petiolatus* has bitter bark, and the sour leaf juice is used to treat fevers and sunstroke.

Based on the Ayush system of medicine, several studies mentioned the therapeutic benefits of using Rudraksha beads against rheumatism, infertility, neurological and cardiovascular illnesses, and other diseases. Furthermore, it was reported that each Rudraksha bead has different pharmacological qualities [5]. As this plant and its fruits have tremendous uses and advantages, its demands gradually increasing day by day. But due to its origin and tropical climate, it is not easy to grow anywhere in the world. The species of Elaeocarpus are considered in the IUCN Red Data List as least concern and endangered species.

To overcome this scarcity, plant tissue culture techniques and methods can show an important role in the *in vitro* propagation of *Elaeocarpus spp.* The study aims to produce somatic embryos of *E. ganitrus*.

Somatic embryogenesis is a biological phenomenon by which somatic cells, instead of germ cell undergoes different stages of embryonic development to form an organism without the fusion of gametes [6]. Somatic embryogenesis and plant tissue culture have become a powerful biotechnological approach with an abundance of applications such as production of true to type plant, genetically homogenous plant propagation, virus-free plant production, plant conservation, germplasm collection, synthetic seed technology, secondary metabolite source and production, etc [7]. Somatic embryos were first reported in carrot and were induced to grow in an unorganized proliferative fashion by the use of coconut milk [8], whereas the somatic embryogenesis in *Elaeocarpus ganitrus* has not been reported yet. Hence, the study shows a protocol to produce somatic embryos by leaf explant of *Eleacarpus ganitrus* under suitable conditions.

Methodology

As explained, young and healthy leaves of *Elaeocarpus ganitrus* were obtained from lawn of Shobhit University, Gangoh, India. The axillary buds were washed under running tap water for 30 min, and then they were thoroughly rinsed with surfactant

(Tween-20) and then washed 4–5 times in running tap water. After general washing, the explants were surface sterilized with 0.1% mercuric chloride ($HgCl_2$) for 5-10 minutes. Subsequently, the explants were washed with sterile double-distilled water in aseptic conditions (inside laminar air flow).

Murashige and Skoog's (1962) media were prepared and with 3% sucrose, 0.8% agar added with IAA 1.0 mg/L and 2, 4-D 0.5 mg/L adjusting pH of the media to 5.5–5.8. The medium was dispensed into culture tubes (15 ml/ tube) and autoclaved at 15 psi and 121 °C for 15 min. The explants were dissected into small pieces under laminar air flow and carefully transferred to the culture tubes.

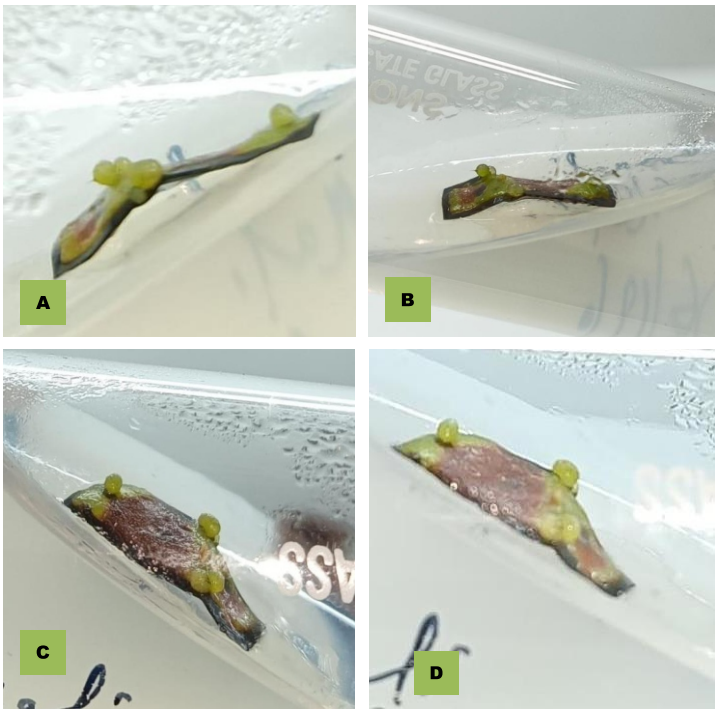
Then the cultures were incubated in the growth chamber at temperature 25+2 °C under 3 klux cool florescent light.

Results and Discussion

The *in vitro* somatic embryogenesis and plant regeneration depend on the different concentrations and combinations of plant growth regulators and photoperiodism (availability of light) [9] The somatic embryos were seen by culturing the leaf cuttings on the MS media supplemented with 1.0 mg/L IAA and 0.5 mg/L 2, 4-D. After an incubation of about 60 days, spherical globular embryos can be clearly seen in the pictures A, B, C and D.

Saklani et al., 2015 has described an efficient micropropagation protocol for *Elaeocarpus ganitrus* developed by from nodal explants of this plant species collected for large scale production of planting material at favorable sites within the country. The shoot buds were germinated to shoots in MS medium accompanied with 2.2 µM BA and 2.2 µM Kn. Addition of Casein Hydrolysate (CH) (100 mg/L) increased the shoot number. Excised microshoots were subcultured on 2.0 µM BA for further enhancement of growth and multiplication. The shoot cultures were further maintained on this concentration for several years by doing regular subculturing at 4-6 weeks time. For root induction in excised shoots were done at MS medium supplemented with 5.0 µM NAA. [10]

The effectiveness of various plant growth regulators (PGRs) with Driver-Kuniyaki-Walnut (DKW) medium for callus induction and regeneration in Elaeocarpus *angustifolius* were experimented for the first time. The tissue culture of Rudraksha has the potential to benefit African communities economically, environmentally, culturally, and educationally. Rudraksha trees can contribute to afforestation projects in Africa, helping to restore degraded lands and combat desertification. [11] *In-vitro* clonal propagation of *Elaeocarpus sphaericus* through axillary bud emergent and succeeding shoot regeneration. The best proliferations from shoots were seen on MS medium supplemented with BAP (1.5 mg/l) and Kn (1.5 mg/l). Shoot multiplication was induced on MS medium fortified with BAP (1.5mg/l), Kn (1.5 mg/l) and CH (100 mg/l). MS medium complemented with 1.0 mg/l BAP and 50 mg/l ADS were used for repetitive sub-culturing of grown shoots at periodic interval of every 3 weeks. [12]. Concentrations and combinations of BAP and Kn unveiled the highest shooting response and number of shoots. WPM with IBA showed better results with the highest number of roots. The rooted plantlets were successfully transferred to vermiculite demonstrated a 37.31 % survival rate [13].



Conclusions

An well-organized and effective plant regeneration protocol was developed via somatic embryogenesis derived from the excised leaf tissue of *Elaeocarpus ganitrus.* The given study indicated that induction of somatic embryogenesis were powerfully exaggerated by the plant growth regulators and culture conditions. The medium containing both natural and synthetic auxin was capable of inducing the formation of somatic embryos in the mentioned woody plant species i.e. *Elaeocarpus ganitrus*. The future perspective of the technique could facilitate the generation of true-to-type plants and allow production of stable transgenic plants from the somatic embryos. This research will pave the way to produce artificial seeds in the woody plants in future.

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