

In vitro Callus Induction from Immature Fruits of *Elaeocarpus ganitrus*: A Tissue Culture Approach

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ABSTRACT

In tissue culture investigations for tree species, the growth medium with a perfect concentration of growth regulators and other components has a crucial role in the *in vitro* callus initiation from explants material. The present study has highlighted the effects of various concentrations of plant growth regulators on callus initiation from immature fruits of *Elaeocarpus ganitrus* in Murashige & Skoog (MS) medium. The immature fruits were collected as explants source during fruiting season and cultured on 1/2, 3/4 and full-strength MS medium. In this study, 1.8mgL⁻¹ of 2, 4-D, and full-strength MS medium was observed best for callus initiation which further supported for the multiplication from immature fruits of *E. ganitrus*. The callus initiation from immature fruits of *E. ganitrus* was the crucial step towards the conservation study of this tree species. This study can be helpful for tissue culture investigations concerning callus development from immature fruit explants material of several other species of *Elaeocarpus* and even for the other endangered tree species.

KEY WORDS: CALLUS, E. GANITRUS, IMMATURE FRUITS, MS.

INTRODUCTION

The genus *Elaeocarpus* consists of a large number of species. Among these many, Rudraksha are popularly known. Rudraksha has tremendous importance in rituals and prayers. Several investigations were carried out by researchers in the past on different species of *Elaeocarpus*. *Elaeocarpus sphaericus* is well known for its antioxidant properties and through several literature reviews, it was inferred that it owns notable biomedical capability. Through GC-MS *E. sphaericus* phytochemicals have been analyzed in the previously published studies (Mahajanakatti et al. 2022). *E. sphaericus* is rich in antimicrobial and anti-inflammatory characteristics. Along with phytochemicals, *E. sphaericus* is a very important and useful source of antibiotics and antioxidants. Findings on *Elaeocarpus ganitrus* fruit set and dispersal may have high implications for species regeneration (Koirala et al. 2022; Khan et al. 2005).

The blue colour is most prevalent in *Elaeocarpus* spp. in its entire distribution. Regeneration failure from seeds of *E. venustus* leads to an approach toward other techniques. Further vegetative propagation was first reported successfully (Lee 1998; Saravanan et al. 2011). The callus initiation using leaf stalk as explants material of *E. grandiflorus* was reported on MS medium (Habibah et al. 2019). Using small and immature soft leaves of *E. ganitrus*, callus was developed (Rishi et al. 2021). A procedure for *in-vitro* propagation was developed for *E. serratus* fruit tree. For the investigation, mature nodal explant material was collected. In the study 1/2, 3/4 strength MS medium was used during the study. Other media like White's, B5 Gamborg and WPM (Woody Plant Medium) were also taken during the investigation (Raji and Siril 2021). For several other trees of different genus, tissue culture has shown its importance and necessity. For Baramasi mango, a variety of micropropagation protocols were developed by nucellar embryogenesis. B5 Gamborg MS minor and major salts along with several other additives were used for basal media. 2,4-D is very essential for callus induction and pro-embryonic calli generation in nucellar embryogenesis (Al-Busaidi 2016). Young soft leaves of *Litchi chinensis* Sonn. were taken as explants for the

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development of callus to get *in vitro* plantlets (Puchooa 2004). Somatic embryogenesis of 'Feizixiao litchi' was first reported. The lactalbumin hydrolysate helped in getting a high frequency of callus initiation when taken in combination with plant growth regulators in MS medium (Wang et al. 2016).

The somatic embryogenesis was achieved in callus cultures of *C. wightii*, a medicinally important woody tree species (Kumar et al. 2003). The MS medium was found suitable for callus initiation from leaf explants of *Ailanthus excelsa* Roxb., and plant growth regulator kinetin was found better than TDZ (Dhaval and Nataraj 2018). For the tropical woody plant, *Parasponia andersonii* Planch the callus initiation was obtained with 0.1-0.2mg L⁻¹ TDZ with 0.05 mgL⁻¹NAA (Knyazev 2018). For direct and indirect shoot organogenesis in *Ficus religiosa* using hypocotyls as explants, two valuable protocols were developed (Hesami and Daneshvar 2018). In zygotic embryos of *Cedrus deodara* formation of adventitious bud was examined. Four different tissue culture growth media variously fortified with and without plant growth regulators were used (Tamta and Palni 2004). In the present study growth regulators and antioxidants were used in MS (Murashige and Skoog 1962) medium for *in vitro* callus initiation from immature fruits of *E. ganitrus*. It is difficult to do tissue culture work with fruits of *E. ganitrus*. Therefore, immature fruits of *E. ganitrus* (Roxb.) were taken into consideration as explants source to get new insights.

Figure 1: [A] Tree of *E. ganitrus* [B] Inflorescence on *E. ganitrus* [C] Growing *E. ganitrus* fruit.



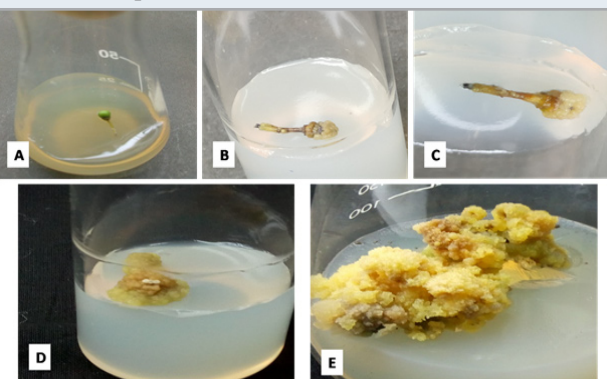
MATERIAL AND METHODS

The samples (Fig. 2A) were obtained from 6-7 years old tree located inside SIET, Meerut, Uttar Pradesh, India (Fig. 1A) which were brought into the laboratory and further washed gently using distilled water. The samples were dipped in distilled water to treat under bavistin (1%) (w/v) for 35mins (Rishi et al. 2021). Under the laminar air hood the surface sterilization of explants was performed using 70% ethanol for time period of 30 seconds. The explants samples were treated with HgCl₂ 0.1% (w/v) for 4 mins (Rishi et al. 2021). The explants were rinsed for 6 times each for 4 min. with sterile double distilled water within the laminar hood. The immature fruits after surface sterilization were cultured on the respected medium of various strength. The temperature of 25±2°C was set for maintaining the *in vitro* cultures with

60-70% humidity. The light and dark conditions of 16h/8h were provided to the respective cultures.

For the investigation MS medium with 1/2, 3/4 and full strength was used and fortified with 2, 4-D (2, 4-Dichlorophenoxyacetic acid) and TDZ which were taken in the concentration range of 0.2 mgL⁻¹ to 2.0 mgL⁻¹. The antioxidants like ascorbic acid (165 mgL⁻¹), citric acid (5 mgL⁻¹) polyvinyl pyrrolidone (PVP,165 mgL⁻¹) were used. The pH of 5.8±0.5 was maintained for the medium and 0.8% agar was added. The medium was autoclaved for 15-20 min. For statistics, analysis of variance, one way ANOVA was carried out for significant difference p≤ 0.05 (n=10).

Figure 2: Different stages of callus development from immature fruit explants on full strength MS medium. [A] Immature fruit; [B]&[C] Callus initiation; [D] Entire immature fruit of *E. ganitrus* converted into callus; [E] Callus multiplication.



RESULTS AND DISCUSSION

The callus induction was started after 5 weeks (Fig.2B) on full strength MS medium. It was observed that the entire explants material converted into callus after 9-10 weeks (Fig.2D). The initial callus appeared to be whitish to light green in colour, but after some time, when it started getting older, the callus started to appear light yellow and green in colour (Fig.2E). From immature fruits of *E. ganitrus* initiation of callus was observed maximum at 1.8mgL⁻¹ of 2,4-D as compared to 1.2mgL⁻¹, 1.4mgL⁻¹, 1.6mgL⁻¹ and 2.0mgL⁻¹ of 2,4-D when considered alone (Fig.3).

On the other hand, with the concentrations of TDZ 0.6mgL⁻¹ analyzed better in comparison with 0.4mgL⁻¹ and 0.8mgL⁻¹ (Fig.4). With combination of both the growth regulators i.e., 2,4-D 1.8mgL⁻¹ and TDZ 0.4mgL⁻¹ concentration, callus initiation was observed (Fig.5).

In this study, it was observed that low concentrations of TDZ responded much better in contrast to higher concentrations. The organogenic callus of *E. robustus* was observed using the MS medium consisting of 2,4-D (Rahman 2004). Callus initiation was reported in *E. tuberculatus* when MS medium was fortified with 2, 4-D (Arshad and Kumar 2006). Callus initiations for *E. sphaericus* were best observed in MS

medium fortified with 1 mgL^{-1} BAP in combination with 0.5 mgL^{-1} 2, 4-D (Dubey and Das 2011). The compact callus induction and basal callus formation were observed in the *in vitro* cultures of *E. blascoi* in WPM using TDZ (Siva et al. 2015). Callus formation was successfully reported in *E. grandiflorus* when 2,4-D was used (Habibah et al. 2019). The sterilization component i.e., 0.1% HgCl_2 was found best for surface sterilization of immature fruit explants. HgCl_2 with 0.1% concentration was analysed as best for surface sterilization of leaf explants of *E. ganitrus* (Rishi et al. 2021).

Figure 3: Different concentrations of 2, 4-D used for callus initiations from explants on full strength MS medium.

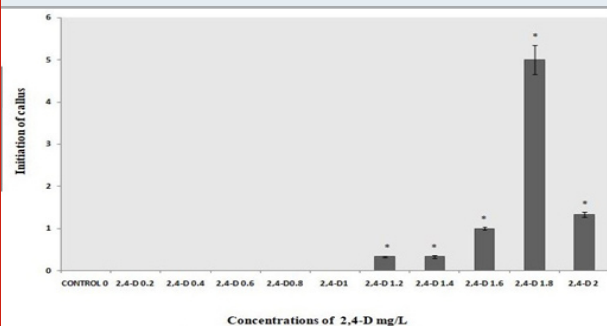


Figure 4: Different concentrations of TDZ used for callus initiations from explants on full strength MS medium

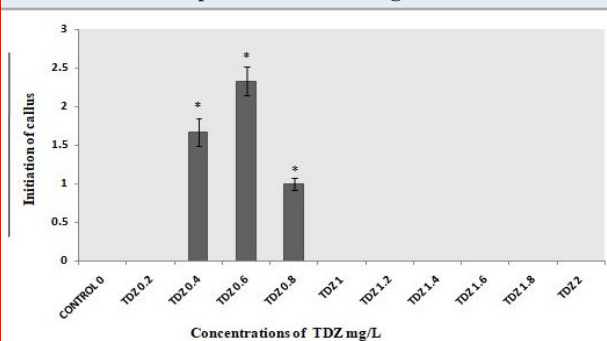
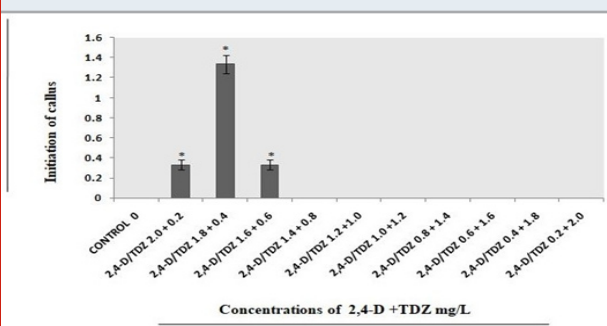


Figure 5: Different concentrations of 2, 4-D+TDZ mg/L used for callus initiations from explants on full strength MS medium



The previously published studies on tissue culture for different tree species including *E. ganitrus*, were observed quite promising based on plant conservation aspects.

The results obtained from such reported studies can be utilized to develop deep insights into the tissue culture of *Elaeocarpus* due to its high importance in several research disciplines. Therefore, the investigation of immature fruits of *E. ganitrus* (Rudraksha) was taken for research purposes which further may open the various ways of holding other studies. The study can become very important in the coming future for the conservation of endangered tree species of not only *Elaeocarpus* but for other species belonging to different genera through plant tissue culture.

CONCLUSION

The findings of the present study have shown that the initiation and multiplication of callus observed best in full strength MS medium with 2, 4-D at the concentration of 1.8 mgL^{-1} . The study indicated that 2,4-D plays a crucial role during callus induction, growth and development to influence the entire explant material. Hence, the procedure may be useful for callus studies of other *Elaeocarpus* species, especially the ones which are known for different types of Rudraksha beads.

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Conflict of interests: Authors declare no conflict of interests to disclose.

Data Availability Statement: The database generated and/or analysed during the current study are not publicly available due to privacy.

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