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ORIGINAL ARTICLE

Effects of growth regulators on callus initiation of *Elaeocarpus ganitrus* (Roxb.)

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ABSTRACT
Elaeocarpus ganitrus (Roxb.) known as Rudraksh belonging to the family of Elaeocarpaceae. The present investigation deals with the effects of plant growth regulators on callus initiation of Elaeocarpus ganitrus (E. ganitrus). For the present study leaves, stem cuttings were collected from the tree as explants source and cultured on different media such as MS (Murashige and Skoog) medium, Anderson medium and WPM (Woody Plant Medium). Plant growth regulators (PGRs) were taken at different concentrations either alone or in combination along with the addition of different antioxidants like Ascorbic acid, Polyvinylpyrrolidone (PVP), Citric acid in the medium. Initiation of callus was observed on several concentrations of PGRs. In the present study 2,4-D with concentration of 2mgL^{-1} was found best for callus initiation and multiplication. Leaf as explant source and MS medium were observed to be the best for callus initiation.
Key words: *E. ganitrus*, Tissue culture, Callus, PGRs, Antioxidants

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INTRODUCTION

Elaeocarpus ganitrus (*E.ganitrus*) popular as Rudraksh and religiously important for endocarp found in Arunachal Pradesh [1]. The species belongs to *Elaeocarpus* can grow comfortably within the climate that is warm and moist. Maximum species belongs to North East India and South India. In India 29 species of *Elaeocarpus* were reported. Few species from this genus found in Andaman and Nicobar Islands of India [2]. In a study MS medium was found best for callus initiation in *Elaeocarpus grandiflorus*. Mostly brown colored callus were obtained and in some treatments green colored callus was observed [3]. Antimicrobial activity was investigated from *E ganitrus* leaves aqueous extract versus clinically isolated bacteria and fungi by using agar well diffusion method on Mueller Hinton agar and Sabouraud Dextrose agar and the outcome shown the potential of *E.ganitrus* leaves for the antimicrobial compounds development [4]. An attempt was made to compile the several features of *E ganitrus* regarding botanical, phytochemical, ethno-medicinal and pharmacological [5]. Olden mythological, medicinal and spiritual aspects of Rudraksha on the base of modern days science was explained in the review [6]. Studies on biology of Rudraksh, and recent research on fruit color found that the blue color is most striking and it is most prevelant in *Elaeocarpus spp.* throughout its distribution [7]. A few unexplored rare, endangered and threatened (RET) plant species from Assam, India taken in consideration because characterization of these (RET) plants are important because of their small population sizes in the native habitat [8]. Phenolics and flavonoids within the leaves of *E.ganitrus* can give considerable antioxidant activity [9]. The plant of *E.ganitrus* has exhibited various biological activities like anti-depressant, anti-microbial, analgesic, antioxidant activity etc. [10]. For *in-vitro* propagation of *Rhododendron griffithianum* Wt. Anderson medium was used [11]. Cytokinine and auxin found effective in shoot multiplication of *Ficus anastasia* [12]. Use of PVP, citric acid, ascorbic acid, bavistin and chloroamphinicol reduced shoot tip explants browning [13]. Several extracts were used for investigating antifungal activity of *E. ganitrus*

using fungal strains [14]. Another study indicated that there was an improvement in the anti-diabetic activity due to chitosan based extracts that shown synergism [15]. A study was performed regarding with *Elaeocarpus ganitrus* nursery seedling survival and growth in the open, dense and sparse canopy of the forest stands and it was suggested that plantation of *E. ganitrus* should be grown understory within slight shaded region for good results [16]. In the previous research studies, protocol for *E. sphaericus in-vitro* propagation was developed using nodal parts [17]. Using MS medium, micropropagation was done in *E. sphaericus* [18]. A study related to *Elaeocarpus robustus in-vitro* propagation was reported [19]. *In-vitro* regenerated plants of *E. robustus* were established and under *ex-vitro* conditions 60% survivals of plants were observed [20]. Efficient protocol of plant tissue culture was developed for *E. robustus* shoot regeneration [21].

The present study is related with effects of PGRs (Plant Growth Regulators) on *in-vitro* callus initiation using leaves and stem segments of *E. ganitrus* (Roxb.) as explants. In our study growth regulators were used at different concentrations alone and in combination. The MS (1962), WPM (1981) and Anderson medium (1984) were also fortified with antioxidants [22-24].

MATERIAL AND METHODS

Plant material and sterilization

The explants (leaves and segments of stem) used for the study collected from approximately 6 years old trees located at SIET, Modipuram, Meerut (UP) India (Fig.1) were brought to the laboratory and washed thoroughly. After proper washing the explants were treated for 30-40 min. with 1% bavistin (*w/v*). Under laminar flow surface sterilization of explants was done by 70% ethanol for time period of 30 seconds and 0.1% (*w/v*) mercuric chloride ($HgCl_2$) for 4 min. The explants materials were rinsed for 4 min. upto 5 times with autoclaved double distilled water under the laminar airflow. The surface sterilization for both leaves and stem cuttings were done separately. The immature leaves with size of 3.5±2 cm were taken. Entire leaves upto 1.5-2.5cm in size were cultured directly but leaves with size of 3.5-5.5cm were taken into 2-3 fragments. Segments of the stems from 3±0.5cm were cultured in the respected medium. The cultures at the temperature of 25±2ºC were maintained under 16 h/8h light/dark conditions to obtain callus initiations and relative humidity maintained 60-70%. The explants materials which were cultured without growth regulators were taken as control.

Culture medium

In the study three different media were used for culturing explants such as MS, Anderson and Woody Plant medium. PGRs such as 2,4-D (2,4-Dichlorophenoxyacetic acid), TDZ (Thidiazuron) were used in the concentration range of 0.1 mgL^{-1} to 2.5 mgL^{-1}. The antioxidants like ascorbic acid (150 mgL^{-1}), citric acid (10 mgL^{-1}) polyvinyl pyrrolidone (PVP, 150 mgL^{-1}) were used and added into the MS, WPM and Anderson medium. 2% Sucrose in WPM and 3% in MS and Anderson medium (HiMedia, India) was added and dissolved thoroughly on a magnetic stirrer. The pH of the medium was adjusted to 5.8±0.5. Agar (0.8%) was added to the medium. Further, media were autoclaved for 15 min at 121º C (at 15 psi). The explants were cultured in the medium having plant growth regulators and antioxidants.

Statistical analysis

The experimental studies were performed in 3 biological replicates and 10 technical replicates. Analysis of variance by one way ANOVA was carried out to estimate significant differences among the means within each treatment ($p \leq 0.05$). Asterisk [*] for showing the significant difference was used.

RESULTS AND DISCUSSION

The cultures were found to be ~95% contamination free. Mercuric chloride of 0.1% *w/v* was observed to be the best for surface sterilization. Initiations of callus were observed only in the leaves cultures after 3 to 4 weeks on MS medium and after 8-9 weeks in WPM and Anderson medium (Fig. 2). The percentage of callus initiation was observed higher in MS medium as compared to WPM and Anderson medium. Callus initiations were not observed in the cultures those were taken as control in all the three media. Less browning was observed in MS as compared to WPM and Anderson medium. The MS was observed to be the best medium in this study because less time period was taken for callus initiation i.e, around 3-4 weeks and for entire tissue to convert into callus 8-9 weeks depending on the size of leaves explants which were taken during the time of culturing. Different stages involved in the *E. ganitrus* callus formation are shown in Fig. 3. It was observed that low concentration may be helpful as compared to higher concentration of TDZ when taken in combination with 2,4-D. But higher concentration of 2,4-D i.e., 2mgL^{-1} was observed much better for the callus initiation for this species of *Elaeocarpus* when taken alone in the MS medium. In this study, callus of different colours like green, light green and very light brown were obtained. Green coloured callus were observed more dominantly in MS medium. It was also

observed that low light helped in callus multiplication. When some callus cultures kept under complete dark conditions, slow callus multiplication was also observed. In earlier studies, 2,4-D was found best for producing organogenic callus of *E. robustus* using MS medium [21]. Shoot and basal callus formation was observed and compact callus induction from nodal explants of *E. blascoi* was observed when TDZ used in WPM [25]. Callus formation was successfully done using 2,4-D and picloram in *E. grandiflorus* [3]. Callus initiation was observed in the tissue culture study of *Elaeocarpus tuberculatus* when 2,4-D was used in MS medium [26]. Findings regarding callus initiation were reported in MS, B5, and WPM for *E.sphaericus*. Better callus initiation was found when leaf was taken as explants source in MS medium fortified with 1 mgL^{-1} BAP in combination with 0.5mgL^{-1} 2,4-D [27].

A B C

Fig. 1 Phenotype of plant material used in this study. A, B- *E. ganitrus* Roxb. grown at SIET, UP, India. C- Leaves of *E. ganitrus* (Roxb.) used as explants in this study.

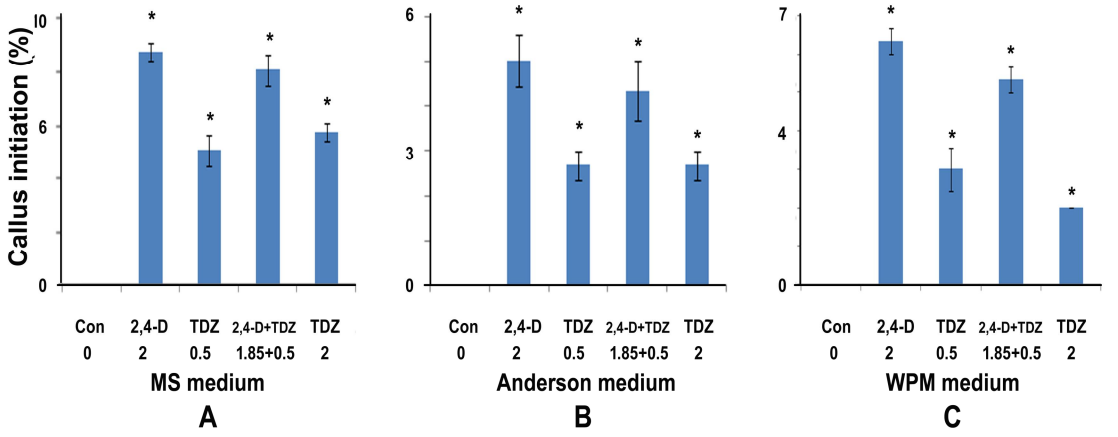

Fig. 2 Percentage of callus formation from leaf explants on different plant medium. A-MS medium, B-Anderson medium, C-WPM medium.

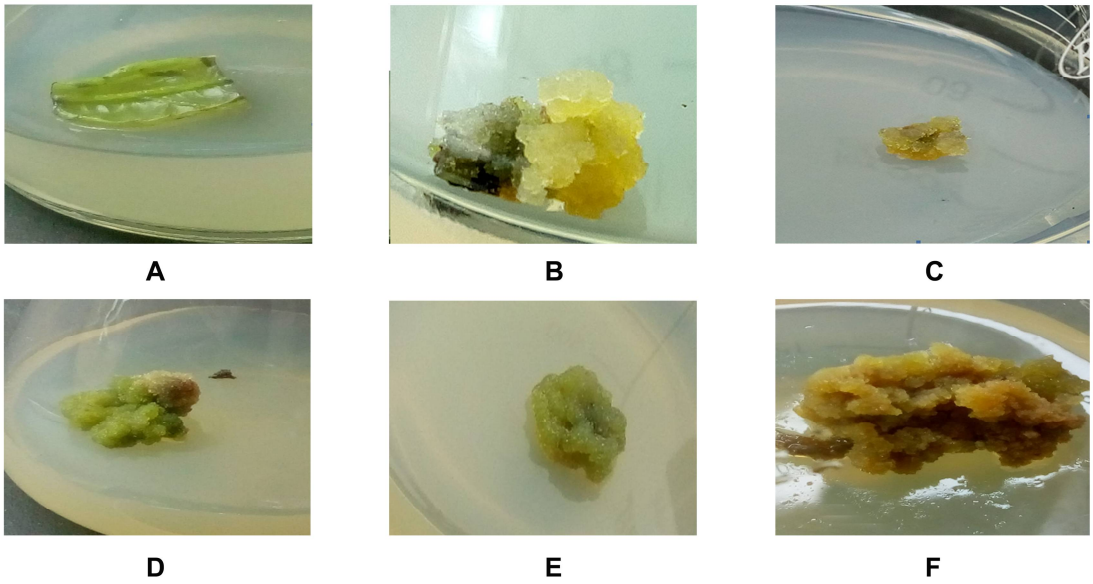

Fig. 3 Different stages of callus formation as observed on MS medium. A-Leaf of *E. ganitrus* in MS medium taken as explants for callus initiation. B- Callus initiated from the leaf and the entire tissue converted into the callus in MS medium. C-E Subcultures of the callus in MS medium. F-Denoting multiplication of light green and brown callus in MS medium.

CONCLUSION

It was observed in our study that 2, 4-D when taken alone or in combination with TDZ played better role in callus initiation from leaf tissue of *E. ganitrus*. Callus initiation and multiplication was best observed in MS medium. This study may be useful for further *in-vitro* propagation experiments.

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CONFLICT OF INTEREST

No conflict of interest exists.

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