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ORIGINAL ARTICLE

Effects of growth regulators and antioxidants on *in-vitro* shoot initiation of *Elaeocarpus ganitrus* (Rudraksh)

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ABSTRACT
Elaeocarpus ganitrus (Roxb.) commonly known as Rudraksh. It is well known for its seeds for rituals in India as well as in some other countries. The present study deals with the in vitro shoot initiation of Elaeocarpus ganitrus (Rudraksh). For the present study nodal segments were collected from the tree 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 for shoot initiation. Initiations of shoots were observed when BAP and Kn were taken individually and in combination, along with the addition of Ascorbic acid, Polyvinylpyrrolidone (PVP), Citric acid in the medium. MS was observed as better medium for shoot initiation while other medium such as Anderson and WPM were equally good in our study.
Key words: *E. ganitrus*, Nodal segment, Shoot initiation, PGRs, Antioxidants.

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INTRODUCTION

Elaeocarpus ganitrus (*E.ganitrus*) commonly known as Rudraksh found in Arunachal Pradesh except Tawang, Upper Subansiri and some high altitude areas [1]. The trees of genus *Elaeocarpus* are of medium to large size, bears good fragrance and colourful fruits. Fruits of several species belonging to this genus are edible and useful in making pickles. Around 29 species of *Elaeocarpus* reported in India. Majority of the species belongs to North East India and South India. Some species of this genus belongs to Andaman and Nicobar Islands of India [2]**.** An attempt was made to compile the information regarding different features and characteristics of *Elaeocarpus sphaericus* [3]. Studies have shown that seeds of *E. ganitrus* are rich source of tannins, carbohydrates, alkaloids, phytosterols, flavonoids and proteins [4]. *E. ganitrus* is observed to possess ethnomedicinal, pharmacological and therapeutic properties [5]**.** Several extracts from different parts of Rudraksh tree having vast therapeutic potential [6]. *In-vitro* clonal propagation of *E. sphaericus* (synonym: *E.ganitrus*) was reported and for the study explants materials were selected from three years old plants [7].

Micropropagation protocol for *E. sphaericus* has been developed from nodal explants. The plants of *E.sphericus* were collected from natural habitat of Arunachal Pradesh [8]. The failure of *Elaeocarpus venustus* regeneration from seeds lead to proceed for the other techniques such as plant tissue culture approaches. Vegetative propagation of *E. venustus* was successfully first ever reported and further research are under progress regarding genetic diversity; *in-vitro* propagation and reintroduction [9]. *Elaeocarpus blascoi* is endemic to Palani hills of Western Ghats in India and need conservation strategies for this valuable tree [10]. *In-vitro* regeneration procedure of *E. blascoi* from nodal segments was developed using Woody Plant Medium (WPM). Hence it is possible to propagate *E. blascoi* which is a species of Western Ghats, through *in-vitro* culture to overcome the extinction threat [11]. It was studied the impact of disturbance on *E. ganitrus* demographic structure by monitoring the flowering, fruiting and

dispersal of seeds during the three consecutive years and at four sites that varied in the degree of disturbance. The findings on fruit set and the dispersal of *E. ganitrus* may have great implications for regeneration of the species [12]. The regeneration of the *Elaecarpus* is poor and there is an urgent need of conservation strategies for this species [13]. The protocol of macropropagation for *Elaecarpus serratus* was obtained by germination of seeds and air layering approach. The saplings were again introduced to the wild environmental conditions selected on ecological niche modelling basis. It was found that the rate of plant survival was higher. This study suggested that the approach is very effective for the conservation of plant and its population status [14]. For rapid clonal propagation of *Elaeocarpus robustus* an investigation was done [15]. Many researchers have attempted to study different species of *Elaeocarpus*. A protocol was developed for *in-vitro* propagation of *Rhododendron griffithianum* Wt. using Anderson medium [16]. Effectiveness of ascorbic acid and polyvinylpyrrolidone in rooting of mini cuttings for 3 clones of *Eucalyptus urophylla* x *Eucalyptus grandis* was studied [17]. Cytokinin supplements and environments were determined for the initiation and establishment of *Quercus robur* seedling tissue shoot cultures [18].

The present study deals with the effects of growth regulators BAP (6-Benzyl Amino Purine) and Kn (Kinetin) for *in-vitro* shoot initiation of *E. ganitrus* (Roxb.). PGRs (Plant Growth Regulators) were taken at different concentration individually and in mixture in three different plant media (MS, WPM and Anderson) along with different antioxidants.

MATERIAL AND METHODS

Plant material and sterilization

Explants (nodal part) used in this study were collected from the trees (6-7years old approx.) from the compounds of Shobhit Institute of Engineering and Technology, Meerut, Uttar Pradesh, India (Fig.1). The collected samples were brought to the laboratory for further experimentation. The explants (3±0.3 cm in length) were treated with (1.5% *w/v*) bavistin for 30min. Surface sterilization of the explants was performed under the laminar air flow by treating with 70% ethanol for 30 seconds and $HgCl_2$ (0.1% *w/v*) (HiMedia, India) for 4 min. The explants were thoroughly rinsed with autoclaved double distilled water for 4 min. (5 times) within the laminar air flow.

Fig. 1: *E. ganitrus* Roxb. at SIET (Deemed to be University), Meerut (UP), India

Culture medium used in the study

In the present investigation different media were used such as MS medium, Anderson medium, Woody Plant medium for culturing *Elaeocarpus ganitrus* explants tissue [19-21]. Cytokinins such as BAP (6-Benzyl aminopurine), Kinetin (Kn) were used in the concentration range of 1.0 to 2.0 mgL^{-1} and added to all growth mediums (Table 1). Antioxidants such as ascorbic acid 130 mgL^{-1}, polyvinyl pyrrolidone (PVP, 130 mgL^{-1}) and citric acid (8 mgL^{-1}) were used and added to MS, WPM and Anderson medium. Sucrose (2% in WPM, 3% in MS and Anderson) (HiMedia, India) was added and dissolved properly on a magnetic stirrer before adjusting pH. The pH of the medium was adjusted to 5.8±0.5. Agar (0.8%) was added to the medium. After melting the agar, medium was autoclaved for 15 min at 121º C (at pressure of 15 psi). Under sterile conditions, the nodal explants were cultured in the respective medium having cytokinins and antioxidants (Table 1). The cultures were maintained at 25±2ºC under photoperiod of 16 h/8h light/dark conditions and relative humidity of 60-70%. Explants cultured without PGRs were used as control.

Statistical analysis

All experiments were conducted in three biological and ten technical replicates. One way analysis of variance (ANOVA) was carried out to estimate significant differences between control and treated experiments $p \leq 0.05$. Asterisk mark (*) was used to designate the significant difference.

RESULTS AND DISCUSSION

The cultures of *E. ganitrus* nodal explants were observed to be ~85% infection free (free from fungal and bacterial contamination). $HgCl_2$ (0.1% *w/v*) was found to be best for surface sterilization of explants. Initiations of shoots were observed after 2.5 weeks on MS medium and after 3-4 weeks on Anderson and WPM medium. Early initiation was observed in MS medium. No shoot initiation was observed in the cultures taken as control. Antioxidants played important role to overcome the problem of phenolics and browning of medium specifically in MS medium. It was observed that the percentage of shoot initiation was better on MS medium in the range of 26.7% to 46.7% (Fig. 3A). The number of shoots per explants was 10% to 13.33% on MS medium (Fig. 3D). On Anderson medium the shoot initiation was observed to be in the range of 30% to 43.33% (Fig. 3B), while the number of shoots per explants were 10% to 13.33% (Fig. 3E). On WPM, the percentage of shoot initiation ranged from 23.33% to 43.33% (Fig. 3C), while the number of shoots per explants were observed to be 10% to 20% (Fig. 3F). So, it may be concluded that PGRs are the key players in driving shoot initiation.

Table 1 Combination of PGRs (mgL^{-1}) used in the study. A total of 16 different combinations used in MS, Anderson and WPM medium.

	BAP used (mgL^{-1})			
Kinetin used (mgL^{-1})	0.0/0.0	1.0/0.0	1.5/0.0	2.0/0.0
	0.0/1.0	1.0/1.0	1.5/1.0	2.0/1.0
	0.0/1.5	1.0/1.5	1.5/1.5	2.0/1.5
	0.0/2.0	1.0/2.0	1.5/2.0	2.0/2.0

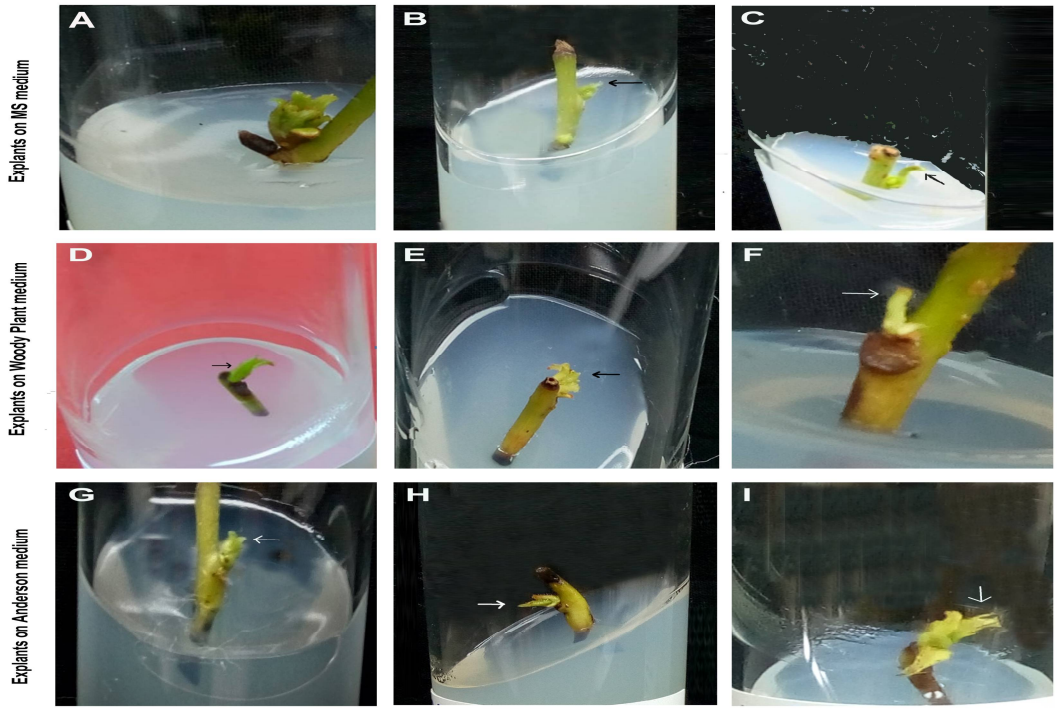

Fig. 2 Different Medium and PGR combination for propagation of *E. ganitrus*. A) BAP (1.5 mgL^{-1})+MS+ Ascorbic acid+ Citric acid+ PVP, B) BAP (2.0 mgL^{-1})+MS+ Ascorbic acid+ Citric acid+ PVP, C)) Kn (1.5 mgL^{-1})+MS+ Ascorbic acid+ Citric acid+ PVP,D) BAP+ Kn (2.0 mgL^{-1} + 2.0 mgL^{-1}) +WPM+ Ascorbic acid + Citric acid + PVP, E) BAP (1.5 mgL^{-1})+WPM+ Ascorbic acid + Citric acid + PVP, F) BAP+ Kn (1.5 mgL^{-1} + 2.0 mgL^{-1}) +WPM+ Ascorbic acid + Citric acid + PVP,G) BAP+ Kn (1.5 mgL^{-1} + 1.0 mgL^{-1}) +Anderson medium+ Ascorbic acid + Citric acid + PVP, H) BAP+ Kn (1.0 mgL^{-1} + 2.0 mgL^{-1})+Anderson medium+ Ascorbic acid + Citric acid + PVP, I) BAP (1.5mg L^{-1}) +Anderson medium+ Ascorbic acid + Citric acid + PVP.

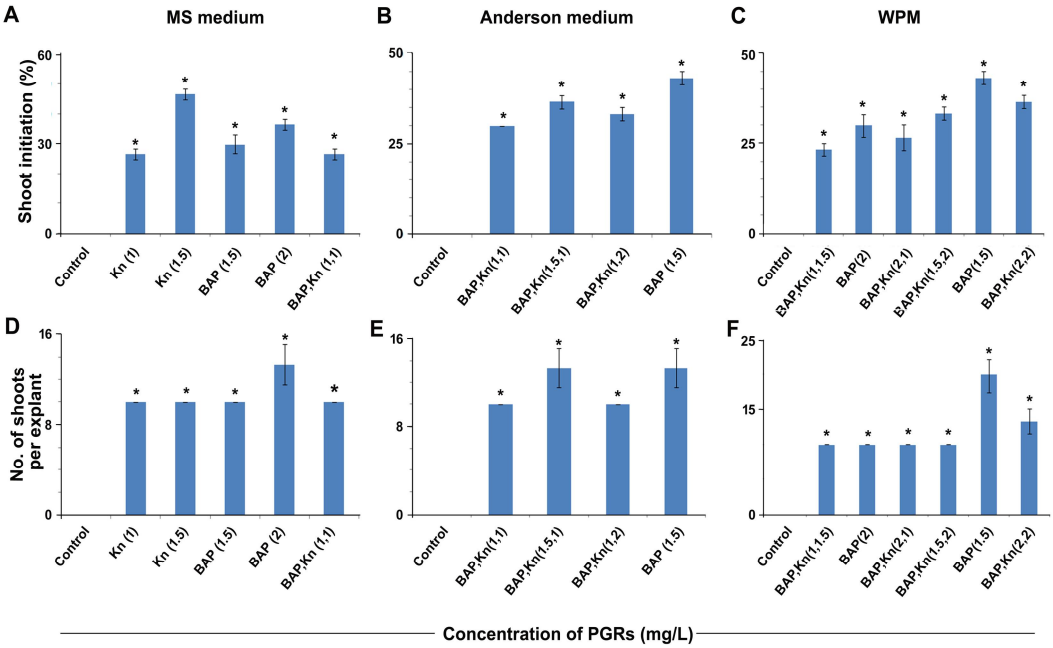

Fig. 3. A-C Shoot initiation on nodal explants in different medium. D-F Number of shoot per explants initiated on different mediums used in this study**.** Asterisk (*) was used to indicate the significant difference at $p \leq 0.05$ (n=10).

In previous studies, similar concentrations of PGRs was used for shoot initiation in *E. sphaericus* but best proliferations of axillary shoot were obtained on MS medium containing equal concentration of BAP (1.5mgL^{-1}) and Kn (1.5mgL^{-1}) [7]. Further best multiplication of shoot was obtained on MS medium supplemented with BAP (1.5mgL^{-1}), Kn (1.5mgL^{-1}) along with Casein hydrolysate (CH) [7]. In another study, researchers reported that best initiation of shoot of *E. sphaericus* were observed in MS medium, when equal concentration of BAP, Kn (2.2 µM each) were used. It was also observed that adding Casein hydrolysate increased the shoot number [8].

In-vitro propagation studies related to *E. robustus* was reported earlier by Roy *et al*., 1998. Shoot tips and nodal explants from 20 years old trees of *E. robustus* were used for the study. Multiple shoots were obtained on MS medium containing 0.5 mgL^{-1}each of BA and Kn. On the same concentrations (0.5 mg L^{1}) of BA and Kn, explants taken from *in-vitro* shoots produced multiple shoots [22]. Efficient plant tissue culture protocol for *E. robustus* has been developed using cotyledon explants for shoot regeneration [23]. In other species of *Elaeocarpus* like *E. blascoi*, *in-vitro* propagation has been standardized using WPM (Woody Plant Medium). PGRs such as BAP, Kn and TDZ (Thidiazuron) were used for micropropagation [11]. *Invitro* propagation study was reported for *Elaeocarpus tuberculatus* in which axillary bud expansion was observed in MS medium when 0.5-1mgL^{-1} BAP was used and further cultures were maintained in MS medium fortified with 0.5mgL^{-1}of BAP and Kn [24]. Investigation regarding *in-vitro* propagation was reported in MS, B5, and WPM for *E. sphericus*. For shoot multiplication MS medium was found better. [25].

CONCLUSION

It was observed that cytokinins like BAP and Kn played key role in the *in-vitro* shoot initiation of *E. ganitrus* in all the three medium (MS, Anderson and WPM). MS medium was observed to be better than the other two medium (Anderson and WPM medium) in comparison during this study. The protocol standardized here may be used in *in-vitro* conservation strategy for this endangered tree species.

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CONFLICT OF INTEREST

No competing interest exists for this research study.

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