

Impact of growth hormones on vegetative propagation of *Elaeocarpus ganitrus* Roxb.

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ARTICLE INFO	ABSTRACT
<p>Received : 19 August 2021 Revised : 15 November 2021 Accepted : 28 November 2021</p> <p>Available online: 11 February 2022</p> <p>Key Words: Air layering Callusing Indole-3-butyric acid Rudraksha Synergist</p>	<p><i>Elaeocarpus ganitrus</i> Roxb. commonly identified as ‘rudraksha’, belonging to <i>Elaeocarpaceae</i> possess a great religious, spiritual and materialistic significance which is placed under the threatened category in the tropical wet evergreen forests of North East India. Because of its over-exploitation, poor germination, low viability rate and hard seed coat, an attempt has been done for mass production of planting materials through cuttings and air layering’s. Cuttings trials were conducted during three different periods, mid - March, mid – July and mid – November by using Indol butyric acid and Naphthalene acetic acid of 1000, 2000, 4000, 6000 ppm where air layering’s were done during mid - May by treating IBA and NAA solution of 500, 1000 and 2000 ppm in combination with para-hydroxybenzoic acid of 2000 ppm as synergist. Application of IBA and NAA of different concentration did not encourage the growth in the cuttings in all three periods. Air layering’s treated with NAA 1000 ppm+2000ppm para- hydroxy benzoic acid and IBA 500 ppm +2000ppm para-hydroxy benzoic acid resulted the highest and same value of callusing and rooting percent (70 and 66.67%), respectively while minimum callusing and rooting percentage (56.67 and 51.67%) was recorded in control. The present study focused on the mass production of planting materials in shorter duration with a reasonable cost for enhancing production as well as socio-economic conditions.</p>

Introduction

The genus *Elaeocarpus* has about 360 species, spread over from the West (Madagascar) to the East (New Zealand); also covering the regions of Southeast Asia, Southern China, Japan, Australia, Malaysia, New Guinea, Fiji and Hawaii (Coode, 2007). Out of the total species, 120 species belonging to this genus recorded in different parts of South and Southeast Asia including foothills of the Himalayas and different parts of India, Nepal, Bhutan, Tibet, Indonesia, Philippines, Myanmar to Bangladesh at an altitude of 900-1800 mean sea level (Bhuyan *et al.*, 2002), about 25 species are found in India (Khan *et al.*, 2003). *Elaeocarpus ganitrus* Roxb. (Synonym: *E. sphaericus* (Gaertn.) K. Schum) belongs to “*Elaeocarpaceae*”, is popularly known as ‘Rudraksha’ for its hard, ornamental stony endocarp and highly recognized for its medicinal and religious value. It is a medium

to large-sized evergreen tree with a large spherical crown attaining a height of 50-200' with a girth of 4'. It is naturally found in evergreen forests of West Bengal, Assam, Bihar, Madhya Pradesh, Arunachal Pradesh excluding Tawang and Upper Subansiri and the Konkana Ghats of Maharashtra states in India. It is also grown as ornamental tree in the homestead in many parts of the country. The Rudraksha is characterized by three-tier structure in tropical evergreen forest but it is usually present in the second storey.

Hindu mythology described about the great significance of religious, spiritual and materialistic aspect of Rudraksha beads. The word Rudraksha is coined from two Sanskrit words: 'rudra' denotes Lord Shiva and 'aksha' means eyes (Ramadurai, 2007). People of Hindu religion are considered the Rudraksha as link between earth and heaven or as a symbol of light and purity. Since ancient times, the beads were threaded together as rosaries or necklaces ('malas') and an important part of Eastern religions particularly in Hindus, Sikhs and Tibetan Buddhists, to ward-off evil spirit and omens and also for meditation and prayer (Li *et al.*, 2014). Wearing of Rudraksha beads around the neck produce a positive effect on nerves, blood pressure and heart ailments (Sakat *et al.*, 2009). The seed have reported in Ayurveda for the treatment of hypertension, insomnia, psychoneurosis and mental diseases. Bhojvaid and Negi (2003) and Hardainiyani *et al.* (2015) reported that the pulp of the fruit is sour in taste and edible and used for epilepsy, headache and mental illness. The aqueous extract of fruit is mainly used as cardio-stimulant, sedative, hypotensive, spasmolytic, anticonvulsant, chloretic, bronchodilatory, where the stem bark is hypoglycemic (Khare, 2007). The paste of stone is externally applied against small-pox, measles and on organs having burning sensation (Pandey and Das, 2004). Rudraksha bead shows various health promoting effects such as antioxidant (Kumar *et al.*, 2008), analgesic (Nain *et al.*, 2012), anti-inflammatory antifungal (Singh *et al.*, 2010), antimicrobial antidiabetic (Hule *et al.*, 2011). Singh *et al.* (2000) also cited that the fruit contains a significant amount of phytochemicals such as iso-elaecarpine, elaeocarpine, quercetin, gallic, ellagic acids and rudrakine. The population of this species in natural as well as planted forest stands is

gradually declining at an alarming rate due to various factors like microbiotic seed nature, considerable period of seed dormancy, poor germination rate along with fungal rot, hard endocarp, long flowering or fruiting intervals etc. The unscrupulous collection of seeds has also adversely affected the natural regeneration. Rising market prices of beads due to religious belief and its many fold importance play a key role for limited distribution of this species in natural habitat (Dafni, 2006), which makes the species under the threatened category on the verge of extinction in near future. Because of the problem in propagation and its ethnic medicinal importance, the conservation approaches mainly *in-situ* and *ex-situ* are to be recommended to synchronize the gap between the demand and supply. In this aspect, the method of vegetative propagation assumes a great significance over sexual propagation (seed) for mass production of planting stock with quick productive gains. The vegetative propagation has not been popularized particularly in forest trees on a substantial scale which become bottleneck for a tree improvement programme in India. However, recently portentous attention has been focused to propagate trees through vegetative means as a strong alternative over seed propagation. Out of the several vegetative methods, two methods namely cutting and air layering are widely used in forest species because of the simplicity and cheap. These methods will be exceptional to give more advantage if the exogenous plant growth regulators are applied alone or in combination with auxin synergist. Few reports on the propagation through vegetative method (Bhojvaid and Negi, 2003); seed propagation (Khan *et al.*, 2003; Saravanan *et al.*, 2011) and *in-vitro* propagation (Saklani *et al.*, 2015) has been carried out but the method should be suitable to agro-climatic conditions for raising planting stock. Thus, the present investigation was undertaken to find the appropriate combination of plant growth regulators with auxin synergist on the response of rooting through cuttings and air layering's.

Material and Methods

The present experiment was conducted in the plantation plot of Buxa-Cooch Behar Research Range (Silviculture - North Division) of

Rajabhatkhawa forest of West Bengal during the year 2018 which is 40 km away from the campus of Uttar Banga Krishi Viswavidyalaya, Pundibari at an altitude of 120 m above mean sea level with 26° 36' N and 89° 40' E in the plains of terai zone.

Preparation of cuttings and hormonal treatments:

The semi hard wood cuttings of 15- 20 cm long and uniform thickness of 1.0-1.5 cm having at least 2- 3 nodes were collected from the disease free and medium sized trees. Each cutting was given a radial cut at the top of the apical bud and a slanting cut at the base with a sharp razor blade to expose maximum absorbing surface for effective rooting. The base portion of the cuttings were dipped for 30 seconds in the solution of IBA (Indole 3-butyric acid) and NAA (Naphthalene acetic acid) of 1000, 2000, 4000, 6000 ppm to a depth of 1 cm and then allowed to dry for 10-15 minutes. After that the cuttings were planted in polybags containing growing media of soil: sand: Farm Yard Manure: coco-pea in the ratio of 2:1:1:1 in three different periods viz., mid-March, mid-July and mid-November and placed in a low cost polyhouse. The upper portion of the cuttings was sealed by either the paste of cow dung or wax to prevent the moisture loss. Irrigation was done twice in a day i.e. in morning and evening. Soil drenching of Bavistin (@2g litre⁻¹) or Captan (@1%) was done in alternate weeks to prevent the disease. The experiment comprised of two growth regulators of four concentrations comprising of nine treatments with a control having three replications. Each replication had twenty numbers of cuttings to the total of sixty cuttings per treatment. The growth attributes like sprouting and rooting percentage, length of the sprout, time taken for sprouting, average length of root and root number per cutting was recorded after 30 days.

Preparation of air-layering and hormonal treatments:

Vigorous and healthy branches of young mature trees of approximately 10 years old were selected for air-layering. Secondary branches of 1.0–1.5 cm diameter was selected randomly from the trees and 2-5 cm length of bark was girdled out cylindrically. Firstly, the girdled portion in the stem were treated with the auxin synergist of p-hydroxybenzoic acid of 2000 ppm by using cotton and allowed to dry for few seconds and then treated with different

concentration of IBA and NAA solution of 500, 1000 and 2000 ppm, respectively. The cut area around the stem was enclosed with a suitable media consisting of soil: cow dung: coco-peat at the rate of 2:1:1 to hold moisture and completely wrapped with polythene sheet (20-25 cm square shaped) and tied at both ends to avoid moisture loss. The experiment had two growth regulators with three concentrations comprising of seven treatments including a control comprising 60 numbers of air layers per treatment (20 numbers in each replication). Each treatment was done separately in separate trees whereas untreated layer served as control. Air layering was done in the month of mid-May with the onset of monsoons. Regular spraying of water once in three days was carried out to keep moisten the rooting media or as and when required and constantly monitored for rooting response. At the end of 45 days, the air layered branches are detached from the parent trees for recording the observation like percentage of callus, percentage of rooting, primary and secondary roots number, root length, weight of fresh and dry root per air-layer. The entire recorded data were subjected to statistical analysis using CRD (Completely Randomized Design) following Gomez and Gomez (1984). The DMRT (Duncan's multiple range test) was also performed to relate the significant difference between the mean values.

Results and Discussion

The response of different treatments on cuttings:

Irrespective of different concentration of IBA and NAA, the results revealed that no growth and sprouting were induced in the cuttings in all of the three periods. Even if, either callus or root formation didn't take place, the cuttings were gradually dried up subsequently and finally failed in all aspect. The present study is well supported by Kumar *et al.* (2014) in *Salvadora oleoides* and Thakur (2014) in *Acacia catechu*. On the contrary, Saravanan *et al.* (2011) reported that 16 % regeneration was achieved in stem cuttings having apical meristem where no regeneration was observed in the stem cuttings devoid of meristems in *E. venustus*. The rejuvenation power in the cutting generally varies with species, age and environment. Cuttings of some species regenerate very easily, some with difficulty while others do not regenerate at all and are thus obstinate. The

present species, Rudraksha was under the last category. The presence of root inhibitors like lignin, tannin and abscisic acid can't be ruled out in the cuttings of this species. The presence of anatomical barriers such as sclerenchymatous tissue may make a barrier for root development. Absence of 'rhizocline' as well as of 'rooting co-factors' may be responsible for the obstinacy of this tree species to root. Nanda and Kochhar (1985) reported the similar findings in *Bougainvillea glabra*, *Echitis caryophyllata* and *Jasminum pubescence*. The capacity of absorption of less or higher concentration of plant growth regulators may have inhibitory effect on sprouting as well as rooting. Non-rooting may be due to sensitivity of plant to auxins or genetic and physiological disruption of auxin transport (Esmon *et al.*, 2006). It can't be ignored that high level of secondary metabolites may prevent root formation in the cuttings (Yadav *et al.*, 2006).

Callusing and rooting in air layerings:

At the end of 45 days of layering, the results exhibited the significant ($p = 0.05$) difference among the various plant growth regulator treatments with respect to the percentage of callusing and rooting, average number of primary and secondary roots, root length and average fresh and dry weight of root (Table 1). Irrespective of the treatments, the average callusing and rooting percentage was 63.33 and 61.67 percent in NAA and IBA, respectively. Maximum callusing (70.00 %) in T₅ (NAA 1000 ppm+2000 ppm phb) was closely followed by 66.67 percent in T₁ (IBA 500 ppm+2000 ppm phb). Callusing (66.67 %) with T₁ (IBA 500 ppm+2000 ppm phb) was statistically at par with T₂ (IBA 1000 ppm+2000 ppm phb) and T₄ (NAA 500 ppm+2000 ppm phb), both recorded same value of 65.00 percent whereas minimum callusing (56.67 %) was recorded in T₇ (Control) which closely preceded by T₃ (IBA 2000 ppm+2000 ppm phb) and T₆ (NAA 2000 ppm+2000 ppm phb) exhibiting 58.33 and 61.67 percent, respectively which showed statistically at par with each other. Similarly, rooting was highest (70.00 %) in T₅ (NAA 1000 ppm+ 2000 ppm phb) statistically at par with T₁ (IBA 500 ppm+2000 ppm phb) having 66.67 % whereas lowest rooting (51.67 %) was exhibited in T₇: Control in air layers (Figure 1). The value of rooting percentage (63.33

%) in T₄ (NAA 500 ppm+2000 ppm phb) was not significantly different with 61.67 % in T₂ (IBA 1000 ppm+2000 ppm phb).

Though, the plant growth regulators exhibited callusing and rooting response but all concentration of NAA slightly showed better results than IBA. Callus and root formation were higher in growth regulator treatment indicating a conduciveness environment in comparison to control and showed the decreasing trend with increase in concentration of IBA while it increased up to certain NAA concentration then declined with further rise in concentration. The present finding is in close agreement with Bhojvaid and Negi (2003) in *E. ganitrus* and Sanchez *et al.* (2009) in *Psidium guajava* where callus formation was reduced with increasing concentration of IBA (2000, 4000 and 6000 ppm) and stopped completely at 6000 ppm. It is proved that IBA and NAA increase elasticity of cell wall and accelerate the cell division but excessive concentrations inhibit the process of formation of callus.

Callus development is common with the totipotency of the meristematic tissue which appears as a thin layer around the wounded end of cuttings of air layers. The newly formed callus consisted of irregular unspecialized parenchymatous cells which further differentiate into root primordial, act as a protective layer for the formation of adventitious root from other tissues of the layered branch (Colodi *et al.*, 2008). Evidently callus formation is an event which generally occurs earlier to the rhizogenesis. Callus and adventitious root formation appear as an independent phenomenon in most tree species but generally occur at the same time and space, resulting competition for same resources. That is the reason why rooting percentage was decreased with increase in hormonal concentration in the present study which contradicts with the results of Tomar and Singh (2011) in *Ficus krishnae* and *F. auriculata* and Bhojvaid and Negi (2003) in *E. ganitrus*. Auxin at lower concentration is found to be more effective for adventitious root formation in *Quercus serrata* (Srivastava *et al.*, 2000). Singh and Ansari (2014) reported that rooting response regulated by a balance of internal translocation of hormonal growth regulators, carbohydrates, nitrogenous substances and co-factors acting

Table 1: Effect of IBA and NAA on callusing percentage, rooting percentage, primary and secondary root number, root length, fresh and dry root weight in air layered branches of *E. ganitrus*.

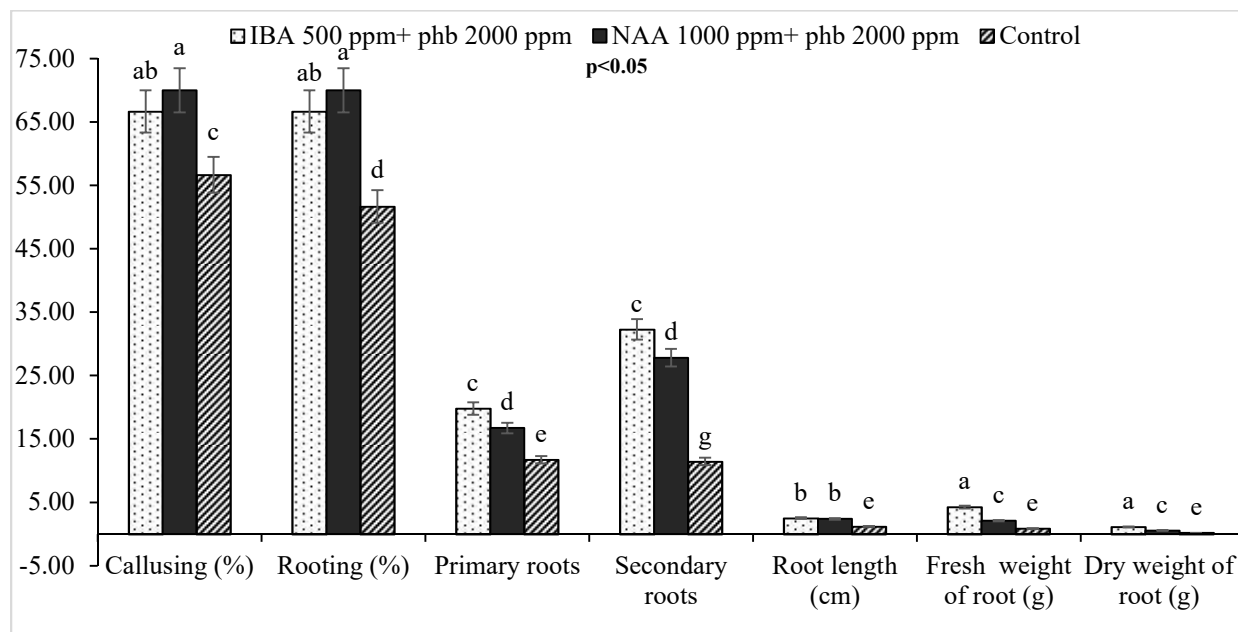
Treatments	Callusing (%)	Rooting (%)	Primary roots	Secondary roots	Root length (cm)	Fresh root weight (g)	Dry root weight (g)
T ₁	66.67 ^{ab} (54.75)	66.67 ^{ab} (54.75)	19.80 ^c	32.27 ^c	2.52 ^b	4.25 ^a	1.12 ^a
T ₂	65 ^b (53.73)	61.67 ^{bc} (51.76)	23.13 ^b	45.33 ^a	3.02 ^a	2.84 ^b	0.74 ^b
T ₃	58.33 ^c (49.80)	58.33 ^c (49.80)	20.33 ^{bc}	40.93 ^b	1.76 ^{cd}	2.00 ^c	0.53 ^c
T ₄	65 ^b (53.73)	63.33 ^{bc} (52.74)	13.07 ^e	22.33 ^e	1.50 ^{de}	0.82 ^e	0.22 ^e
T ₅	70 ^a (56.84)	70 ^a (56.84)	16.73 ^d	27.80 ^d	2.38 ^b	2.11 ^c	0.56 ^c
T ₆	61.67 ^{bc} (51.76)	60 ^c (50.77)	36.33 ^a	17.87 ^f	1.92 ^c	1.36 ^d	0.36 ^d
T ₇	56.67 ^c (48.84)	51.67 ^d (45.96)	11.73 ^e	11.45 ^g	1.18 ^e	0.87 ^e	0.23 ^e
Mean	63.33 (52.78)	61.67 (51.80)	20.16	29.14	2.04	2.03	0.54
SE(m) (±)	1.01	1.07	0.95	0.77	0.14	0.14	0.037
CD(p=0.05)	3.07	3.27	2.89	2.33	0.41	0.43	0.11

Values in parentheses are arc-sine value

Mean with same letter is not significantly different among the treatments of the respective parameters

T₁ = IBA 500 ppm + 2000 ppm para-hydroxy benzoic acid
T₂ = IBA 1000 ppm + 2000 ppm para-hydroxy benzoic acid
T₃ = IBA 2000 ppm + 2000 ppm para-hydroxy benzoic acid
T₇ = Control

T₄ = NAA 500 ppm + 2000 ppm para-hydroxy benzoic acid
T₅ = NAA 1000 ppm + 2000 ppm para-hydroxy benzoic acid
T₆ = NAA 2000 ppm + 2000 ppm para-hydroxy benzoic acid



*Mean with same letter is not significantly different among the treatments of the respective parameters

Figure 1: Growth and survival of *E. ganitrus* air layered treatments (control, NAA 1000 ppm+2000 ppm phb and IBA 500 ppm+2000 ppm phb).

synergistically with auxins. Auxins prop up the differentiation of meristematic tissue and helps for the formation of root primordial/callus above the girdling zone of the branch (Tchoundjeu *et al.*, 2002). Besides that, girdling in air layering block in the acropetal and basipetal flow of photosynthates in the phloem which causes the accumulation of secondary metabolites such as organic compounds, carbohydrates, auxins etc. above this region (Alves-de Oliveira *et al.*, 1999) which favours the formation of callus and development of adventitious root primordial to develop into roots (Singh *et al.*, 2004). This finding shows close conformity with the findings of Eganathan *et al.* (2000). The different concentration of IBA and NAA had significant variation in average number of primary and secondary roots per air-layering (Table 1). Maximum mean number of primary root (36.33) was exhibited in T₆ (NAA 2000 ppm+2000 ppm phb) in comparison to all the other treatments where as T₇ (Control) was showed minimum mean number roots (11.73). Among the IBA treatments, T₂ (IBA 1000 ppm+2000 ppm phb) showed the highest (23.13) number of primary roots followed by 20.33 number of roots in T₃ (IBA 2000

ppm+2000 ppm phb acid). Overall, it was observed that a greater number of primary roots was noticed in higher concentration of NAA whereas it was exhibited increasing trend up to certain concentration of IBA and then declined. Similarly, the mean number of secondary roots increased with increase in IBA and NAA concentration but up to certain level. Maximum mean number of secondary root (45.33) was observed in T₂ (IBA 1000 ppm+2000 ppm phb) which was significantly differed among all treatments whereas minimum (11.45) was recorded with T₇ (Control). Both IBA and NAA enhanced the number of secondary roots only up to certain concentration indicating optimum concentration is favorable while higher concentration may be toxic to root regeneration. It is proved that exogenous application of auxins increased lateral roots initiation on the basis of auxin concentration and geotropic response of roots. The present study is in close agreement with the results of Swamy *et al.* (2002) in *Grewia optiva* and *Robinia pseudoacacia*.

IBA and NAA significantly affected the average length of the root in air layers. The root length varied from 1.18 cm to 3.02 cm among the

treatment with a mean value of 2.04 cm. Air layering's treated with T₂ (IBA1000 ppm+2000 ppm phb) exhibited highest root length (3.02 cm) followed by 2.52 cm in T₁ (IBA 500 ppm+2000 ppm phb) which was statistically at par with T₅ (NAA 1000 ppm+2000 ppm phb) recording 2.38 cm whereas control (T₇) was recorded minimum root length (1.18 cm). The fresh and dry root weight differed significantly among the treatments. Among the treatments, T₂ (IBA 1000 ppm+2000 ppm phb) significantly recorded highest (4.25 g) fresh weight of roots followed by 2.84 g in T₁ (IBA 500 ppm+2000 ppm phb) and lowest (0.87 g) was in T₇ (Control). Among the NAA treatments, T₅ (NAA 1000 ppm+2000 ppm phb) showed the maximum fresh weight of the roots (2.11 g) followed by 1.36 g in T₆ (NAA 2000 ppm+2000 ppm phb). Dry weight of roots was followed the similar pattern like fresh weight in all treatments. T₂ (IBA 1000+2000 ppm phb) and T₅ (NAA 1000 ppm+2000 ppm phb) recorded the maximum dry weight of the roots (1.12 and 0.56 g) among the IBA and NAA, respectively. The season and effective translocation of the food materials in the form of starch and nitrogen to the girdled zone of the air layers might have encouraged the growth of

the root by means the production of more number and lengthier roots. Subsequently, the root absorbed water and nutrients from the media leading to more growth, subsequently showed increasing trend in the fresh and dry root weight. This finding is well in line with the results of Reddy (2004) in *Ficus carica*.

Conclusion

The study indicated that *E. ganitrus* amenable to vegetative propagation by air layering. Exogenous application of NAA 1000 ppm+2000 ppm phb and IBA 500 ppm+2000 ppm phb induced the root initiation in air layers successfully in comparison to control as difficult-to-root in cuttings. This technique provides an efficient, minimum cost and efforts in natural conditions for producing massive superior planting stocks for clonal seed orchard or direct planting in the field to obtain the products in short duration.

Conflict of interest

The authors declare that they have no conflict of interest.

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