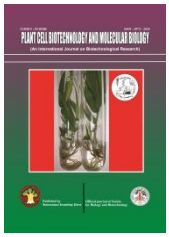

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# Effects of Nitrogen Content and pH on Micropropagation of Rudraksha (*Elaeocarpus ganitrus* Roxb.)

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

This study evaluated the effects of pH and inorganic nitrogen sources ($KNO_3$ and $NH_4NO_3$) on the regeneration of *Elaeocarpus ganitrus* Roxb. The pH and nitrogen concentration in the medium were crucial for the successful micropropagation of *E. ganitrus*. The concentration of nitrogen in the medium significantly affected the development and growth rate of cultures. As a basal medium, Murashige and Skoog's medium (MS) was employed together with various nitrogen concentrations and plant growth hormones. After 4-5 weeks of inoculation, BAP (0.5 mg/l) and NAA (0.1 mg/l)

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produced the maximum number of shoots (4.42 ± 2.17) among all the growth hormones tested. To obtain the maximum number of shoots and shoot length, different strengths of $KNO_3$ (500-3500 mg/l), $NH_4NO_3$ (500-3500 mg/l), and pH (5-7.5) were adjusted in the medium. The largest number of roots was contained in MS medium with 1.0 mg/l NAA (4.47 ± 1.38) after root induction was carried out on MS with NAA, IBA, and IAA (0.2-2.0 mg/l). When the medium contained 1500 mg/l $KNO_3$ and 2000 mg/l $NH_4NO_3$ (3.02 ± 1.60), the highest root initiation was observed. The rooting medium with a pH of 6.0 had the most influence and produced the maximum number of roots (4.8 ± 0.48). The plantlets were allowed to harden and acclimate in a greenhouse. A 76% survival rate was noted in the field.

*Keywords: Ammonium nitrate; Elaeocarpus ganitrus; Endangered flora; In vitro; micropropagation; Potasium nitrate; pH level; Rudraksha.*

## 1. INTRODUCTION

The genus *Elaeocarpus* encompasses approximately 360 species, with a distribution range extending from Madagascar in the west to New Zealand in the east. This extensive range also includes regions such as Southeast Asia, Southern China, Japan, Australia, Malaysia, New Guinea, Fiji, and Hawaii [1]. Among these, 120 species are recorded in various parts of South and Southeast Asia. These regions include the foothills of the Himalayas and areas in India, Nepal, Bhutan, Tibet, Indonesia, the Philippines, Myanmar, and Bangladesh, typically at altitudes of 900-1800 meters above sea level [2]. India alone is home to about 25 species of this genus [3]. Within this genus, *Elaeocarpus ganitrus* Roxb. (also known as *E. sphaericus* (Gaertn.) K. Schum) is a noteworthy species that is a member of the Elaeocarpaceae family. Known by most as "Rudraksha," this species is well-known for its rocky endocarp, which is hard and decorative and has important medicinal and religious properties. This is a medium- to big-sized evergreen tree that grows to a height of 50-200 feet with a girth of 4 feet. It has a large spherical crown. It occurs naturally in the Konkana Ghats of the Maharashtra states of India, as well as the evergreen forests of West Bengal, Assam, Bihar, Madhya Pradesh, and Arunachal Pradesh, with the exception of Tawang and Upper Subansiri. In many regions of the nation, it is also grown as an ornamental tree on homesteads. Rudraksha beads hold significant religious, spiritual, and materialistic value, as depicted in Hindu mythology. The Sanskrit terms "rudra," which signifies Lord Shiva, and "aksha," which means eyes, are the origin of the word "rudraksha" [4].

Hindus view the rudraksha as a sign of purity and light or as a bridge connecting earth and heaven. Beads have been twisted into prayers or necklaces (called "malas") for millennia. This art form is significant in Eastern religions, particularly among Hindus, Sikhs, and Tibetan Buddhists, where it is used for meditation and prayer, as well as to fend off evil spirits and omens [5]. Heart conditions, blood pressure, and nerves can all benefit from wearing Rudraksha beads around the neck [6]. Ayurveda describes using the seeds to cure mental problems, sleeplessness, hypertension, and psychoneurosis. According to Hardainiyan et al. [7], the flesh has a sour flavor and can be consumed. It is used to treat mental health issues, headaches, and epilepsy. Whereas the stem bark is hypoglycemic, the fruit's aqueous extract is mainly used as a cardio-stimulant, sedative, hypotensive, spasmolytic, anticonvulsant, chloretic, and bronchodilatory drug [8]. Stone paste is applied externally to cure measles, smallpox, and organs that burn [9]. The rudraksha bead has been shown to offer a number of health benefits, including anti-inflammatory, antifungal, antioxidant activity [10], and analgesic[11]. The fruit has a notable concentration of phytocomponents, including gallic, ellagic, quercetin, isoelaeocarpine, and rudrakine [12].

Due to a number of factors, including the hard endocarp, long flowering or fruiting intervals, fungal rot, microbiotic seed nature, extended periods of seed dormancy, and poor germination rates, the population of this species is steadily declining in both natural and planted forest stands at an alarming rate. The natural regeneration has also been negatively impacted by the dishonest seed collection. The limited distribution of this species in its native habitat is mostly caused by the rising market prices of beads because of religious belief and their multiplication in value [13], putting the species under fear of extinction in the near future. Due to the difficulty in propagation and the significance of the plant for ethnic medicine, the primary conservation methods are *in vitro* and *in vivo*.

Plant tissues use and make nitrogen available in different ways, depending on its supply and environmental factors such as temperature, plant genotype, and substrate pH [14 and15]. Specifically, adding $NH_4^+$ to the tissue culture medium releases $H^+$ ions, which has an impact on the pH of the medium [16]. The physiological processes that influence roots [17] and shoot growth rate and condition [18] may be impacted by this pH change. It has been observed that in stevia, providing $NH_4^+$ to the rooting medium influences the quantity of roots developed from micropropagated shoots and the plant's subsequent survival in soil [19].

*Elaeocarpus ganitrus* micropropagation has been included into conservation initiatives to help this species become resistant to insects and diseases [20 and 21]. Conventionally grown plants and micropropagated plants have both been successfully planted in the field and grow normally in comparison to seedlings [22 and 23]. However, the heterogeneity of clones in their ability to form adventitious branches and roots presents a considerable obstacle to the program's micropropagation element. This diversity raises the cost of producing clones significantly and restricts access to some elite genotypes. Here, we describe how varied nitrogen concentrations, medium pH, and medium buffering affect the growth of shoots and roots during rudraksha micropropagation.

## 2. MATERIALS AND METHODS

The Rudraksha explants were sourced from the University garden at Shobhit Institute of Engineering and Technology in Modipuram, Meerut, Uttar Pradesh, India. Young plants provided the shoots, which were then processed into explants. These shoots were sectioned into 2-3 cm long nodal segments, thoroughly rinsed with distilled water, and swabbed with cotton soaked in 70% ethyl alcohol for initial sterilization. Following this, the segments underwent a 30-minute treatment with a 0.2% Bavistin solution to eliminate fungal contamination. After the Bavistin treatment, the segments were rinsed thoroughly with sterile distilled water to remove any chemical residues.

In order to guarantee complete eradication of any microbiological impurities, the explants were subjected to a 0.1% (w/v) solution of mercuric chloride ($HgCl_2$) in a laminar flow hood for duration of three minutes. To guarantee total $HgCl_2$ clearance, three further rinses with sterile distilled water were performed after this. The nodal segments were then cultivated on Murashige and Skoog's (1962) medium [24], which was enhanced with different amounts of auxins, cytokinins, and nitrogen to promote plant growth. Before the medium was autoclave-sterilized, its pH was adjusted to the appropriate level. After that, the cultures were kept in a controlled environment at 25°C with a photoperiod of 8 hours of darkness and 16 hours of light at an intensity of 90 mmol $m^{-2}$ $s^{-1}$[21].

### 2.1 Effects of Auxin-cytokinin on Shoot Initiation

The influence of various cytokinin-auxin combinations on the initiation and multiplication of in vitro cultured shoots was examined. Nodal segments were cultured on a medium supplemented with different concentrations of BAP (0.1-5.0 mg/l), Kn (0.1-5.0 mg/l), and NAA (0.1-0.2 mg/l). Specifically, a medium with 0.5 mg/l BAP and 0.1 mg/l NAA was used to evaluate the interaction effects between cytokinin and auxin on shoot multiplication. This combination aimed to optimize the multiplication of axillary shoot buds, increasing both the number and length of shoots.

### 2.2 Effects of pH Levels on Shoot Multiplication

To assess the impact of pH on shoot multiplication, the MS medium was supplemented with 0.5 mg/l BAP and 0.1 mg/l NAA. The pH of the medium was adjusted to six different levels: 5, 5.5, 6, 6.5, 7, and 7.5, using either 1M NaOH or 0.25M HCl before autoclaving. The cultures were observed regularly over a period of four weeks to distinguish healthy growing cultures from non-growing ones, with any infected cultures being discarded. After 40 days, the number of shoots per explant and the length of the shoots were recorded to determine the optimal pH for shoot multiplication.

### 2.3 Effect of $NH_4NO_3$ and $KNO_3$ on Shoot Multiplication

The study utilized MS medium with 3% sucrose (Hi-Media), supplemented with varying concentrations of $NH_4NO_3$ and $KNO_3$ (0, 500, 1000, 1500, 2000, 2500, 3000, and 3500 mg/l). The pH of the medium was adjusted to 5.8-6.0 using a pH meter. In vitro sterilized shoots were

subcultured on induction and multiplication MS media, with various combinations of $NH_4NO_3$ and $KNO_3$ added. A control group used MS medium without $NH_4NO_3$ and $KNO_3$.

## 2.4 Effect of Auxins on Root Initiation

For complete plantlet regeneration, elongated shoots (2.0-3.0 cm) were subjected to various hormone regimes to induce adventitious root formation. The study examined the effects of different factors on root induction. Auxins, specifically NAA, IBA, and IAA, were added to the MS medium at concentrations of 0.2, 0.5, 1.0, and 2.0 mg/l, either individually or in combination, to evaluate their impact on rooting of the healthy shoots.

## 2.5 Effect of Nitrogen and pH Level on Root Multiplication

To investigate the effects of different nitrogen concentrations and pH levels on root induction, individual shoots (2-3 cm) were excised after a four-week proliferation period on MS medium. The rooting medium consisted of MS medium solidified with 0.8% plant agar, supplemented with 3% sucrose and 1.0 mg/l NAA. This medium varied in inorganic nitrogen levels, as previously described for shoot multiplication. Each experiment was conducted in triplicate, using 20 ml of medium and ten explants per replication. Initially, cultures were maintained in dark conditions at 25 ± 1°C for one week, then exposed to the same light and temperature conditions as described earlier. After 28 days on the rooting medium, the percentage of rooted shoots, number of roots, and root lengths were recorded.

## 2.6 Hardening of Plantlets

After carefully removing the rooted plantlets from the culture bottles, all adhering agar was properly cleaned under running tap water. Next, a 0.1% Bavistin solution was applied to them. The plantlets were washed and placed in tiny paper cups with a mixture of autoclaved soilrite, keeping their root systems intact. Once a week for 15 days, these plantlets received ¼x strength MS media (free of organics and sucrose) and were housed in the culture room. Following a period of four to six weeks, the plantlets were transferred to pots and polybags filled with a 1:1:1 mixture of sand, farmyard manure, and soil. The temperature and relative humidity were kept at 28 ± 2°C and 70-80%, respectively.

## 2.7 Data Analysis

The data represent the average results of three experiments, each conducted three times. Regular observations were made to record the percentage of culture response, the number of shoots per explant, and the rooting. Results were illustrated using the mean ± standard error of three replicates.

Induction rate (%) = (number of induced explants / total number of initial explants) × 100%.

Average shoot or root number = total number of shoots or roots / number of shoots or rooted plantlets.

Rooting rate (%) = (number of rooted plantlets / total number of shoots) × 100%.

# 3. RESULTS AND DISCUSSION

## 3.1 Effect of Auxin-Cytokinin for Shoot Initiation

Nodal segment explants underwent direct regeneration through organogenesis. The highest shoot development rate (4.42 ± 2.17) was achieved when these explants were cultured on MS medium supplemented with antioxidants, 0.5 mg/l BAP, and 0.1 mg/l NAA [25]. In comparison to low cytokinin levels (0.1 mg/l BAP), it was discovered that high cytokinin levels (5.0 mg/l BAP) greatly increased shoot development from explant cultures [Fig. 1]. When nodal segments were growing, there were notable variations in shoot proliferation amongst the various BAP and NAA concentrations. However, developed was not substantially impacted by the combination of the medium and Kn dosages. Nodal segments were significantly affected by MS medium containing BAP and NAA, leading to the regeneration of elongated shoots. The highest number of elongated shoots was seen on MS medium. Thus, the best media for Rudraksha shoot multiplication was found to be MS medium enhanced with BAP and NAA [Figs. 7A and B].

The selection of media and growth regulators is vital for successful tissue culture, with the selection of medium dependent on the specific objectives of the tissue culture technology used for the particular plant species or variety [26, 27,]. Various studies have compared different media for their impacts on *in vitro* shoot multiplication [28-30]. Different plant species typically have varying nutritional requirements, leading to different responses to various basal media.

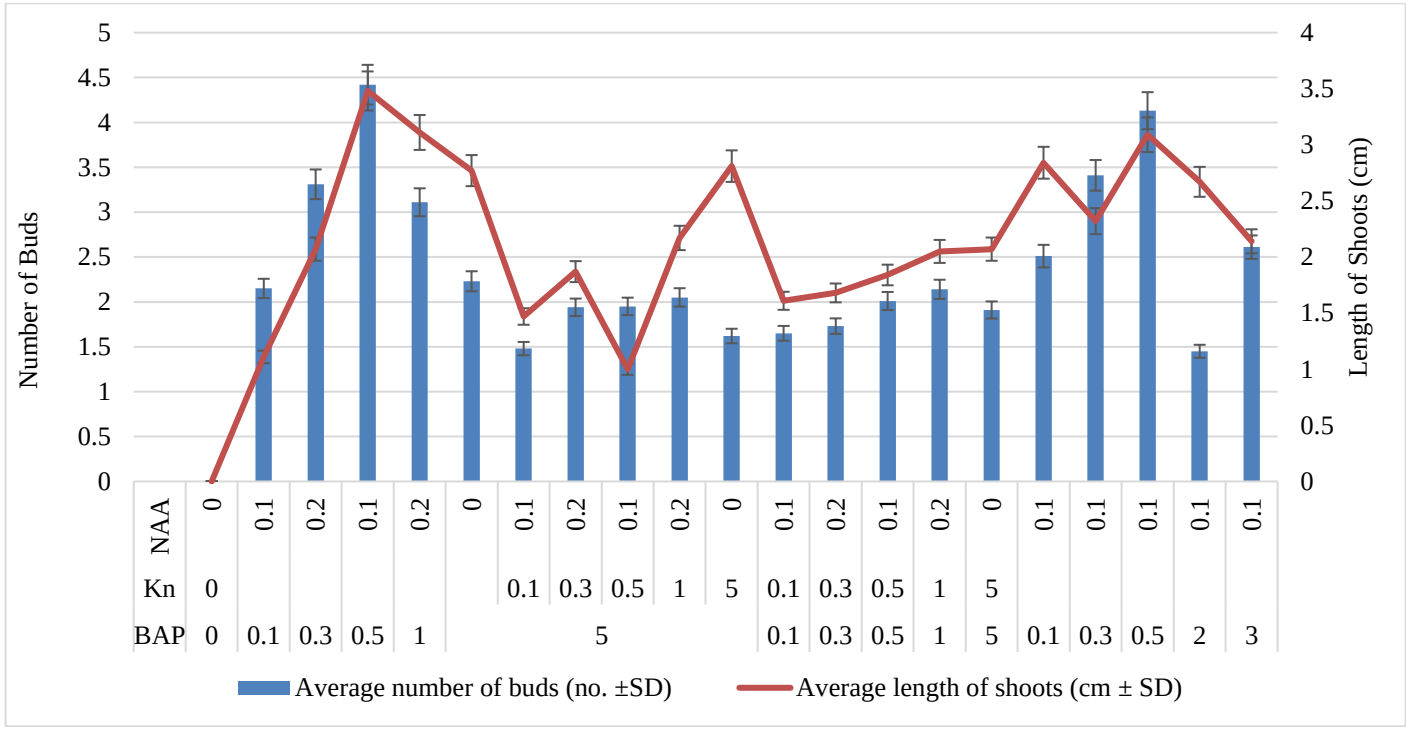


**Fig. 1. Impact of the combination of auxins and cytokinins added to MS medium on the induction and growth of *Elaeocarpus ganitrus* shoots cultured *in vitro*. (Data collected following a 5-week culture period).**

Tissue culture technology is widely applicable to all plant species since any plant part can serve as an explant. However, research has shown that herbaceous plants typically respond better and are more easily propagated via tissue culture protocols compared to woody plants. Abbot [31] observed that woody plants have lower propagation potential due to their complex and lengthy life cycles. Additionally, Mott [32] highlighted the challenges of clonal multiplication from mature tissues or organs of woody plants. These findings align with [33], who reported that the shoots of woody plants have longer dormancy periods than those of herbaceous plants, contributing to the limited success of *in vitro* propagation of woody plants.

### 3.2 Effect of Nitrogen and pH Levels on Shoot Multiplication

Shoot development from nodal segment explants was successfully achieved through culture in media supplemented with varying concentrations of BAP, Kn, and NAA. BAP and NAA were identified as essential auxin and cytokinin for direct organogenesis in rudraksha. The most effective shoot induction occurred on MS medium enriched with 0.5 mg/l BAP and 0.1 mg/l NAA, resulting in an average number of 4.42 shoots per explant and a high frequency of shoot formation, as shown in the accompanying graph. This optimized nitrogen composition in MS medium, containing BAP (0.5 mg/l) and NAA (0.1 mg/l), was consistently maintained through successive subcultures to enhance morphological responses [Fig. 2].

When $NH_4NO_3$ was used as the sole nitrogen source, the multiplication rate decreased significantly across all concentrations. Higher concentrations, notably 2500 mg/l $NH_4NO_3$, led to increased hyperhydricity and callus formation, resulting in poorer shoot quality with reduced multiplication rates and shorter shoot lengths. In contrast, media supplemented exclusively with $KNO_3$ produced high-quality shoots with dark green leaves. Combining lower concentrations of $NH_4NO_3$ with higher concentrations of $KNO_3$ effectively reduced hyperhydricity and callusing while improving the multiplication rate, as depicted in the graph. The highest multiplication rate (4.21 ± 1.82) and maximum shoot length (3.50 ± 1.54) were achieved with 2500 mg/l $KNO_3$ and 1000 mg/l $NH_4NO_3$ [Fig. 7 C].

Optimal shoot multiplication was observed in media containing both $NH_4NO_3$ and $KNO_3$, where varying concentrations significantly influenced key parameters such as average shoot number, shoot length, hyperhydricity, and callusing of rudraksha after four weeks in culture. The most healthy shoot formation occurred with 2500 mg/l $NH_4NO_3$ and 1000 mg/l $KNO_3$, resulting in healthy shoots with green leaves. However, exceeding these optimal levels led to reduced shoot proliferation and length, along with increased hyperhydricity and callusing, which peaked at 43% with 3000 mg/l $NH_4NO_3$ and 500 mg/l $KNO_3$. Moreover, longer shoots progressively declined with higher $NH_4NO_3$ and $KNO_3$ concentrations.

The current study showed that, in comparison to employing individual nitrogen sources, a combination of nitrogen sources in MS medium was more beneficial for shoot regeneration and growth. Researchers [34-36] reported similar results. Nitrogen was found to cause hyperhydricity in *Prunus avium* [37], *Castanea sativ*a [38], *Salix babylonica* [39], and *Aloe polyphylla* [40] at a concentration of 60 mM (standard MS medium). However, because $NH_4$ ion can set off processes that result in this situation, this hyperhydricity was linked to it instead of nitrogen itself [41]. Inorganic nitrogen levels eliminated hyperhydricity and healthy growth of shoots [37, 42], which aligns with our own findings [Fig. 7 D]. Statistical analysis indicated that shoot length was significantly influenced by different concentrations of $NH_4NO_3$ and $KNO_3$, particularly the $NH_4$ ion ratio. The longest shoots were observed at a 1:3 ratio (2500 mg/l $KNO_3$ and 1000 mg/l $NH_4NO_3$), which also slightly increased total nitrogen content in the medium of *E. ganitrus*. In contrast, the shortest shoots occurred at higher concentration in $NH_4NO_3$. Compared to the higher concentration of ammonium ions and total nitrogen likely contributed to reduced shoot length, possibly due to inhibited activity of enzymes like Nitrate Reductase and Glutamate Synthases involved in amino acid production [43].

Previous research supports our finding that nitrogen in the medium affects pH levels, with ammonium increasing acidity while nitrates tend to make it more alkaline [44, 45]. Ammonium had a more pronounced effect on pH even when the media were buffered. Although nitrate-only media slightly raised pH, the change was less significant than in ammonium media. The ability to maintain pH is unlikely to be solely due to nitrate uptake. The results obtained here agree

with De Jong and co-workers [46], who found that pH favored floral organ growth using a specialized medium. They determined that a pH range of 5-7.5 was optimal for growth and shoot length, with maximum shoot production recorded at pH 6 [Fig. 3]. These findings highlight the importance of carefully managing pH levels in tissue culture media to optimize plant growth and development [47].

### 3.3 Effect of Auxin on Root Initiation

Rooting induction from nodal explants and the production of multiple shoots are illustrated in Fig. 4. Healthy multiple shoots cultured on MS media with 0.2 mg/l-2.0 mg/l of IAA, IBA, and NAA showed prompt root formation. Root explants demonstrated the highest responsiveness, producing roots within 14-18 days of culture, whereas other auxins took approximately four weeks to induce rooting. Additionally, the study found that optimal concentrations of NAA (1.0 mg/l) accelerated rooting to 14-18 days, compared to the 28 days required when using lower concentrations of NAA and other auxins. These findings align with [2], who found that NAA effectively stimulates root induction in tree species like apple and *Cassia angustifolia* Vahl, while IAA and IBA are less effective. IAA and IBA also showed poor results for inducing roots in woody plants.

### 3.4 Effect of Nitrogen and pH Levels on Root Multiplication

Root development was notably influenced by nitrogen concentration and buffering conditions. Optimal rooting occurred at lower nitrogen levels, with a marked decline in the number of roots per shoot at zero and only higher concentration of nitrate and ammonium nitrogen. However, this trend was less apparent in percentage rooting data, where 2000 mg/l $KNO_3$ and 1500 mg/l $NH_4NO_3$ nitrogen yielded similar rooting percentages to those at lower nitrogen levels. Additionally, media containing $NH_4NO_3$ (2000 mg/l) and $KNO_3$ (1500 mg/l) produced significantly higher root numbers per shoot (maximum of 3.02±1.60) compared to media with higher concentrations of these combination [Fig. 5].

Research shows that the pH of the growing medium can influence some plants' ability to develop roots *in vitro*, with most species favoring a slightly acidic pH. In *Vitis*, *Ribes nigrum*, and *Aronia melanocarpa* shoots, Zatkyo and Molnar [48] observed a substantial relationship between medium acidity (pH 7.0 to 3.0) and rooting. They attributed this to the requirement of acidity for auxin activity. [49] reported minimal impact of pH on rooting in apple stem slices: when the pH was set to 5.5 before autoclaving (resulting in a pH of 5.54 post-autoclaving), the root number was 5.5, while setting the pH to 8.0 before autoclaving (dropping to 5.65 post-autoclaving) increased the root number per slice to 7. With a MES-buffered medium, the highest root count occurred at pH 6.4 post-autoclaving. This experiment highlighted connects between pH effect on NAA uptake and root number, a phenomenon also noted by [50].

In the case of *Lilium auratum* bulb scales, direct root formation was successfully achieved when the pH of the MS medium ranged from 4 to 7, with optimal results at pH 6. The optimal pH range for adventitious bulblet formation was between 4 and 8, with the majority forming at an initial pH of 5 to 7 [51]. This comprehensive analysis underscores the critical role of pH in root formation across various species, indicating that slight adjustments in pH can significantly influence rooting efficiency and overall plant development.It was discovered that reducing the pH of the growth medium to 6.5 significantly enhanced the rooting of *Bougainvillea* shoots [52]. Similarly, as present study, accordance to [53] found that a pH of 6.0 in MS medium and incubating in darkness were essential for consistent root development in two *Santalum* species, as well as in *Correa decumbens* and *Prostanthera rotundifolia* [54, 17]. In contrast, other Australian woody species exhibited optimal rooting at a pH of 5.5, with a pH of 4 proving inhibitory. The carnation meristem tips reported better rooting at pH 5.5 compared to pH 6.0 [55]. In potato, buds rooted best at pH 5.7, with inhibition occurring at pH levels of 4.8 and 6.2 or higher and leaf segments showed delayed root formation when the medium containing IAA was initially set to either an acidic pH (5.5 or 6.0) or a neutral pH (6.5)[56].

In our study, we found that effective and rapid root formation in rudraksha was achieved at a medium pH level of 6.0 to 6.5, resulting in a higher number of roots. We observed that in vitro induction and multiplication of roots in rudraksha were healthy and increased in number at a pH level of 6.0. Conversely, lower pH levels combined with auxin resulted in recalcitrant roots [Figs. 6 and 7D].

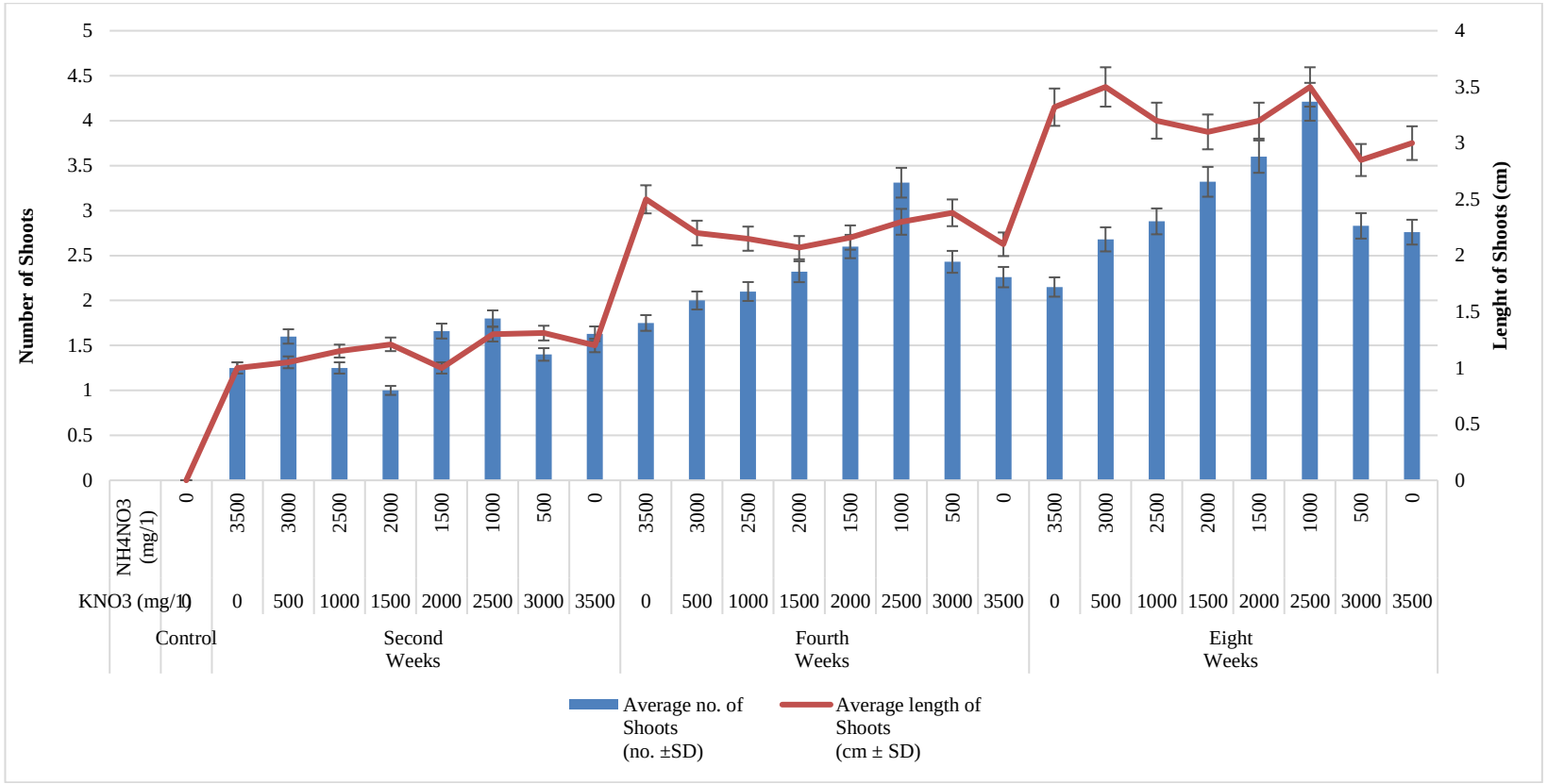


**Fig. 2. Influence of nitrogen on the induction and growth of shoots on MS medium containing 0.5 mg/l of BAP and 0.1 mg/l of NAA**

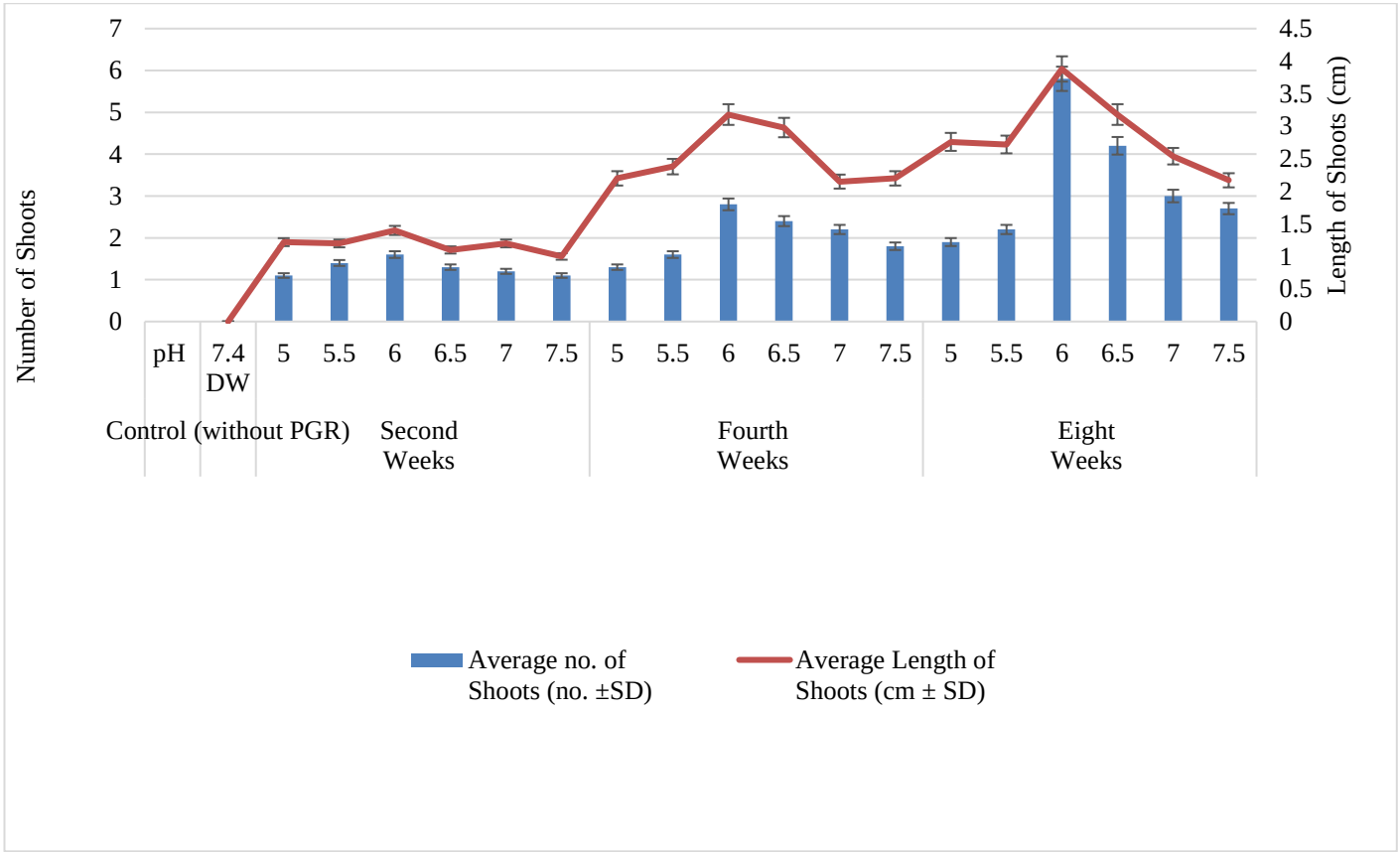


**Fig. 3. Effect of pH on multiplication of shoots on MS Media + BAP (0.5mg/l) + NAA (0.1mg/l)**

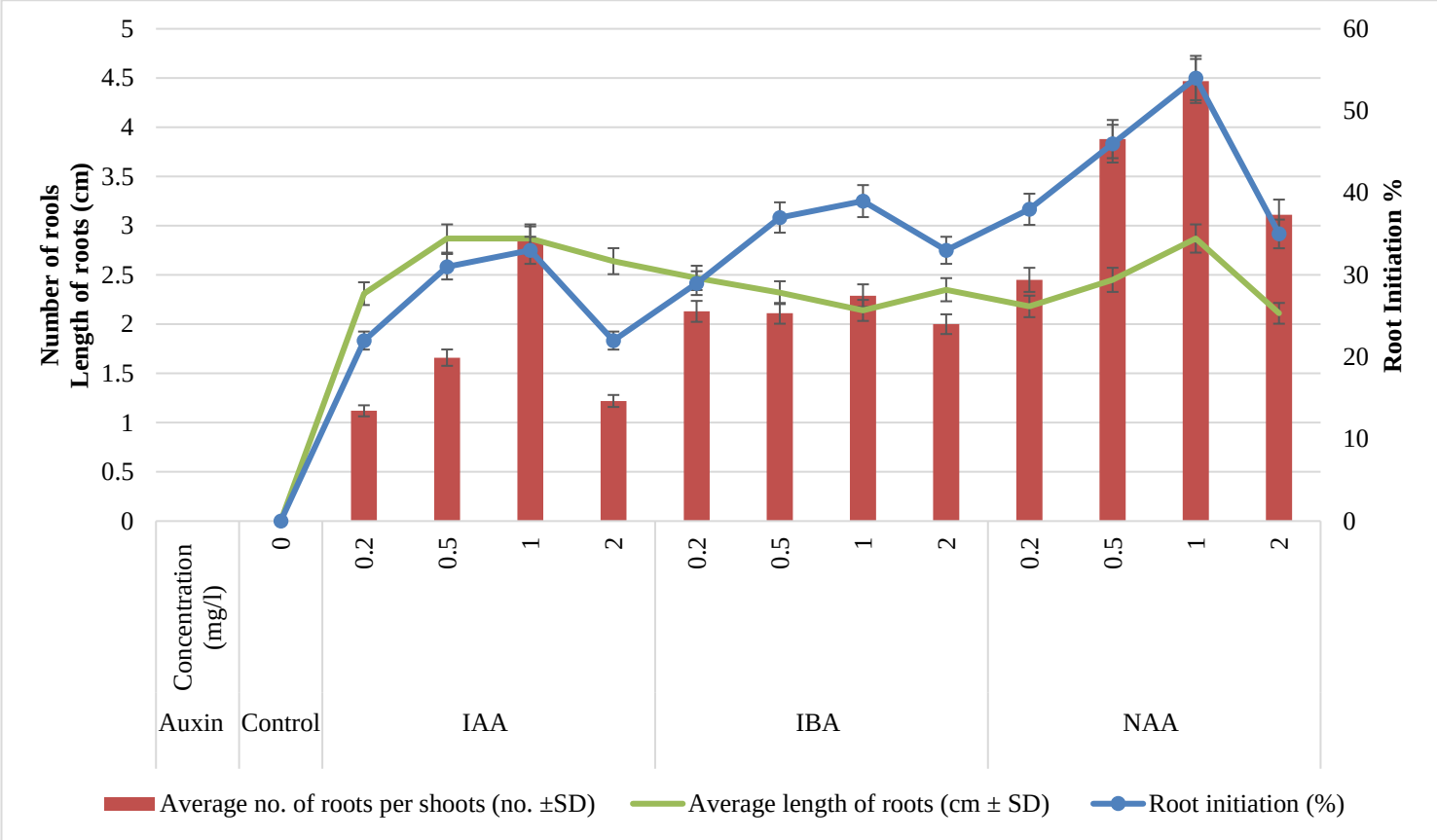


**Fig. 4. Effect of auxins on induction of roots on MS medium (recorded 6 weeks old cultures)**

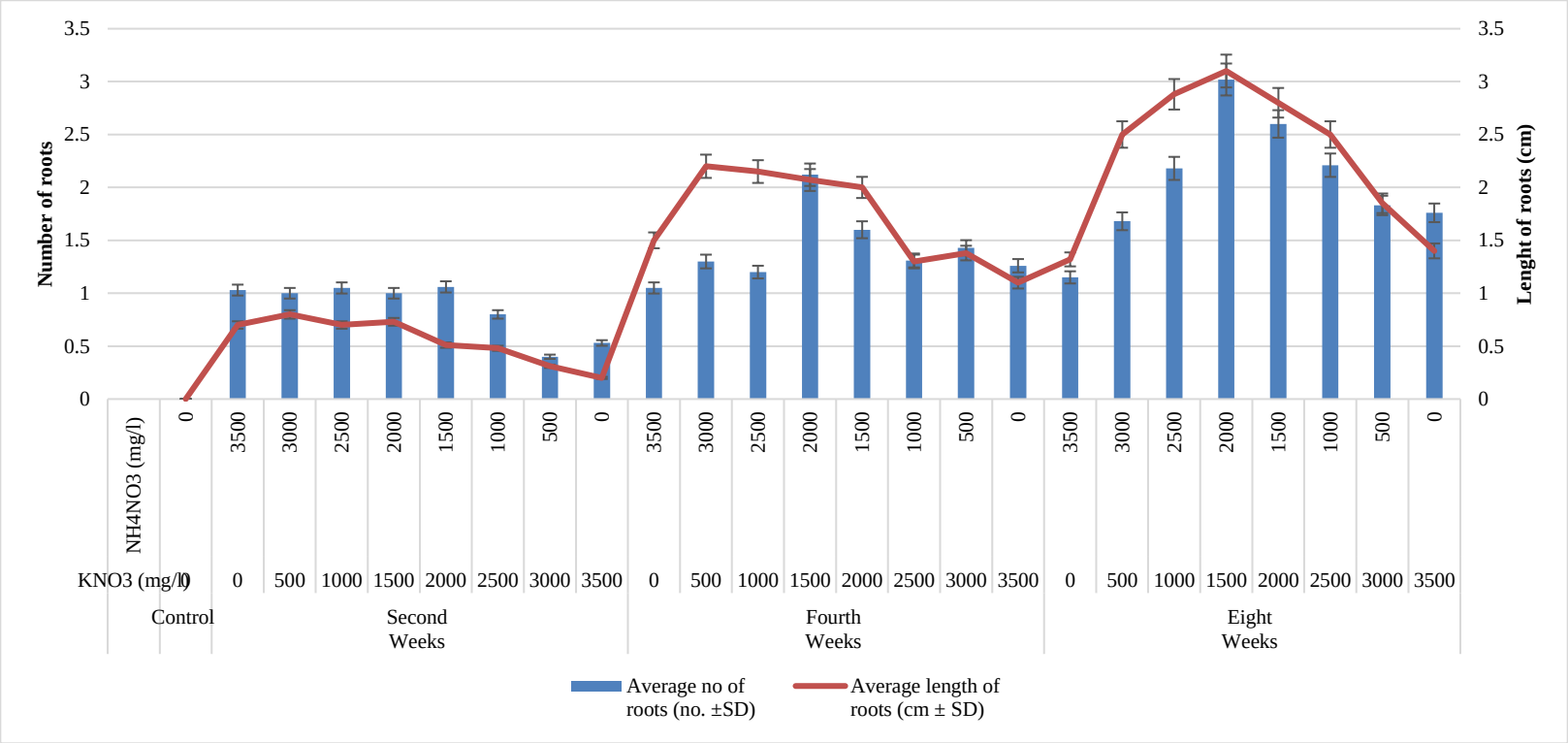


**Fig. 5. Effect of nitrogen on induction of roots on MS medium supplemented with NAA (1.0mg/l).**

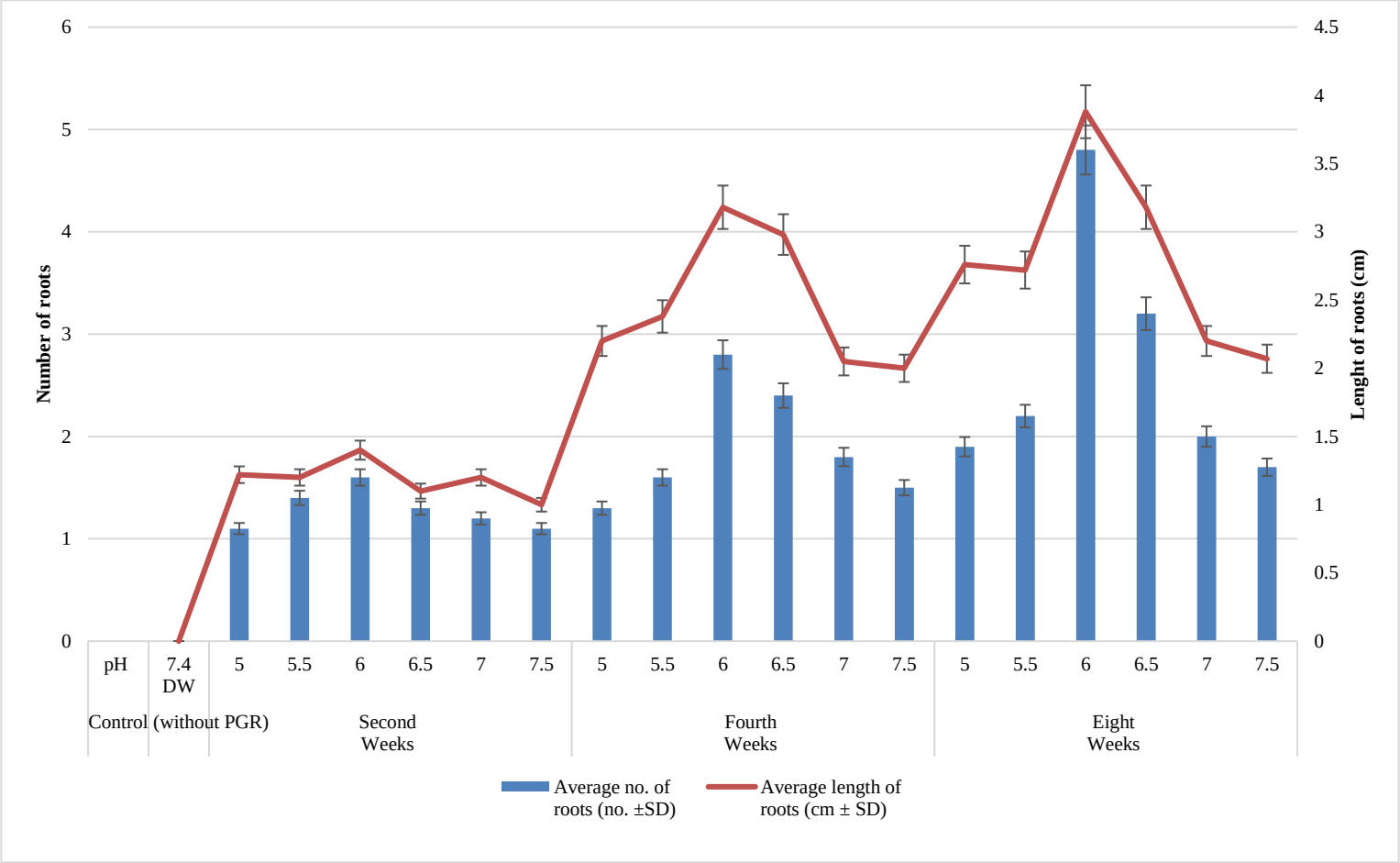


**Fig. 6. Effect of pH on induction and proliferation of roots MS media with NAA (1.0mg/l)**

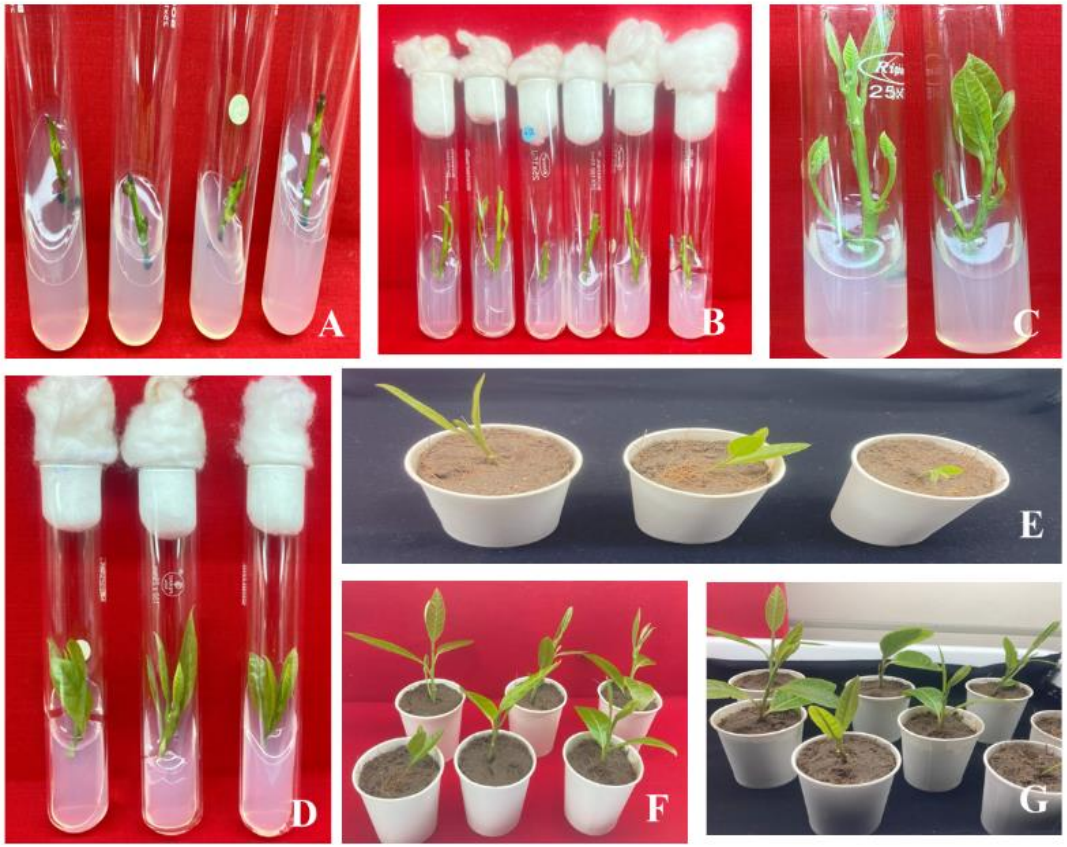


**Fig. 7. Micropropagation of *E. ganitrus* from explants of the nodal segment: (A) Nodal segment explants were used to induce shoots on MS media containing 0.5 mg/l of BAP and 0.1 mg/l of NAA. (B) Shoot induction and multiplication on MS medium supplemented with BAP and NAA after eight weeks. (C) Multiplication of shoots on MS medium supplemented with pH 6.0, BAP, NAA, $KNO_3$ (2500 mg/l), and $NH_4NO_3$ (1000 mg/l). (D) The regenerated shoots' initiation of rooting on MS medium supplemented with 1.0 mg/l NAA, 1500 mg/l $KNO_3$, 2000 mg/l $NH_4NO_3$, and pH 6.0. (E-G) Sturdy plantlets are moved to a plastic pot that is filled with a 1:1:1 ratio of farm yard manure, sand, and garden soil**

### 3.5 Hardening of Plantlets

These plants need to gradually acclimate in order to successfully adapt to field settings. In this investigation, a careful process for hardening and acclimating the plantlets was used. The plants were progressively exposed to greater temperatures through a methodical, step-by-step procedure, which helped to avoid the shock that comes with sudden temperature fluctuations. To harden the rooted plantlets *in vitro*, they were initially put in root trainers filled with soilrite. They were given on a half-strength MS liquid medium without sucrose for fifteen days [Fig. 7 E]. The acclimated plants were then transferred to clay pots and polybags that were filled with soil, farmyard manure, and sand in a 1:1:1 volumetric composition [Fig. 7 F and G]. This method ensured that there were no observable phenotypic variations and that all planted plants were highly homogeneous.

## 4. CONCLUSION

The findings from this study highlight the *E. ganitrus* varied responses to different inorganic nitrogen concentrations and pH levels. Specifically, shoot proliferation and growth are minimal at low concentrations of ammonium nitrate ($NH_4NO_3$) and high concentrations of potassium nitrate ($KNO_3$). High concentrations of ammonium nitrate combined with low concentrations of potassium nitrate maximized root multiplication. We concluded that nitrate nitrogen is most effective for shooting, while ammonium nitrogen is most effective for rooting, with an optimal media pH of 6. This approach is valuable for both the enhancement and conservation of the endangered species of *Elaeocarpus ganitrus* Roxb., ensuring its survival and promoting biodiversity.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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