






In vitro Effect of Phytohormones on the Regeneration Potential in Nodal Explant of *Rosa hybrida* L.

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Abstract: This study investigates the impact of diverse phytohormone compositions and concentrations on the growth and regeneration of *Rosa hybrida* L. nodal explants for micropropagation. Nodal explants were cultivated on five distinct Murashige Skoong (MS) media incorporating varying levels and combinations of indole butyric acid (IBA), benzyl aminopurine (BAP), and naphthalene acetic acid (NAA). Treatments included A-1 (IBA 400 ppm, BAP 125 ppm), A-2 (IBA 650 ppm, BAP 175 ppm), A-3 (IBA 900 ppm, BAP 225 ppm), B-1 (BAP 900 ppm, NAA 90 ppm), and B-2 (BAP 450 ppm, NAA 45 ppm). Under optimized culture conditions, explants were monitored for growth and contamination over 30 days. Results analysis revealed that the A-2 hormone combination exhibited the highest regeneration potential in *R. hybrida* L. nodal explants among the five treatments (A-1, A-2, A-3, B-1, and B-2). This suggests that the specific concentration of IBA and BAP in A-2 treatment significantly influenced the regenerative capacity of the nodal explants. These findings contribute valuable insights into optimizing micropropagation protocols for *R. hybrida* L., offering potential applications in large-scale production and conservation efforts.

Keywords: nodal explant; micropropagation; phytohormones; *Rosa hybrida* L.; Murashige Skoog media.

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1. Introduction

The quick multiplication of cultivars with desirable traits and the production of disease-free, healthy plants have both been made possible through *in vitro* rose propagation. Over the past few years, various techniques for *in vitro* rose propagation using apical buds or nodal segments have been developed [1]. Modern processes have been developed to meet the demands of the cut flower industry in the twenty-first century. Gene transfer techniques could substantially speed up breeding processes and address a wide range of agronomic and environmental problems that are otherwise intractable using conventional methods [2].

Young and healthy shoot explants from the first to fifth nodes were taken out and cultivated on Murashige and Skoog basal media with different concentrations of indole butyric acid (IBA) and naphthalene acetic acid (NAA) [3]. The most widely used cut flower in the planet is the rose. Rose tissue culture has evolved over the past 20 years and is now employed for a wide range of purposes, including micropropagation from auxiliary buds, nodal sections, branch ends, leaf explants, and other sources, as well as basic anatomical and physiological research [4,5].

Single bud nodal stem segments that had been surface sterilized were grown on MS media that had been supplemented with 30 g/l sugar, 6 g/l agar, and a range of BAP concentrations (1, 2, 3, 4, and 5 mg/l). The most efficient BAP concentration for shoot initiation and multiplication was found to be 3 mg/l. The best results for shoot elongation were found to be achieved using MS medium supplemented with BAP 3 mg/l and GA3 1 mg/l [6].

An experiment was undertaken using several types of explants and various combinations of phytohormones in Murashige Stock culture media in order to discover a standard and quick methodology for micropropagation of Rose (*Rosa indica* L.) investigation. Rose explants with nodal segments were found to be more sensitive than shoot-tip explants. Among the phenolics, secretion was a concern with nodal explants, which slowed plant growth in cultured conditions and caused disease. The media has become brown. The condition was solved by taking antioxidant and antiphenolic supplements, such as charcoal, PVP, AgNO₃, and other substances. The addition of growth hormone had a good impact on shoot proliferation as well. Other considerations include pH, temperature, and light influencing the plant growth in media [7,8].

A plant's natural growth requirements are entirely met by the microenvironment of the culture vessel, which is used for micropropagation, a controlled and aseptic vegetative propagation method. Recent advances in propagation techniques may aid in enhancing species diversity and large-scale plant output [9]. It has been demonstrated that micropropagation is a highly successful technique for producing uniform, disease-free rose plants quickly [10]. It has been demonstrated that the multiplication of rose, based on the division of *in vitro* plantlets into branches, terminal buds, and nodal sections, gives multiplication rates of two- to nine-fold during a period of four weeks. Up to 4,00000 plants can be produced annually from a single rose mother plant utilizing *R. hybrida* micropropagation [11]. Hence, the main objective of this study is to identify the best growth hormone treatment combination for micropropagation of nodal explant of *R. hybrida* L, which shows better regeneration potential and should be efficient for mass micropropagation.

2. Materials and Methods

2.1. Selection and collection of the explants.

The explants used in this study were sourced from the Braj Nursery in Mathura, Uttar Pradesh, known for its cultivation of healthy and disease-free *R. hybrida* plants, ensuring the quality of the source materials for propagation. Specifically, nodal sections of the plant were selected as explants. Nodal sections found on the stem are the areas from which leaves or stems emerge that contain essential meristematic tissue crucial for initiating new growth and supporting tissue culture processes [1].

The collected nodal sections from five *R. hybrid* plants adhered to a systematic approach aimed at ensuring the quality and integrity of the explants for further propagation. Meticulous attention was paid during the harvesting process to select healthy and mature nodal segments free from any evident signs of disease, damage, or infection. Subsequently, the collected nodal sections underwent an extensive surface sterilization procedure to eliminate potential external contaminants thoroughly. This rigorous sterilization step played a pivotal role in preventing the introduction of microbial or fungal elements that could compromise subsequent culture processes. Post-sterilization, these nodal sections, rich in meristematic cells capable of initiating new growth, were selected as the primary explants for micropropagation. Their inherent capacity to generate new shoots facilitated the development of new plants through tissue culture techniques, forming a foundational step in the propagation process [3].

2.2. Sterilization of the explant.

The sterilization procedure for the healthy rose plant explants involved a series of steps aimed at ensuring their cleanliness and free from contaminants. Initially, the plants were carefully collected and stored in poly bags with distilled water to preserve their vitality. The cleaning process began with a gentle 10 to 15-minute wash under slow-running tap water to remove surface impurities [12]. Subsequently, the explants underwent a 10-minute treatment with Tween 20 detergent, which enhanced the cleansing process. To ensure thorough sterilization, the explants underwent sequential chemical treatments. They were immersed in a 1.0 % Bavistin solution for 12 min to remove any fungal contamination, followed by exposure to a solution containing 2.0% streptomycin and 0.1% mercuric chloride for 5 to 10 minutes, effectively eliminating potential pathogens. The final step involved disinfecting the explants using 70% ethanol for 1 to 2 min, eradicating any residual microbes [13]. To conclude, the sterilized explants underwent thorough rinsing with autoclaved distilled water, repeated four to five times. This careful rinsing process was designed to eliminate any residual sterilizing agents, ensuring the comprehensive eradication of contaminants. The holistic sterilization protocol, incorporating diverse treatments and rinsing steps, was implemented to establish an ideal and sterile environment conducive to subsequent tissue culture experiments and propagation initiatives.

2.3. Media composition and preparations.

The nodal explants of Rose were cultivated using five distinct MS media formulations with varying compositions of growth hormones. The preparation of the media mix involved the following process. Initially, powdered MS media was gradually dissolved in water to achieve a volume of 400 ml. Subsequently, 15 grams of sucrose were added, and the pH was adjusted to 5.8 using either 0.1 N HCl or 0.1 N NaOH. To solidify the media, 4 grams of agar were incorporated, along with the requisite hormones (IBA, BAP, and NAA) combinations and concentrations [14]. The volume of the media was adjusted to 500 ml by adding an additional 100 ml of distilled water. The resulting mixture was dispensed into sterile flasks and subjected to autoclaving at 121°C and a pressure of 15 psi for a duration of 35 mins to ensure the sterilization of the medium. We prepare five media compositions of 500 ml each [15].

Following sterilization, the media was left in a sterile environment, undergoing monitoring until it reached a suitable state for use in culturing the explants. This careful preparation and sterilization process aimed to create a conducive and uncontaminated growth medium, essential for the successful cultivation and growth of the nodal explants in subsequent tissue culture experiments. Different types of media compositions used to treat nodal explant of *R. hybrida* L. for micropropagation are as follows:

Control A: It is utilized for micropropagation and comprises 2.15 grams of MS Media, 15.0 gm of sucrose, and 1.6 mg of ascorbic acid. It is diluted with 500 ml of distilled water.

Control B: It comprises of 2.15 grams of MS Media, 15.0 grams of sucrose, and 500 milligrams of charcoal and is diluted in 500 ml of distilled water.

2.4. Optimization of media and cultured conditions.

Different concentrations of plant growth-promoting regulators (PGPRs), such as IBA, BAP, and NAA, were integrated into the MS medium to stimulate shoot proliferation from the explants. The initial step involved adjusting the pH of the medium to 5.8 using either 0.1 N HCl or 0.1 N NaOH before the addition of agar. Subsequently, the medium was sterilized by autoclaving at 121°C and 15 psi pressure for a duration of 20 minutes. Following the inoculation of explants, all cultures were carefully maintained under aseptic conditions. Specifically, the cultures were kept in dark conditions at 21°C and in light at 21°C to ensure optimal growth conditions. Further optimization included maintaining a light cycle of 16 hours of light and 8 hours of darkness, a consistent temperature of $24 \pm 2^\circ\text{C}$, and adequate relative humidity levels throughout the duration of the experiment [15].

Regular assessments were conducted at weekly intervals to monitor the progress of shoot proliferation under various culture conditions. These assessments involved examining the development and growth of shoots in relation to the specific cultural conditions provided, allowing for comprehensive documentation of the shoot proliferation rates over time.

3. Results and Discussion

A comprehensive and successful culturing endeavor was undertaken with *R. hybrida* L. employing an array of growth hormone combinations. The primary aim was to investigate the regeneration efficiency of explants when cultured in a spectrum of BAP, NAA, and IBA concentrations. The assessment of regenerative capacity was conducted at 15day intervals, after which the flasks were placed under the standardized protocol. This protocol ensured a consistent environment comprising 16 hours of light and 8 hours of darkness, maintaining a stable temperature of 24°C throughout the culturing process [16].

Effect of IBA and BAP-enriched media supplemented with ascorbic acid on the growth of nodal explants in *R. hybrida* L. The effect of IBA and BAP-containing media supplemented with ascorbic acid on the growth of nodal explants of *R. hybrida* L. was assessed through various treatments. In Treatment A-1, utilizing a combination of IBA and BAP at concentrations of 400ppm and 125ppm, respectively, growth was observed in 3 out of 5 flasks, indicating a 60% success rate with a total plant regeneration growth length of 3 cms (figure 1). Treatment A-2, featuring IBA and BAP at concentrations of 650ppm and 175ppm, respectively, exhibited growth in 4 out of 5 flasks, showcasing an 80% success rate with a total growth length of 4 cms. Conversely, Treatment A-3, incorporating IBA and BAP at

concentrations of 900ppm and 225ppm, respectively, displayed growth in 2 out of 5 flasks, resulting in a 40% success rate with a total growth length of 2 cms (figure 1). Notably, the Control group (Treatment A) devoid of hormones showed minimal growth, with only 1 out of 5 flasks exhibiting growth, achieving a 20% success rate with a total growth length of 1 cm [17].

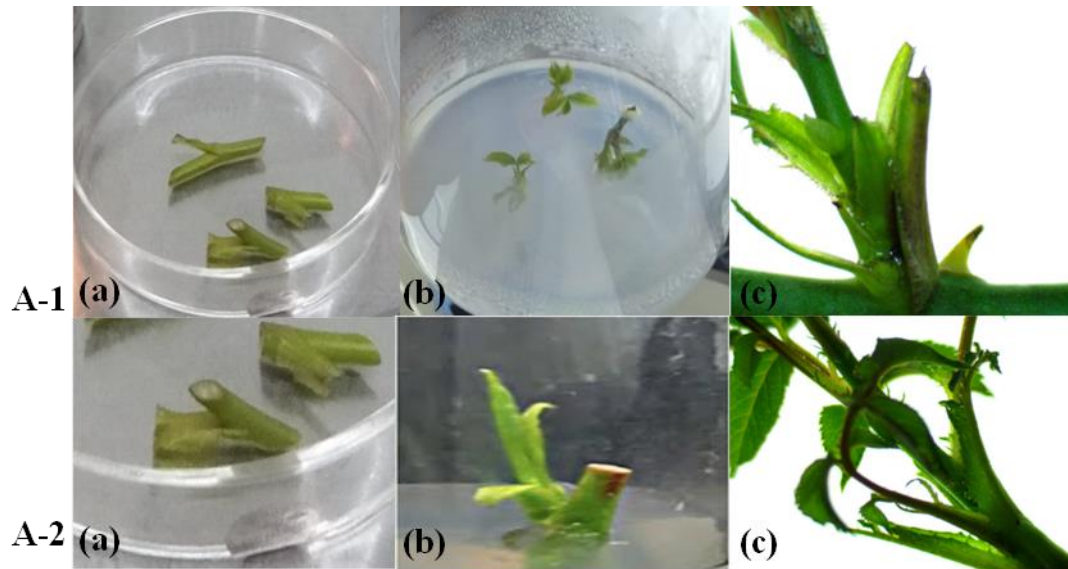


Figure 1. Plant growth regeneration in A-1 and A-2 treated explants: (a) Explant on day 1; (b) Growth after 15 days; (c) Growth after 30 days.

This detailed assessment demonstrates the varying effects of different hormone concentrations on the growth and regeneration potential of nodal explants in *R. hybrida* L., illustrating the significance of hormone ratios in influencing successful plant regeneration.

The resulting growth statistics, encapsulating the observations made in media fortified with varying hormone ratios, are succinctly presented in Table 1.

Table 1. Showing the effect of IBA and BAP-containing media with ascorbic acid on the growth of nodal explants of *Rosa hybrida* L.

Treatment	Hormones	Concentration		Number of flasks showing growth	Rate of growth	Total plant regeneration growth length in cms
A-1	IBA+BAP	400ppm+125ppm		3	60%	3
A-2	IBA+BAP	650ppm+ 175ppm		4	80%	4
A-3	IBA+BAP	900ppm+ 225ppm		2	40%	2
A	Control	-		1	20%	1

Effect of NAA and BAP-enriched media with charcoal on the growth of nodal explants in *Rosa hybrida* L. The evaluation of NAA and BAP-containing media, supplemented with charcoal, on the growth of nodal explants of *R. hybrida* L., involved several treatments. In Treatment B-1, featuring NAA and BAP at concentrations of 90ppm and 900ppm, respectively, growth was observed in 2 out of 5 flasks, representing a 40% success rate, with a total plant regeneration growth length of 4 cms (figure. 2).

Contrastingly, Treatment B-2, incorporating NAA and BAP at concentrations of 45ppm and 450ppm respectively, exhibited growth in 1 out of 5 flasks, resulting in a 20% success rate with a total growth length of 2 cms (figure. 3). Notably, the Control group (Treatment B), which lacked hormones, displayed no growth in any of the 5 flasks, yielding a 0% success rate.

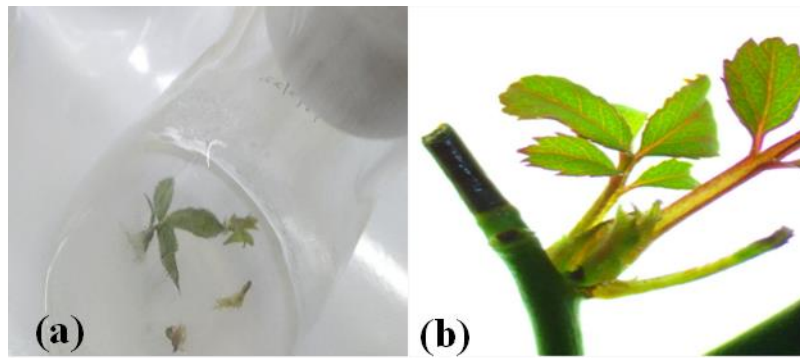


Figure 2. Plant growth regeneration in A-3 treated explant: (a) Explant after 15 days; (b) Explant after 30 days.

This comprehensive evaluation sheds light on the influence of varying concentrations of NAA and BAP in media supplemented with charcoal, showcasing their impact on nodal explants' growth and regeneration potential in *R. hybrida L* [17]. The resulting growth statistics, encapsulating the observations made in media fortified with varying hormone ratios, are succinctly presented in Table 2.

Table 2. Showing the effect of NAA and BAP-containing media with charcoal-containing media on the growth of nodal explants of *Rosa hybrida L*.

Treatment	Hormones	Concentration	Number of flasks showing growth	Rate of growth	Total plant regeneration growth length in cms
B-1	NAA+BAP	90 ppm+ 900ppm	2	40%	4
B-2	NAA+BAP	45 ppm+ 450ppm	1	20%	2
B	Control	-	0	0%	No growth

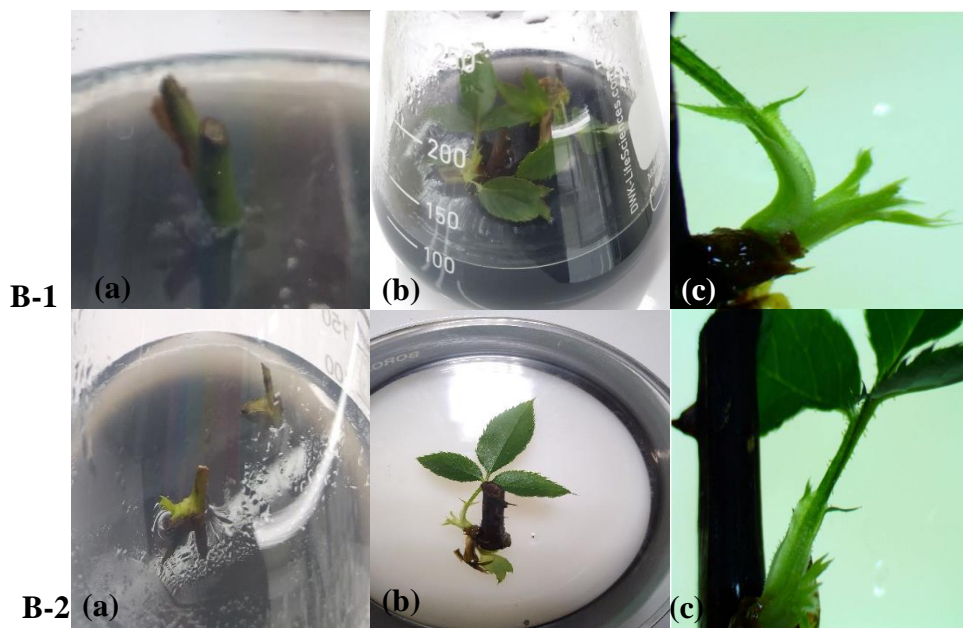


Figure 3. Plant growth regeneration in B-1 and B-2 treated explants: (a) Explant on Day 1; (b) Explant after 15 days; (c) Explant after 30 Days.

So, the above-discussed finding suggested that among the five media compositions used to study the potential of phytohormones, we observed that the combination of indole butyric acid and benzylaminopurine have more potential to induce efficient and faster regeneration in nodal explant than the phytohormone combination of naphthalene acetic acid and benzylaminopurine. It is also found that the (A-2 Basal MS media) having an IBA concentration of 650 ppm and BAP concentration of 175 ppm, shows better shoot

regeneration potential in Nodal explants of *R. indica* L. under controlled lab conditions. Therefore, it is also suggested that a lesser amount or concentration of BAP (175 ppm) used in combination with IBA has more potential for explant regeneration than a higher amount of BAP (900 ppm) used with NAA. In the future, we will try to find out whether this type of media formulation also has better growth regeneration potential in other flowering/ornamental plants or not.

4. Conclusions

The current study aimed to ascertain the effects of varying phytohormone compositions and concentrations on the growth and regeneration of nodal explants of *R. hybrida* L., as well as to optimize the culture conditions for *in vitro* micropropagation of the plant from the nodal segment. To achieve this goal, different concentrations of growth hormones IBA, BAP, and NAA were used in combination with one another. Out of the five media compositions used in the study, we observed that the phytohormone combination of IBA + BAP was found to be more potent in inducing quick and efficient regeneration in nodal explants than the BAP + NAA. The lesser amount or concentration of BAP (175 ppm) used in combination with IBA has more potential for explant regeneration than the higher amount of BAP (900 ppm) used with NAA. Among all the combinations used for the treatment of explants, A-2 Basal Media + IBA (650 ppm) + BAP (175 ppm) shows the best results. So, from this experiment, it was concluded that the A-2 media hormone combination of indole butyric acid and benzyl amino urea and their concentrations increase the nodal explant's proliferation rate in aseptic conditions.

Author Contributions

Conceptualization, F.H.N. and U.S.; methodology, F.H.N.; original draft preparation, F.H.N., P.S., P.K., and S.S.; F.H.N.; D.J. revised the manuscript thoroughly and finalized it. All authors have read and agreed to the published version of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

Abbreviation	Definition
Ppm	parts per million
g/l	grams per liter
mg/l	milligrams per liter
PVP	polyvinylpyrrolidone
AgNO ₃	Silver nitrate
HCl	Hydro chloride
NaOH	Sodium Hydroxide

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