



Role of BAP in *In-vitro* Conditions for Promoting Shoot Growth in the Nodal Segment of *Rosa indica* L.

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Rosa indica L. is the world's most popular ornamental flowering plant. Since the last two decades, rose tissue culture has been enhanced and used for a variety of objectives, ranging from fundamental anatomical and physiological studies to micropropagation from auxiliary buds, shoot tips, leaf explants, and so on. In *in vitro* conditions, surface sterilized nodal segments of *Rosa indica* L. were cultivated in MS media supplemented with 30 g/l sugar as a carbon source, 6 g/l agar as a solidifying agent, and various concentrations of BAP (1, 2 and 3 mg/l). Maximum growth of shoots in nodal segments of *Rosa indica* L. was observed on MS medium having 2.0 mg/l of BAP concentration as compared to other concentrations. The results showed that BAP was considered to be the best phytohormone for *in vitro* shoot multiplication and proliferation of *Rosa indica* L.

Keywords: *Rosa indica* L.; micropropagation; nodal segment; BAP.

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1. INTRODUCTION

A Rose is a perennial plant of the genus *Rosa* in the Rosaceae family. There are over 100 different species and 2000 herbaceous to woody plant species. They are a collection of tall shrubs and climbing or trailing plants with stinging prickles on their stems. Flowers are enormous and spectacular, and come in a variety of hues ranging from white to yellow and red. Roses may be referred to as therapeutic herbs due of their medicinal qualities. A rose plant's petals, stem, leaves, roots, and hip all contain different secondary metabolites and nutrition in the form of vitamins and minerals. There have also been reports of significant antibacterial and antifungal activity in extracts from various rose plant parts. There exist three primary cultivars cultivated for commercial purposes, specifically for the synthesis of rose essential oil and rose water [1]. The majority of species are found in Asia, with a minor number found in Europe, North America, and Northwest Africa. For its beauty and smell, species, cultivars, and hybrids are all commonly produced. Rose bushes differ in size from small, miniature roses to climbers that can grow to be 7 metres tall. Species from all around the world easily hybridize, giving rise to the many diverse forms of garden roses [2].

"*Rosa indica* L. is a plant of lovely flower with significant horticultural value. It may be employed in a variety of sectors, including medicine, ceremonies, and social gatherings. Eventually, 20000 rose varieties emerged as a result of crop improvement programmes such as selection and hybridization. It is produced as cut flowers and potted plants in home gardens all over the world. According to world health organization (WHO) any plant having substances that can be used for therapeutic healing of chemo pharmaceutical semisynthetic new drug is reoffered as medicinal plant" [3]. "Disease susceptibility, such as bacterial blight, black spot, and powdery mildew, is a major limitation in Rose output. The in vitro culture technique is an alternate way for plant multiplication; millions of plants must be seeded each year. There are a few studies on the effect of growth regulators and culture environment on shoot multiplication of commercial rose cultivars" [4].

"Plant growth regulators play a role in determining the development of plant cells in

micro-propagation. Cytokinins and auxins are the most commonly used plant growth regulators in tissue culture, with a wide range of these regulators, both natural and synthetic, and a wide range of responses depending on the type used" [5]. "The cytokinins promote amplification of the photosynthetic apparatus of dappled plants, resulting in chloroplasts with larger granular systems and greater accumulation of chlorophylls and photosynthetic enzymes, implying that cytokinins, along with other factors such as light and plant nutrition, regulate the synthesis of pigments and proteins" [6]. "Cytokinins, in conjunction with auxins, regulate the rate of cell division during leaf development. Cytokinin activity predominates during the early stages of foliar ontogeny, determining the maximum rate of chloroplast and cell division, membrane formation, and protein synthesis. Following this linear growth phase, there is a decrease in cytokinin activity and an increase in auxin activity, which stimulates mesophyll cell lengthening" [7].

"Cytokinins such as 6-benzylaminopurine (BAP) and kinetin are usually used to reduce apical dominance and induce the meristematic form and stimulate sprouts in banana meristematic explants" [8]. "BAP strongly stimulates growth of axillary, adventitious and foliar buds" [9]. "Although BAP stimulates the shoot proliferation in bananas, if used in high concentrations it can cause high somaclonal variation rates becoming unviable its use for in vitro seedlings production" [10].

The objective of the present study was to establish an efficient protocol on micropropagation by manipulating growth regulators and culture condition.

2. MATERIALS AND METHODS

2.1 Plant Material

Rosa indica L. branches in order to use as explants were collected from college garden of DUVASU, Mathura. Nodal explants (3-4cm in size) was selected from the healthy mother plant of *Rosa indica* L. and used as plant material for analyzing shoot growth at the different concentrations of BAP (6- Benzyl amino purine) through micropropagation.



Fig. 1. *Rosa indica* L. in garden of College of Biotechnology, COB, DUVASU, Mathura

2.2 Sterilization Technique

The collected explants were initially washed with tap water for 10 minutes to remove dust particles. After washing with the tap water the nodal segments of *Rosa indica* L. were treated with the liquid detergents (Tween-20) for 5 minutes and then washed with sterilized distilled water 2-3 times to remove the residues of Tween 20. After that nodal segments were transferred to 20% solution of sodium hypochlorite for 5 minutes. Further, the explants were treated with 70% ethanol for 25– 30 seconds and washed with sterile water two to three times. Finally, 0.01 % of mercuric chloride was treated for 1-2 minutes and finally washed with sterile distilled water two to three times to remove traces of mercuric chloride. Now nodal segments of *Rosa indica* L. were used to culture [11].

2.3 Media Preparation

Culture Media Preparation used in this work is Murashige and Skoog's medium (MS medium) in semisolid form. All the required chemicals were added in sterilized distilled water. Agar was used as solidify agent to the media. The pH of the media was maintained to be 5.8 by using 1N NaOH and boil the content until it attains homogeneity. In addition, different concentrations of BAP as 1mg/lit, 2mg/lit & 3mg/lit were supplemented with the basal MS medium. "The MS culture medium was modified by the reduction of KNO_3 and NH_4NO_3 salts by $\frac{1}{4}$ and

$CaCl_2$ increased two-fold from the original concentration of MS, maintaining 3% sucrose, myo-inositol 0.1 g L^{-1} , and 0.5 mg L^{-1} Benziladenine (BA). There were observed that reduction of nitrogen concentration and increased Calcium could present benefits to in vitro plant development of some woody species" [12,13]. All phytajars were placed in a culture room under a white fluorescent light with a brightness of 2000 Lux. The incubation temperature was set to $25^\circ\text{C} \pm 2$ with an 18/6 h light/dark cycle [14]. The data was taken for shoot initiation on initial cultures after 4 weeks of cultures and for root induction after 8-12 week of culture period.

3. RESULTS AND DISCUSSION

An axillary bud is a successful explant in the in vitro propagation of roses. The most difficult step in the successful establishment of cultures is surface sterilization of explants, which comes after the preparation of explants for micropropagation [15].

The percentage of shoot growth was varied among the different concentration of BAP (6-Benzyl Amino Purine) as shown in Table 1. The development of axillary shoot from the nodal explants was observed after 7 to 25 days. Contamination was also observed during the culture. The presence of cytokinin in the culture medium helped in the multiplication of shoots in rose and auxin help in multiplication of roots.

Table 1. Observation of growth in explants of *Rosa indica* L. (BAP control, 1 mg/L, 2 mg/L, 3 mg/L)

Days of observation	No. of Phytajar showing infection	Observation (growth of explants)	Shoot growth
7 days	0	Axillary shoot initiation	4
10days	0	Shoot growth	4
15days	0	Shoot growth	4
25 days	3	Newly developed leaf	3

Table 2. Shoot proliferation of *Rosa indica* L. at various concentration of BAP

Concentration of BAP (mg/l)	No. of explants (per culture)	No. of explants forming shoots (%)
Control	2	45%
1mg/L	2	75%
2mg/L	2	95%
3mg/L	2	65%

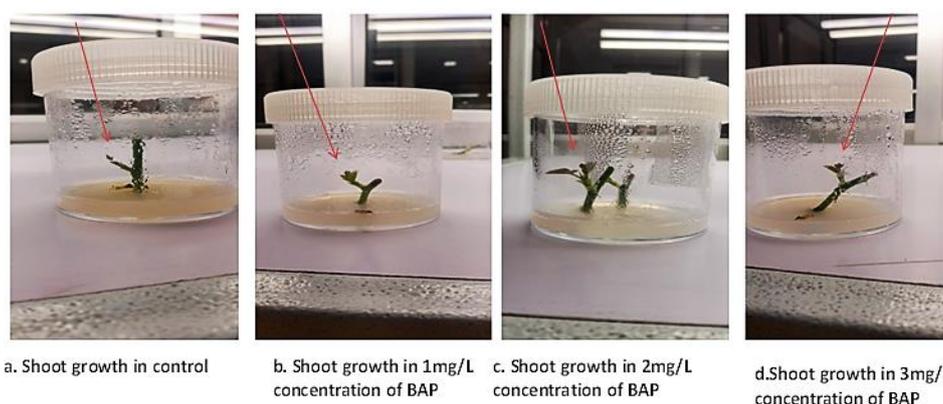


Fig. 2. After 25 days shoot growth in explants of *Rosa indica* L. at different concentrations of BAP (control, 1mg/L, 2mg/L, 3 mg/L)

Nodal explants of *Rosa indica* L. were seen to develop shoots at varying concentrations of BAP (6-Benzyl Amino Purine). After 7 days of culture, shoots began to grow on the nodal side of the explants. Table 2 shows the concentrations at which the largest proportion of explants developing shoots (95%) was observed: 1mg/l (75%), 2 mg/l (95%), and 3 mg/l (65%). At control concentration, (45%) shoot development was seen (Fig. 2). In order to choose for the culturing of nodal explants in order to produce a large number of shoots, the concentration of BAP (6-Benzyl Amino Purine) with MS media at 2 mg/l was therefore the best for multiplication. In the experiment, nodal explants with lateral buds grown on MS media produced shoots after 25 days of initial cultivation. So from above findings it can be concluded that BAP worked as the best growth regulator for promoting shoot proliferation in various plant species [16,17,18].

4. CONCLUSION

In the present study, the shoot proliferation and development shows better response at phytohormone concentration (BAP) at 2 mg/l. So this phytohormone concentration should be used to be followed in micropropagation culture protocol for *Rosa indica* L. In developed nations like India, protocol optimization for medicinal plants has generally made good progress.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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