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# Micropropagation from nodal explants of rose (*Rosa hybrida* L.) at different concentration of BAP (6-Benzyl Amino Purine)

**Dipti Shradha Tirkey, Diwakar Prasad Nirala and Phallo Kumari**

**Abstract**
The nodal explants from young healthy shoots were cultured on basal medium of BAP (6-Benzyl Amino Purine) containing several concentrations. It was reported as most effective growth regulator in stimulating shoot proliferation of many plant species. The aim of the study was to analyze the effectiveness of different concentration of BAP (6-Benzyl Amino Purine) in micropropagation of rose (*Rosa hybrida* L.). The maximum percentage of number of explants forming shoots (95%) was observed in 3 mg/l concentration of BAP (6-Benzyl Amino Purine). The shoot elongation was considerably slow. Findings could be utilized as an efficient tissue culture technique to yield large number of shoots from nodal explants of roses.

**Keywords:** Rose, Explants, BAP, Shoots etc.

## Introduction
Rose is one of the most important commercial crops of genus *Rosa* that belongs to family Rosaceae. Roses have been one of the world's most popular ornamental plants for a long time. They are grown worldwide as cut flowers and potted plants and in home gardens. The flowers vary greatly in size, shape and colour. It is generally propagated by vegetative methods like cutting, layering, budding and grafting. Although propagation by vegetative means is a predominant technique in roses, yet it does not ensure healthy and disease-free plants. Moreover, dependence on season and slow multiplication rates are some of the other major limiting factors in conventional propagation. Significant features of in vitro propagation procedure are its enormous multiplicative capacity in a relatively short span of time; production of healthy and disease free plants; and its ability to generate propagules around the year (Bhojwani, 1981) [1]. Tissue culture derived dwarf roses used for pot plant production have a faster rate of growth, early flowering, and exhibit shorter shoots and more laterals than conventionally produced plants.

## Materials and Methods
### Plant materials
Nodal explants containing lateral buds of actively field-grown *Rosa hybrid L. var. 'Perfume Delight'* rose were used for multiplication experiments. Each node contains an area of meristematic tissue called an axillary bud. They were cut in 3-4 cm length segments.

### Explants Sterilization
For the sterilization, the explants were first washed thoroughly in running tap water for 10-15

minutes. After that they were again washed with liquid detergent. After washing with detergent, explants were again washed with running water to remove traces of detergent for 10-15 minutes. These washing explants were dipped in 70% ethyl alcohol for 30 seconds. After alcohol dip, explants were surface sterilized with freshly prepared 0.1% Mercuric chloride ($HgCl_2$) for 5 minutes. After that, explants were thoroughly washed for 3-4 times with sterile water to remove any traces of mercuric chloride.

**Culture Media**

Murashige and Skoog's (1962) [7] medium (MS) was used for rose propagation in semi solid form.

**Composition of MS**

| **Macronutrients** | | **Micronutrients** | |
|---|---|---|---|
| $MgSO_47H_2O$ | 370 mg/l | $AlCl_2$ | 0.025 mg/l |
| $KH_2PO_4$ | 170 mg/l | $H_3BO_3$ | 6.2 mg/l |
| $KNO_3$ | 1900 mg/l | $MnSO_44H_2O$ | 22.3 mg/l |
| $NH_4NO_3$ | 1650 mg/l | $ZnSO_47H_2O$ | 8.6 mg/l |
| $CaCl_2$ | 440 mg/l | $Na_2MoO_42H_2O$ | 0.25 mg/l |
| **Vitamins** | | $CuSO_45H_2O$ | 0.0025 mg/l |
| Thiamine HCl | 0.1 mg/l | KI | 0.83 mg/l |
| Pyridoxine | 0.5 mg/l | $FeSO_47H_2O$ | 27.85 mg/l |
| Myoinositol | 100 mg/l | $Na_2EDTA.2H_2O$ | 37.25 mg/l |
| Glycine | 2 mg/l | **Chemicals required** | |
| Thiamine HCl | 0.1 mg/l | BAP (6-Benzyl Amino Purine) | |
| **Carbon source** | | Coconut milk | |
| Sucrose | 30 m/l | | |

**Final Preparation of semi solid media:** All the chemicals were added in distilled water. Media are made semi solid by using solidifying agent agar-agar powder (8%) was added in the liquid media and pH (5.8) was checked. It was boiled till the homogenous was obtained. Finally, the media was sterilized by Millipore filter.

**Lab instruments**

**a) Laminar air flow** – It is the most suitable, convenient and reliable instrument for aseptic work. All contaminants are blown away by the ultraclean air and thereby an aseptic environment is maintained over the working area.

**b) pH meter**- It is necessary for the measurement and adjustment of pH of the nutrient medium.

**c) Autoclave**- It is very important for sterilization of nutrient media, glass goods, instruments etc.

**d) Hot air oven**- It is necessary for drying the washed glass goods.

**Lab goods**: Test tubes, Measuring cylinder, Conical flasks, Pipettes, Beaker, Petri dishes were well washed and then dried in hot air oven. Scalpel, Forceps, Scissors, Cotton plugs (wrapped in gauge cloth) were also used during the micropropagation.

All the equipments were first sterilized in autoclave under steam at a pressure of 15lb/in² and a temperature of 120˚C for 15 minutes. The semisolid media (Murashige and Skoog, 1962) [7] was used with BAP and coconut milk. Explants (*Rosa hybrida* L.) were cultured to the sterilized medium and culture tubes were capped with sterilized cotton plugs. Inoculation is done under laminar air flow very carefully. All the equipments are used is sterilised. The explants are inoculated in semisolid media. After inoculation, culture tubes are kept in the culture room and observed every day. Cultures were maintained at 25 ± 3˚C air temperatures in a culture room. After 2 weeks the explants were examined for axillary shoot growth.

**Results and Discussions**

**Observation**

The percentage shoot formation 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 9 to 14 days. There infections were also observed during the culture. The presence of cytokinin in the culture medium helped in the multiplication of shoots in hybrid roses and auxin help in multiplication of roots.

**Table 1:** Observation of growth of explants (BAP conc. 3mg/l)

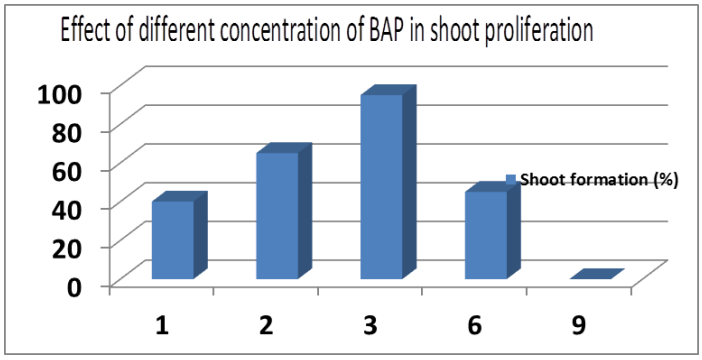
| Days of observation | No. of cultures | No. of culture showing infection | Observation (growth of explants) | Callus | Shoot growth |
|---|---|---|---|---|---|
| 3 days | 20 | 0 | No growth | 0 | 0 |
| 1 week | 20 | 4 | No growth | 0 | 0 |
| 10 days | 16 | 4 | Callusing | 0 | 0 |
| 2 week | 12 | 0 | Axillary shoot growth | 9 | 9 |
| 20 days | 12 | 0 | Shoot growth | 11 | 11 |
| 3 weeks | 12 | 0 | Shoot developed | 12 | 12 |

**Table 2:** Effects of different concentration of BAP in the shoot proliferation

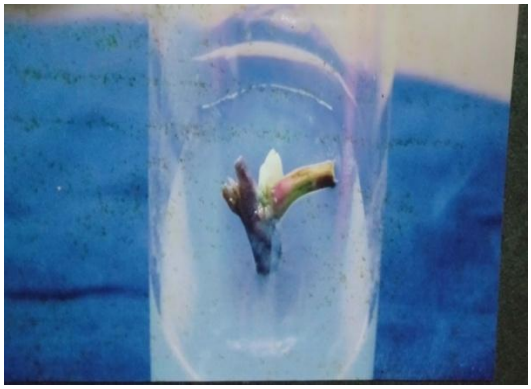
| Concentration of BAP (mg/l) | No. of cultures | No. of explants | No. of explants forming shoots (%) |
|---|---|---|---|
| 1 | 20 | 20 | 40% |
| 2 | 20 | 20 | 65% |
| 3 | 20 | 20 | 95% |
| 6 | 20 | 20 | 45% |
| 9 | 20 | 20 | 0% |

The formation of shoots was observed from nodal explants of Rose (*Rosa hybrida* L.) at different concentration of BAP (6-Benzyl Amino Purine). Shoots formation were taken place from nodal side of explants after 20 days of culture. The maximum percentage of number of explants forming shoots (95%) was observed in the concentration of 3 mg/l followed by 2 mg/l (65%), 6 mg/l (45%), and 1 mg/l (40%) as shown in Table-2. No any shoot formation was observed in the concentration of 9 mg/l. Thus the concentration 3 mg/l of BAP (6-Benzyl Amino Purine) with MS media was best for multiplication and it was selecting for culturing of nodal explants for production of large number of shoots. Tissue culture system in roses has been established (Hsia and

Korban, 1996; Ibrahim and Debergh, 2001; Kim *et al*., 2003; Hameed *et al*., 2006; Drefahl *et al*., 2007) [4, 5, 6, 3, 2].




After 3 weeks of initial culture, nodal explants containing lateral buds cultured on MS medium in the experiment developed shoots. BAP was the most effective growth regulator in stimulating shoot proliferation.



**Initial stage callusing**

**Callusing**

**Growth- emergence of shoot**

**Emergence of multiple shoots (20 days)**

**Growth after 30 days**

**Growth after 36 days**

## Conclusion

From the above results, it can be concluded that the maximum percentage of number of explants forming shoots (95%) was observed in 3 mg/l concentration of BAP (6-Benzyl Amino Purine). It was the most effective concentration for growth regulator in stimulating shoot proliferation.

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