

## Original Research Article

# Standardization of *In vitro* seed culture technique for Six Rose varieties

### ABSTRACT

Research was conducted at Biotechnology-cum-Tissue Culture Centre under Department of Floriculture and Landscaping, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, during the year 2021-2022 to rescue the embryo of rose seeds in Six Rose varieties like Pusa Baramasi, Gold Strike, Ravel, Sadabahar, Summer Fragrance and Orange Sensation with the objectives to study the effect and efficacy of plant bioregulators on *in vitro* germination of rose seeds. The *in vitro* experiment was laid out in Completely Randomized Design (CRD) with 8 treatments and 3 replications by using different combination of BAP, GA<sub>3</sub> and AdS in MS media. The results of the study revealed that seeds of the rose variety Pusa Baramasi was the best explants for the experiment. The MS medium when supplemented with treatment T<sub>5</sub> i.e., {BAP (2.5 mg/l) + GA<sub>3</sub> (0.5 mg/l) + AdS (50 mg/l)} resulted in early shoot initiation in Pusa Baramasi (7.15 days) followed by Ravel (11.63 days), Gold Strike (11.89 days), Sadabahar (12.38 days), Summer Fragrance (12.95 days) and Orange Sensation (13.32 days). Maximum available days for shoot development was also observed in Pusa Baramasi (36.02 days) when MS medium supplemented with same concentration of BAP, GA<sub>3</sub> and AdS followed by Ravel (18.17 days), Gold Strike (17.72 days), Sadabahar (17.19 days), Summer Fragrance (16.53 days) and Orange Sensation (15.85 days) in the treatment T<sub>5</sub> significantly delayed the cessation of the growth in Pusa Baramasi (43.17 days) followed by Ravel (29.79 days), Gold Strike (29.61 days), Sadabahar (29.57 days), Summer Fragrance (29.48 days) and Orange Sensation (29.71 days) and produced maximum number of leaves in Pusa Baramasi (3.40 leaves) followed by Ravel (1.51 leaves), Gold Strike (1.50 leaves), Sadabahar (1.23 leaves), Summer Fragrance (1.07 leaves) and Orange Sensation (0.92 leaves). Pusa Baramasi showed light green leaves with the treatment T<sub>4</sub> {BAP (2.0 mg/l) + GA<sub>3</sub> (0.5 mg/l) + AdS (50 mg/l)} and T<sub>5</sub> {BAP (2.5 mg/l) + GA<sub>3</sub> (0.5 mg/l) + AdS (50 mg/l)} and other treatments showed whitish green leaves. Ravel, Gold Strike and Sadabahar varieties also showed light green leaves when MS medium was supplemented with treatment T<sub>5</sub> and rest of the treatments showed whitish green colour leaves. Summer Fragrance and Orange sensation showed whitish green colour leaves in all the treatments. Early callus initiation was observed in Pusa Baramasi (12.53 days) followed by Ravel (16.59 days), Gold Strike (17.69 days), Sadabahar (18.26 days), Summer Fragrance (18.48 days), Orange Sensation (19.21 days) and Pusa Baramasi showed light green callus when MS medium was supplemented with the treatment T<sub>5</sub> and other treatments showed whitish green callus. Ravel, Gold Strike, Sadabahar, Summer Fragrance and Orange sensation also showed whitish green colour callus in all the treatments.

**Keywords:** Callus, *In vitro*, Seed culture, Embryo rescue, Shoot initiation, MS medium, Rose varieties, Pusa Baramasi

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## 1. INTRODUCTION

Rose (*Rosa spp.*) is known as the 'Queen of Flowers' and is one of nature's most beautiful creations. It belongs to the family Rosaceae. Rose is England's national flower, Alberta's provincial flower and the state flower of Iowa, North Dakota, Georgia and New York. It has become an integral part of life, intertwined with all stages of life from birth to death. The cultivation of this lovely flower in India began around 100 A.D with the distillation of roses, as mentioned in Ayurveda by Charaka.

Rose is now a popular ornamental shrub used for landscaping as well as the most important commercial cut flower. Its beautiful flowers are of exquisite shape, various sizes, available in bewitching colours and its delightful fragrance has made it an important flower which is used for various purposes. Rose is a primary raw material used in the extraction of rose water, rose oil, gulkand and other products. Rose water has been valued from ancient times for use in making sherbets and for various medicinal purposes. Rose water is still used in eye-lotions and eye-drops for its soothing properties. Rose water is sprinkled on guests at weddings and other social functions and it is also used in drinking water. Rose oil (*otto of roses*) has medicinal properties and is frequently used in Ayurvedic medicine. It is primarily used in the production of perfumes, soaps and cosmetics. Rose oil is also used to flavour soft drinks and alcoholic beverages in small quantities. Gulkand is considered as a laxative and a tonic. For making cold beverages in the summer, dried rose petals known as Pankhuri are utilized.

Rose is a flower without which love's emotions, a poet's thoughts and a writer's words are incomplete. There are numerous places where roses are grown. Rose is renowned for its capacity to survive a wide range of environmental conditions. Low yields are the result of rose culture being somewhat neglected and lack of awareness with respect to its cultivation. With these concerns in mind, it is necessary to implement some appropriate production technology that will allow farmers to receive the required information regarding the scope and methods of rose cultivation.

India ranked top in terms of area and production of cut roses, but only exported 0.20 to 0.40 percent of the world's total exports. India, China, Ecuador, Colombia, Kenya, Mexico, Italy, Thailand, Japan, and the Netherlands are the top 10 producers of cut roses worldwide. India takes the top position which supplies 46.54 percent of the world's area planted with cut roses. India is contributing 0.20 percent of the global export of cut roses and is placed on 15th position among top rose producing countries.

In India, the rose was referred in old Sanskrit literature as Tarunipushpa, Atimanjula, Simantika etc. and was used for adorning not just royal palaces but also saints' ashrams (Rode and Ogale, 1984). Rose cultivation elicits a wide range of surprising attributes from the grower, including the skill of the artisan, the creative vision of the artist, the perseverance of the lover and the trained eye of persistent observation.

Despite the fact that *in vitro* germination is frequently used to assess the ideal pollination conditions, particularly temperature, in certain other species (Yates and Sparks, 1989), data on roses are few (Gudin *et al.*, 1991). The expansion of the pollen tube after a certain point depends on the nutrients present in the pistil. The most crucial component is sucrose, which serves as a nutrition for the formation of the pollen tube and maintains the osmotic pressure of the pollen grain. Rose breeding began long before the nature of genes and the laws of Mendel were understood.

The primary goals of modern rose breeding have been to create new and novel rose varieties with vigorous growth, recurrent flowering, exquisite flower colour, shape, size, fragrance, floriferousness, winter hardiness, resistance to diseases and pests and good keeping quality. Amateur and professional rose breeders as well as various institutions in the country and abroad, have been working for many years to achieve these goals. However, rose breeders have faced a significant threat to their hybridization programme due to high pollen sterility, poor hip setting resulting in poor and irregular seed germination. It is a reality

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that rose's true genetic potential has not yet been completely realized. Modern rose cultivars are normally associated with variable and often low fertility which could be due to their interspecific origin as well as intensive inbreeding (Pipino *et al.*, 2011).

Every year, about 8 billion rose stems, 80 million potted rose plants and 220 million garden rose plants are sold. In India, flower cultivation covers approximately 1,67,000 ha, with a total production of 9.87 lakh metric tonnes of loose flowers and 4794 million cut flowers. With the advent of modern technologies, the area under rose cultivation in India has increased. Karnataka, Tamil Nadu, Maharashtra, West Bengal, Andhra Pradesh and Kerala are important rose growing states in India.

In roses the swollen receptacle is known as the hip and the fruit consisting of the seed and pericarp wrapping is called the achene. An achene is a one seeded fruit consists of a mature embryo which is protected by a seed coat called the testa to produce the actual seed that is surrounded by the pericarp made of an epicarp, woody and fibrous layers. The ring fibers are the innermost part of the fibrous layer. The pericarp surrounds the embryo from that portion it will grow into a plant.

Inside the pericarp, the embryo is wrapped in a tough, thin sack called the testa. The testa is generally brown or tan. The embryo inside is white if only it is viable. The testa has two layers from which ABA is formed. The pericarp is not easily removed and most of the inhibitor chemicals are present in the testa. So, it must be removed for the embryo to germinate.

Big seeds usually have thicker pericarps than small seeds and required more effort to open them. But the smaller seeds have thinner pericarp so can be more easily damaged. Seeds that have been thoroughly desiccated are most likely to contain viable embryos after freezing than those with higher moisture content.

Roses are propagated commercially by T-budding and nowadays by stenting but these methods are laborious, time consuming and more costly. Thus, this research was carried out to develop a protocol for mass propagation of rose seedlings through *in vitro* culture of achenes obtained from the rose hips by using MS media and specific type of plant bioregulators with objectives like effect of Plant Bioregulators on *in vitro* culture establishment of rose seeds and efficacy of Plant Bioregulators on *in vitro* germination of rose seeds.

## **2. METHODOLOGY**

### **2.1 Experimental Site**

Research was carried out in the Tissue Culture Laboratory of the Biotechnology - cum-Tissue Culture Centre, Baramunda, Department of Floriculture and Landscaping, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar during 2021-2022.

### **2.2 Plant Material**

The explants required for the study were collected from the rosarium of AICRP on Floriculture at Biotechnology-cum-Tissue Culture Centre

### **2.3 Explants**

The explants used in the present study were achenes obtained from hips of rose plants. Explants from the cultivars Summer Fragrance, Ravel, Orange Sensation, Pusa Baramasi, Gold Strike and Sadabahar were collected from the rose mother block maintained at Biotechnology-cum-Tissue Culture Centre, Bhubaneswar.

### **2.4 Source of Chemicals**

The chemicals and Plant Bioregulators used for the study mainly consisted of MS media (with  $\text{CaCl}_2$  & vitamins; without sucrose & agar powder), plant growth hormones (BAP,

NAA, AdS, Kinetin, TDZ, GA<sub>3</sub> and IBA), sucrose and agar-agar, which were of Analytical grade and were procured from Hi-Media Laboratories Pvt. Ltd., India.

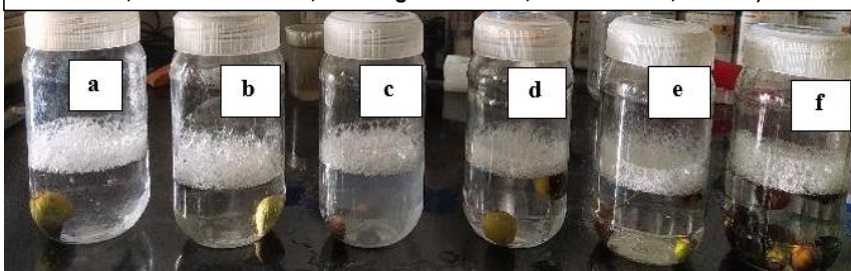
## 2.5 Varietal Performances

**Table 1. Hip characters of seed setting rose varieties**

Name of Variety	Weight of Hips (g)		Width of Hips (cm)		Length of Hips (cm)		Maturity of Hips (Days)	Number of Seeds/hips
	Range	Mean	Range	Mean	Range	Mean	Mean	Mean
<b>Summer Fragrance</b>	3.28-6.22	4.75	1.95-2.71	2.33	2.92-4.12	3.52	156.66	11.24
<b>Ravel</b>	2.65-3.40	3.02	1.64-1.92	1.78	2.42-2.69	2.56	126.33	8.45
<b>Orange Sensation</b>	3.81-5.22	4.51	2.17-2.43	2.30	3.06-3.83	3.45	121.37	10.53
<b>Pusa Baramasi</b>	2.52-4.11	3.32	1.27-1.68	1.47	2.12-2.48	2.30	112.33	9.13
<b>Gold Strike</b>	2.42-4.26	3.34	1.34-1.56	1.45	2.23-2.41	2.32	121.12	8.21
<b>Sadabahar</b>	3.28-4.86	4.07	1.83-2.19	2.01	2.48-2.73	2.61	131.24	10.42

	
<p><b>Fig 1. Rose cv. Summer Fragrance</b></p>	<p><b>Fig 2. Rose cv. Ravel</b></p>
	
<p><b>Fig 3. Rose cv. Orange Sensation</b></p>	<p><b>Fig 4. Rose cv. Pusa Baramasi</b></p>
	
<p><b>Fig 5. Rose cv. Gold Strike</b></p>	<p><b>Fig 6. Rose cv. Sadabahar</b></p>

**Fig 7. Sterilization of explants in 0.02% Bavistin (a. Summer Fragrance, b. Sadabahar, c. Pusa Baramasi, d. Orange Sensation, e. Gold Strike, f. Ravel)**



## **2.6 Execution of Experiment**

### **2.6.1 Nutrient MS media formulation**

The formulation of MS medium (Murashige and Skoog, 1962) was used as a basal medium for the entire study and composition of the same is given below. Inorganic and organic constituents of MS medium were used. However  $(\text{NH}_4)_2\text{SO}_4$  is used in place of  $\text{NH}_4\text{NO}_3$ , hence it is modified MS medium for macro nutrients.

### **2.6.2 Preparation of stock solution**

The stock solution for macro and micro nutrients were prepared separately by carefully dissolving the above-mentioned chemicals in sterile distilled water against each stock. During the media preparation  $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$ ,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , Myo- Inositol and Sucrose were added immediately. Fresh stock solution of vitamins such as Glycine (200 mg), Nicotinic acid (50mg), Pyridoxine-HCl (50mg) and Thiamine- HCl (10 mg) were

separately dissolved in 100 ml of sterile distilled water and during media preparation, 1 ml from each solution was added to the media. The prepared stock solution was stored in the refrigerator at a low temperature ranging from 4°C to 20°C.

#### **2.6.3 Preparation of stock solution for Plant Growth Regulators (PGRS)**

(i) **GA<sub>3</sub>**: GA<sub>3</sub> (50 ppm) solution was prepared by dissolving 50 mg of GA<sub>3</sub> in a little quantity of ethanol and the volume is made up to 1000 ml with distilled water. GA<sub>3</sub> (25 ppm) solution was prepared in similar method by taking 25 mg GA<sub>3</sub> instead of 50 mg.

(ii) **BAP** (Benzyl Amino Purine): BAP (50 ppm) solution was prepared by dissolving 50 mg of BAP in a little quantity of ethanol and the volume is made up to 1000ml with distilled water. BAP (25 ppm) solution was prepared in similar method by taking 25 mg BAP instead of 50 mg.

(iii) **Adenine Sulphate**: It was first dissolved in a little quantity of hot water then added to the MS medium.

#### **2.6.4 Preparation of culture medium**

All the stock solutions of macro nutrients, micronutrients, iron compounds, vitamins and plant bio-regulators of required quantities were mixed in a small amount of distilled water to prepare the required culture medium. The required quantity of sucrose was dissolved in distilled water and was added fresh to the medium. The pH of the medium was adjusted to  $5.7 \pm 0.1$  using with 0.1 N NaOH or 0.1 N HCl. The final volume was made up by adding sterile distilled water. Agar (0.8% w/v) was added to the medium and boiled in a beaker and stirred with a glass rod until it completely dissolved. The hot medium was then immediately poured into the sterilized culture tubes and plugged with non-absorbent cotton plugs.

#### **2.6.5 Sterilization of culture medium**

Autoclave sterilization was performed on plugged culture tubes containing culture medium at 121°C and 15 psi (1.05 kg/cm<sup>2</sup> pressure) for 20 minutes (Dodds and Roberts, 1982). After being autoclaved, the medium was placed in a Laminar air flow bench to cool. After waiting for 2-3 days to check for contamination in the culture medium, the medium was then used for culturing.

#### **2.6.6 Sterilization of glassware and instruments**

All the Glasswares were dipped in detergent solution overnight and washed under running tap water. The Glasswares were then treated with chromic acid overnight and then they were washed under running tap water. These were washed with distilled water and dried in an oven for two hours at 150°C. Petri dishes, forceps, scalpel, and blade were thoroughly cleaned with alcohol, wrapped with a paper and kept in a clean sterilized base followed by sterilization in an autoclave at 15 psi and 121°C for 30 minutes.

#### **2.6.7 Sterilization of laminar air flow cabinet**

The laminar air flow cabinet working chamber was properly wiped with absolute ethyl alcohol. Filtered air (80-100 cft/min) was blown into the working area for around 5 minutes to remove dirt particles. The sterilized materials to be used (except living tissue) were kept inside the chamber and were exposed to the UV lights for about 25 minutes. While working with laminar air flow cabinet, the filter air flow was continuously passed through it.

#### **2.6.8 Culture room**

The culture room was provided with ideal condition. The room temperature was maintained at 25°C with a relative humidity of 80%. The room was provided with air conditioner and fluorescent tubes exclusively to provide 11-hour photoperiod to maintain the light requirements.

### **2.7 Stages of *in vitro* culture**

#### **2.7.1 Explants**

Healthy disease-free Rose plants were selected for the collection of hips which were grown at the Rose Mother Block of the Biotechnology-cum-Tissue Culture Centre, Department of Floriculture and Landscaping, OUAT, Bhubaneswar. These explants were properly washed under running water for 1 min and then with distilled water to remove all the adhering dirty particles and dried at ambient conditions to remove the water droplets.

#### **2.7.2 Surface sterilant for sterilization of explants**

Under aseptic conditions intact rose hips and seeds were placed in 500 ml beaker containing liquid detergent solution called Labolene and washed for 10 minutes followed by washing under running tap water. After that the intact rose hips and seeds were kept in 0.02% Bavistin solution for about 15 minutes. They were then removed and washed gently under running tap water followed by distilled water. Thereafter the hips and seeds were surface sterilized with 0.1% HgCl<sub>2</sub> for 5 minutes and 3 minutes respectively. After surface sterilization, the hips and seeds were rinsed 3-4 times in sterilized distilled water and dried on autoclaved filter paper. All of these were carried out inside laminar air flow chamber.

#### **2.7.3 Media supplements for callus formation**

After sterilization of hips and seeds with the sterilant, they were placed on sterile filter paper to get rid of the adhering water droplets. Then they were carefully transferred to the culture tubes after breaking the achenes and were inoculated with MS medium containing different concentration of BAP, GA<sub>3</sub> and AdS for initial culture establishment.

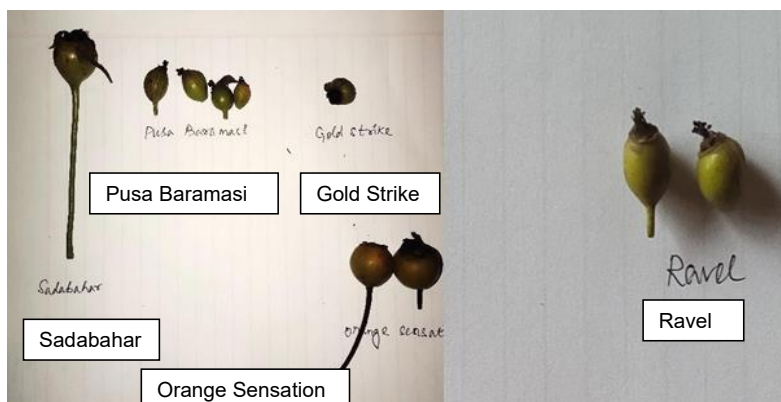
#### **2.7.4 Standardization of media supplements for callus proliferation**

After callus formation the calli mass was divided into 4 equal pieces with a sharp blade and each part (1/4th of the calli mass) was carefully transferred to the shoot proliferation media containing different concentrations of Cytokinin (BAP) and GA<sub>3</sub> which was without AdS (Table 4) and BAP and GA<sub>3</sub> which was with AdS (Table 3). Three replications per treatment and 4 cultures per treatment were used during the culturing.

**Table 2. Impact of Plant Bioregulators on seed germination and growth of *Rosa* achenes (Without AdS)**

Treatments	Concentration of Plant Bioregulators (mg/l)	
	BAP	GA <sub>3</sub>
T1 (Control)	-	-
T2	1.0	0.5
T3	1.5	0.5
T4	2.0	0.5
T5	2.5	0.5
T6	3.0	0.5
T7	3.5	0.5
T8	4.0	0.5





**Fig 8. Different hips of Rose cultivar**

**Table 3. Effect of Plant Bioregulators on seed germination and growth of Rose achenes (With AdS)**

Treatment	Concentration of Plant Bioregulators (mg/l)		
	BAP	GA <sub>3</sub>	AdS
T <sub>1</sub> (Control)	-	-	-
T <sub>2</sub>	1.0	0.5	50
T <sub>3</sub>	1.5	0.5	50
T <sub>4</sub>	2.0	0.5	50
T <sub>5</sub>	2.5	0.5	50
T <sub>6</sub>	3.0	0.5	50
T <sub>7</sub>	3.5	0.5	50
T <sub>8</sub>	4.0	0.5	50

## 2.8 Observations to be recorded

Observation recorded within 45 days of inoculation during culture establishment up to callus and 28 days for shoot proliferation study:

### i. For shoot proliferation study

- Days to shoot initiation: It is the time period between inoculation of calli mass to initiation of shoot.
- Days to shoot development: It is the duration between inoculation of calli mass to complete development of shoot.
- Shoot length (cm): It is the length of shoots after total proliferation.
- Number of leaves/ shoots: It is the total number of leaves formed after entire proliferation.
- Leaf colour: It is the colour of leaves during entire growth phase.

### ii. For callus development study

- Days to callus initiation: It is the duration between explant inoculation to initiation of callus.
- Days to callus development: It is the duration between inoculation of explant to initiation of callus.
- Callus spread: Length of callus (E-W) (cm) × Breadth of callus (N-S) (cm).
- Colour of callus: Colour of callus in entire growth phase.

## 2.9 Statistical Analysis

The data recorded from the experiments were analyzed by using one-way ANOVA in a Completely Randomized Design (CRD) with three replications each consisting of 4 cultures for shoot proliferation and callus induction, as described by Gomez and Gomez (1984). The treatment effects were tested by 'F' test at 5% level of significance. The critical difference at 5% level was calculated for comparing the treatment means.

$CD = SE(m) \cdot t$ , where  $SE(m) \pm$  = Standard Error Mean =  $\sqrt{(2 \cdot \text{Error Mean Square}) / \text{Number of replications}}$ .

T=two sides table value at error degree of freedom at 5% level of significance.

## 3. RESULTS

### 3.1 Effect of Plant Bioregulators on *in vitro* culture establishment of rose seeds

#### 3.1.1 Effect of growth regulators on shoot initiation

The data presented in the Table 4 indicated that there were significant differences among media and the varieties. Data from interactions between media and varieties of rose revealed that MS media without any growth regulators in test tube (T<sub>1</sub>V<sub>4</sub>) Orange Sensation took the highest number of days for shoot initiation (15.62 days) and (T<sub>1</sub>V<sub>1</sub>) Pusa Baramasi took the lowest number of days for shoot initiation (10.83 days).

Shoot initiation was achieved on MS medium supplemented with various concentration of BAP, GA<sub>3</sub> and AdS. BAP and GA<sub>3</sub> favored shoot initiation among the different hormones tested. Adenine sulphate helped in inducing early shoot initiation in the treatment T<sub>5</sub> in combination with BAP and GA<sub>3</sub>. Early shoot initiation was observed in Pusa Baramasi (7.15 days) when MS medium was supplemented with BAP (2.5 mg/l), GA<sub>3</sub> (0.5 mg/l) and AdS (50

mg/l) in treatment T<sub>5</sub> followed by Ravel (11.63 days), Gold Strike (11.89 days), Sadabahar (12.38 days), Summer Fragrance (12.95 days) and Orange Sensation (13.32 days).

**Table 4. Effect of plant bioregulators on shoot initiation in 6 varieties of Rose.**  
Duration: 30 days Basal medium: MS medium

Treatment	MS+ growth regulator concentration (mg/l)			Pusa Baramasi (V <sub>1</sub> )	Summer Fragrance (V <sub>2</sub> )	Gold Strike (V <sub>3</sub> )	Orange Sensation (V <sub>4</sub> )	Ravel (V <sub>5</sub> )	Sadabahar (V <sub>6</sub> )
	BAP	GA <sub>3</sub>	AdS						
T <sub>1</sub>	-	-	-	10.83	15.14	13.87	15.62	14.65	15.15
T <sub>2</sub>	1.0	0.5	50	9.71	14.73	13.40	14.88	13.96	14.56
T <sub>3</sub>	1.5	0.5	50	8.96	14.14	13.05	13.88	13.08	13.81
T <sub>4</sub>	2.0	0.5	50	7.83	13.36	12.45	13.50	12.46	13.06
T <sub>5</sub>	2.5	0.5	50	7.15	12.95	11.89	13.32	11.63	12.38
T <sub>6</sub>	3.0	0.5	50	8.46	13.87	12.67	13.44	12.69	13.08
T <sub>7</sub>	3.5	0.5	50	10.07	14.26	13.45	14.53	13.29	13.49
T <sub>8</sub>	4.0	0.5	50	11.61	14.60	14.36	15.18	14.54	14.18
SE(m) ±				0.31	0.22	0.24	0.23	0.30	0.23
CD (5%)				0.92	0.65	0.72	0.69	0.89	0.69
CV				0.92	2.66	3.16	2.80	3.88	2.89

### 3.1.2 Effect of growth regulators on shoot development

The data presented in the Table 5 indicated that there were significant differences among media and the varieties. Data from interactions between media and varieties of rose revealed that MS media without any growth regulators in test tube (T<sub>1</sub>V<sub>6</sub>) Sadabahar produced the lowest number of days for shoot development (11.91 days) and (T<sub>1</sub>V<sub>1</sub>) Pusa Baramasi produced the highest number of days for shoot development (30.14 days). Shoot development was achieved on MS medium supplemented with various concentration of BAP, GA<sub>3</sub> and AdS. BAP and GA<sub>3</sub> favored shoot development among the different hormones tested. Adenine sulphate helped in inducing shoot development in the treatment T<sub>5</sub> in combination with BAP and GA<sub>3</sub>. The shoot development was observed to be the highest in Pusa Baramasi (36.02 days) when MS medium was supplemented with BAP (2.5 mg/l), GA<sub>3</sub> (0.5 mg/l) and AdS (50 mg/l) in treatment T<sub>5</sub> followed by Ravel (18.17 days), Gold Strike (17.72 days), Sadabahar (17.19 days), Summer Fragrance (16.53 days) and Orange Sensation (15.85 days).

**Table 5. Effect of plant bioregulators on shoot development in 6 varieties of Rose after 2-6 weeks of shoot initiation**

Duration: 30 days

Basal medium: MS medium

Treatment	MS + growth regulator concentration (mg/l)			Pusa Baramasi (V <sub>1</sub> )	Summer Fragrance (V <sub>2</sub> )	Gold Strike (V <sub>3</sub> )	Orange Sensation (V <sub>4</sub> )	Ravel (V <sub>5</sub> )	Sadabahar (V <sub>6</sub> )
	BAP	GA <sub>3</sub>	AdS						
T <sub>1</sub>	-	-	-	30.14	12.42	13.19	11.94	12.59	11.91
T <sub>2</sub>	1.0	0.5	50	31.70	13.23	14.36	13.01	13.88	13.29
T <sub>3</sub>	1.5	0.5	50	32.98	14.11	15.14	14.70	15.50	14.72
T <sub>4</sub>	2.0	0.5	50	34.87	15.60	16.41	15.41	16.76	15.72
T <sub>5</sub>	2.5	0.5	50	36.02	16.53	17.72	15.85	18.17	17.19
T <sub>6</sub>	3.0	0.5	50	33.87	14.80	15.67	15.03	16.40	15.86
T <sub>7</sub>	3.5	0.5	50	31.65	13.85	14.31	13.41	14.70	14.31
T <sub>8</sub>	4.0	0.5	50	29.69	13.08	12.79	11.88	12.78	12.55
SE(m) ±				0.35	0.28	0.40	0.26	0.29	0.40
CD (5%)				1.04	0.83	1.20	0.78	0.88	1.19
CV				1.85	3.38	4.64	3.23	3.35	4.77

### 3.1.3 Effect of growth regulators on cessation of growth

The data presented in the Table 6 indicated that there were significant differences among media and the varieties. Data from interactions between media and varieties of rose revealed that MS media without any growth regulators in test tube (T<sub>1</sub>V<sub>3</sub>) Gold Strike took the lowest number of days for the cessation of growth (27.06 days) and (T<sub>1</sub>V<sub>1</sub>) Pusa Baramasi took the highest number of days for cessation of growth (40.97 days). Cessation was observed on MS medium supplemented with various concentration of BAP, GA<sub>3</sub> and AdS. BAP and GA<sub>3</sub> favored cessation for longer times among the different hormones tested. Adenine sulphate helped in increasing cessation in the treatment T<sub>5</sub> in combination with BAP and GA<sub>3</sub>. The cessation was observed highest in Pusa Baramasi (43.17 days) when MS medium was supplemented with BAP (2.5 mg/l), GA<sub>3</sub> (0.5 mg/l) and AdS (50 mg/l) in treatment T<sub>5</sub> followed by Ravel (29.79 days), Gold Strike (29.61 days), Sadabahar (29.57 days), Summer Fragrance (29.48 days) and Orange Sensation (29.71 days).

**Table 6. Effect of plant bioregulators on cessation in 6 varieties of Rose after 3-7 weeks of culture**

**Duration: 30 Days**

**Basal medium: MS**

Treatment	MS + growth regulator concentration (mg/l)			Pusa Baramasi (V <sub>1</sub> )	Summer Fragrance (V <sub>2</sub> )	Gold Strike (V <sub>3</sub> )	Orange Sensation (V <sub>4</sub> )	Ravel (V <sub>5</sub> )	Sadabahar (V <sub>6</sub> )
	BAP	GA <sub>3</sub>	AdS						
T <sub>1</sub>	-	-	-	40.97	27.56	27.06	27.55	27.24	27.07
T <sub>2</sub>	1.0	0.5	50	41.40	27.96	27.75	27.89	27.84	27.85
T <sub>3</sub>	1.5	0.5	50	41.95	28.24	28.19	28.58	28.58	28.54
T <sub>4</sub>	2.0	0.5	50	42.70	28.95	28.86	28.90	29.22	28.78
T <sub>5</sub>	2.5	0.5	50	43.17	29.48	29.61	29.17	29.79	29.57
T <sub>6</sub>	3.0	0.5	50	42.34	28.67	28.35	28.47	29.09	28.94
T <sub>7</sub>	3.5	0.5	50	41.72	28.11	27.76	27.94	27.98	27.80
T <sub>8</sub>	4.0	0.5	50	41.30	27.68	27.15	27.07	27.32	26.73
SE(m) ±				0.20	0.27	0.35	0.32	0.26	0.23
CD (5%)				0.60	0.80	1.04	0.97	0.77	0.70
CV				0.83	1.62	2.14	1.98	1.56	1.43

### 3.1.4 Effect of growth regulators on length of the shoots (cm)

The data presented in the Table 7 indicated that there were significant differences among media and the varieties. Data from interactions between media and varieties of rose revealed that MS media without any growth regulators in test tube (T<sub>1</sub>V<sub>2</sub>) Summer Fragrance had the lowest length of the shoot (0.72 cm) and (T<sub>1</sub>V<sub>1</sub>) Pusa Baramasi produced the highest length of the shoot (1.12 cm).

Length of the shoots was observed on MS medium supplemented with various concentration of BAP, GA<sub>3</sub> and AdS. BAP and GA<sub>3</sub> favored the length of the shoots among the different hormones tested. Adenine sulphate helped in increasing shoot length in the treatment T<sub>5</sub> in combination with BAP and GA<sub>3</sub>. The highest shoot length was observed in Pusa Baramasi (2.80 cm) when MS medium was supplemented with BAP (2.5 mg/l), GA<sub>3</sub> (0.5 mg/l) and AdS (50 mg/l) in treatment T<sub>5</sub> followed by Ravel (1.94 cm), Gold Strike (1.35 cm), Sadabahar (1.17 cm), Summer Fragrance (0.96 cm) and Orange Sensation (0.75 cm).

### 3.1.5 Effect of growth regulators on number of leaves per explant

The data presented in the Table 8 indicated that there were significant differences among media and the varieties. Data from interactions between media and varieties of rose revealed that MS media without any growth regulators in test tube (T<sub>1</sub>V<sub>2</sub>) Summer Fragrance produced a smaller number of leaves (0.77 leaves) and (T<sub>1</sub>V<sub>1</sub>) Pusa Baramasi produced a greater number of leaves (1.15 leaves).

The number of leaves per explant was observed on MS medium supplemented with various concentration of BAP, GA<sub>3</sub> and AdS. BAP and GA<sub>3</sub> favored the number of leaves per explant among the different hormones tested. Adenine sulphate helped in increasing the number of leaves per explant in treatment T<sub>5</sub> which had a combination with BAP and GA<sub>3</sub>. The number of leaves per explant was observed to be the highest in Pusa Baramasi (3.40 leaves) when MS medium was supplemented with BAP (2.5 mg/l), GA<sub>3</sub> (0.5 mg/l) and AdS (50 mg/l) in

treatment T<sub>5</sub> followed by Ravel (1.51 leaves), Gold Strike (1.50 leaves), Sadabahar (1.23 leaves), Summer Fragrance (1.07 leaves) and Orange Sensation (0.92 leaves).

**Table 7. Effect of plant bioregulators on length of the shoots (cm) in 6 varieties of Rose after 1-7 weeks of culture**

Treatment	MS + growth regulator concentration (mg/l)			Pusa Baramasi (V <sub>1</sub> )	Summer Fragrance (V <sub>2</sub> )	Gold Strike (V <sub>3</sub> )	Orange Sensation (V <sub>4</sub> )	Ravel (V <sub>5</sub> )	Sadabahar (V <sub>6</sub> )
	BAP	GA <sub>3</sub>	AdS						
T <sub>1</sub>	-	-	-	1.15	0.77	1.05	0.37	1.15	0.92
T <sub>2</sub>	1.0	0.5	50	1.41	0.81	1.20	0.54	1.25	0.97
T <sub>3</sub>	1.5	0.5	50	1.86	0.85	1.27	0.72	1.33	1.07
T <sub>4</sub>	2.0	0.5	50	2.53	0.95	1.41	0.85	1.46	1.15
T <sub>5</sub>	2.5	0.5	50	3.40	1.07	1.50	0.92	1.51	1.23
T <sub>6</sub>	3.0	0.5	50	2.25	0.96	1.36	0.81	1.45	1.17
T <sub>7</sub>	3.5	0.5	50	1.66	0.90	1.24	0.65	1.36	1.05
T <sub>8</sub>	4.0	0.5	50	1.21	0.81	1.09	0.44	1.25	0.99
SE(m) ±				0.16	0.02	0.05	0.03	0.06	0.03
CD (5%)				0.47	0.07	0.15	0.10	0.19	0.09
CV				14.04	4.25	6.90	8.53	7.99	4.78

**Table 8. Effect of plant bioregulators on number of leaves per explant in 6 varieties of Rose after 2-7 weeks of culture**

Treatment	MS + growth regulator concentration (mg/l)			Pusa Baramasi (V <sub>1</sub> )	Summer Fragrance (V <sub>2</sub> )	Gold Strike (V <sub>3</sub> )	Orange Sensation (V <sub>4</sub> )	Ravel (V <sub>5</sub> )	Sadabahar (V <sub>6</sub> )
	BAP	GA <sub>3</sub>	AdS						
T <sub>1</sub>	-	-	-	1.12	0.72	1.06	0.43	1.10	0.95
T <sub>2</sub>	1.0	0.5	50	1.29	0.76	1.13	0.53	1.31	1.00
T <sub>3</sub>	1.5	0.5	50	1.50	0.83	1.16	0.63	1.47	1.05
T <sub>4</sub>	2.0	0.5	50	1.82	0.88	1.26	0.70	1.67	1.08
T <sub>5</sub>	2.5	0.5	50	2.80	0.96	1.35	0.75	1.94	1.17
T <sub>6</sub>	3.0	0.5	50	2.14	0.87	1.23	0.67	1.67	1.09
T <sub>7</sub>	3.5	0.5	50	1.61	0.83	1.13	0.55	1.34	1.02
T <sub>8</sub>	4.0	0.5	50	1.23	0.73	1.03	0.43	1.07	0.97
SE(m) ±				0.10	0.03	0.04	0.04	0.04	0.02
CD (5%)				0.29	0.10	0.12	0.12	0.12	0.06
CV				9.81	7.32	5.91	11.62	4.83	3.27

### 3.1.6 Effect of growth regulators on colour of leaves per explant

The data presented in the Table 9 indicated that there were significant differences among media and the varieties. Data from interactions between media and varieties of rose revealed that MS media without any growth regulators in test tube (T<sub>1</sub>V<sub>1</sub>) Pusa Baramasi, (T<sub>2</sub>V<sub>2</sub>) Summer Fragrance, (T<sub>3</sub>V<sub>3</sub>) Gold Strike, (T<sub>4</sub>V<sub>4</sub>) Orange Sensation, (T<sub>5</sub>V<sub>5</sub>) Ravel and (T<sub>6</sub>V<sub>6</sub>) Sadabahar showed whitish green leaves.

The colour of the leaves per explant was observed on MS medium supplemented with various concentration of BAP, GA<sub>3</sub> and AdS. BAP and GA<sub>3</sub> favored the colour of leaves per explant among the different hormones tested. Pusa Baramasi showed light green leaves when MS medium was supplemented with BAP (2.5 mg/l), GA<sub>3</sub> (0.5 mg/l) and AdS (50 mg/l) with the treatment T<sub>4</sub> and T<sub>5</sub> and other treatments showed whitish green leaves. Ravel, Gold Strike and Sadabahar also showed light green leaves when MS medium was supplemented with BAP (2.5 mg/l), GA<sub>3</sub> (0.5 mg/l) and AdS (50 mg/l) in treatment T<sub>5</sub> and rest of the treatments showed whitish green colour leaves. Summer Fragrance and Orange sensation showed whitish green colour leaves in all the treatments.

#### **3.1.7. Effect of growth regulators on callus initiation**

The data presented in the Table 10 indicated that there were significant differences among media and the varieties. Data from interactions between media and varieties of rose revealed that MS media without any growth regulators in test tube (T<sub>1</sub>V<sub>4</sub>) Orange Sensation took the highest number of days for callus initiation (22.60 days) and (T<sub>1</sub>V<sub>1</sub>) Pusa Baramasi took the lowest number of days for callus initiation (15.68 days).

Callus initiation was achieved on MS medium supplemented with various concentration of BAP, GA<sub>3</sub> and AdS. BAP and GA<sub>3</sub> favored shoot initiation among the different hormones tested. Adenine sulphate helped in inducing early callus initiation in the treatment T<sub>5</sub> in combination with BAP and GA<sub>3</sub>. Early callus initiation was observed in Pusa Baramasi (12.53 days) when MS medium was supplemented with BAP (2.5 mg/l), GA<sub>3</sub> (0.5 mg/l) and AdS (50 mg/l) in treatment T<sub>5</sub> followed by Ravel (16.59 days), Gold Strike (17.69 days), Sadabahar (18.26 days), Summer Fragrance (18.48 days) and Orange Sensation (19.21 days).

#### **3.1.8 Effect of growth regulators on spread of the callus (cm)**

The data presented in the Table 11 indicated that there were significant differences among media and the varieties. Data from interactions between media and varieties of rose revealed that MS media without any growth regulators in test tube (T<sub>1</sub>V<sub>4</sub>) Orange Sensation took the lowest length (0.08 cm) and (T<sub>1</sub>V<sub>1</sub>) Pusa Baramasi took the highest length (0.53 cm).

Length of the callus was observed on MS medium supplemented with various concentration of BAP, GA<sub>3</sub> and AdS. BAP and GA<sub>3</sub> favored the spread of the callus among the different hormones tested. Adenine sulphate helped in increasing callus spread in the treatment T<sub>5</sub> in combination with BAP and GA<sub>3</sub>. The callus spread was observed to be the highest in Pusa Baramasi (1.14 cm) when MS medium was supplemented with BAP (2.5 mg/l), GA<sub>3</sub> (0.5 mg/l) and AdS (50 mg/l) in treatment T<sub>5</sub> followed by Ravel (0.91 cm), Gold Strike (0.80 cm), Sadabahar (0.68 cm), Summer Fragrance (0.58 cm) and Orange Sensation (0.35 cm).

#### **3.1.9 Effect of growth regulators on callus colour**

The data presented in the Table 12 indicated that there were significant differences among media and the varieties. Data from interactions between media and varieties of rose revealed that MS media without any growth regulators in test tube (T<sub>1</sub>V<sub>1</sub>) Pusa Baramasi, (T<sub>2</sub>V<sub>2</sub>) Summer Fragrance, (T<sub>3</sub>V<sub>3</sub>) Gold Strike, (T<sub>4</sub>V<sub>4</sub>) Orange Sensation, (T<sub>5</sub>V<sub>5</sub>) Ravel and (T<sub>6</sub>V<sub>6</sub>) Sadabahar showed whitish green callus.

The colour of the callus was observed on MS medium supplemented with various concentration of BAP, GA<sub>3</sub> and AdS. BAP and GA<sub>3</sub> favored the colour of callus among the different hormones tested. Pusa Baramasi showed light green callus when MS medium was

supplemented with BAP (2.5 mg/l), GA<sub>3</sub> (0.5 mg/l) and AdS (50 mg/l) with the treatment T<sub>5</sub> and other treatments showed whitish green callus. Ravel, Gold Strike, Sadabahar, Summer Fragrance and Orange sensation also showed whitish green colour callus in all the treatments.

### 3.1.10 Effect of Adenine Sulphate, BAP and GA<sub>3</sub> on the explants

Best shoot proliferation was observed when explants were inoculated on Murashige and Skoog (MS) medium supplemented with adenine sulphate (AdS) 50 mg/l. Adenine sulphate acts as a precursor for natural cytokinin synthesis and enhances biosynthesis of natural cytokinin, the compounds produced could be more effective in causing the physiological response than the cytokinins added to the Murashige and Skoog (MS) culture medium. The benefits of adenine sulphate are noticed when it is associated together with cytokinins such as benzyl aminopurine (BAP) as reported by Van Staden *et al.*, (2008). The results suggest that BAP and GA<sub>3</sub>, in combination with AdS, improves the process of seed germination.

The problem of seed germination and shoot initiation was considerably increased by the application of an adjuvant, adenine sulphate (AdS) in the optimal medium which led to the development of shoots. In the present study higher levels of BAP (more than 2.5 mg/l) resulted in delay of shoot initiation and poor seed germination.

During the studies adenine sulphate (AdS) has been used as an experimental hormone for the *in vitro* seed germination of rose. This resulted in early shoot initiation, more shoot length and callus induction from rose explants variety Pusa Baramasi when supplemented on MS medium supplemented with BAP (2.5 mg/l) + GA<sub>3</sub> (0.5 mg/l) + AdS (50 mg/l). Same concentration of BAP (2.5 mg/l) + GA<sub>3</sub> (0.5 mg/l) + AdS (50 mg/l) also helps in *in vitro* seed germination of rose in other varieties also. In the explant cultures adenine sulphate played an important role for improving the frequency of multiple shoot induction when compared to culture media without adenine sulphate. Thus, adenine sulphate exerts a synergistic effect when used along with BAP and GA<sub>3</sub>.

**Table 9: Effect of plant bioregulators on colour of leaves per explant in 6 varieties of Rose 2-7 weeks of culture**

Treatment	MS + growth regulator concentration (mg/l)			Pusa Baramasi (V <sub>1</sub> )	Summer Fragrance (V <sub>2</sub> )	Gold Strike (V <sub>3</sub> )	Orange Sensation (V <sub>4</sub> )	Ravel (V <sub>5</sub> )	Sadabahar (V <sub>6</sub> )
	BAP	GA <sub>3</sub>	AdS						
T <sub>1</sub>	-	-	-	Whitish Green	Whitish Green	Whitish Green	Whitish Green	Whitish Green	Whitish Green
T <sub>2</sub>	1.0	0.5	50	Whitish Green	Whitish Green	Whitish Green	Whitish Green	Whitish Green	Whitish Green
T <sub>3</sub>	1.5	0.5	50	Whitish Green	Whitish Green	Whitish Green	Whitish Green	Whitish Green	Whitish Green
T <sub>4</sub>	2.0	0.5	50	Light Green	Whitish Green	Whitish Green	Whitish Green	Whitish Green	Whitish Green
T <sub>5</sub>	2.5	0.5	50	Light Green	Whitish Green	Light Green	Whitish Green	Light Green	Light Green
T <sub>6</sub>	3.0	0.5	50	Whitish Green	Whitish Green	Whitish Green	Whitish Green	Whitish Green	Whitish Green
T <sub>7</sub>	3.5	0.5	50	Whitish Green	Whitish Green	Whitish Green	Whitish Green	Whitish Green	Whitish Green
T <sub>8</sub>	4.0	0.5	50	Whitish Green	Whitish Green	Whitish Green	Whitish Green	Whitish Green	Whitish Green



**Table 10. Effect of plant bioregulators on callus initiation in 6 varieties of Rose after 2-3 weeks of culture**

Treatment	MS+ growth regulator concentration (mg/l)			Pusa Baramasi (V <sub>1</sub> )	Summer Fragrance (V <sub>2</sub> )	Gold Strike (V <sub>3</sub> )	Orange Sensation (V <sub>4</sub> )	Ravel (V <sub>5</sub> )	Sadabahar (V <sub>6</sub> )
	BAP	GA <sub>3</sub>	AdS						
T <sub>1</sub>	-	-	-	15.68	22.54	21.01	22.60	19.55	21.51
T <sub>2</sub>	1.0	0.5	50	14.51	21.39	20.55	22.22	18.56	20.55
T <sub>3</sub>	1.5	0.5	50	13.63	20.40	19.37	21.56	17.89	19.95
T <sub>4</sub>	2.0	0.5	50	12.87	19.39	18.64	20.18	17.24	19.40
T <sub>5</sub>	2.5	0.5	50	12.53	18.48	17.69	19.21	16.59	18.26
T <sub>6</sub>	3.0	0.5	50	13.54	19.49	18.30	20.51	17.51	19.53
T <sub>7</sub>	3.5	0.5	50	14.43	20.40	19.34	21.12	18.28	20.24
T <sub>8</sub>	4.0	0.5	50	15.22	21.43	20.30	21.60	19.82	21.43
SE(m) ±				0.13	0.15	0.19	0.29	0.13	0.12
CD (5%)				0.38	0.44	0.35	0.87	0.40	0.35
CV				1.58	1.26	1.06	2.39	1.28	1.00

**Table 11. Effect of plant bioregulators on length of the callus (cm) in 6 varieties of Rose after 2-7 weeks of culture**

Treatment	MS + growth regulator concentration (mg/l)			Pusa Baramasi (V <sub>1</sub> )	Summer Fragrance (V <sub>2</sub> )	Gold Strike (V <sub>3</sub> )	Orange Sensation (V <sub>4</sub> )	Ravel (V <sub>5</sub> )	Sadabahar (V <sub>6</sub> )
	BAP	GA <sub>3</sub>	AdS						
T <sub>1</sub>	-	-	-	0.53	0.16	0.31	0.08	0.41	0.22
T <sub>2</sub>	1.0	0.5	50	0.60	0.28	0.45	0.15	0.62	0.34
T <sub>3</sub>	1.5	0.5	50	0.72	0.35	0.50	0.20	0.75	0.44
T <sub>4</sub>	2.0	0.5	50	0.91	0.47	0.64	0.27	0.83	0.56
T <sub>5</sub>	2.5	0.5	50	1.14	0.58	0.80	0.35	0.91	0.68
T <sub>6</sub>	3.0	0.5	50	0.89	0.45	0.62	0.23	0.82	0.53
T <sub>7</sub>	3.5	0.5	50	0.67	0.34	0.43	0.17	0.61	0.39
T <sub>8</sub>	4.0	0.5	50	0.58	0.21	0.35	0.09	0.43	0.28
SE(m) ±				0.04	0.02	0.03	0.02	0.03	0.02
CD (5%)				0.12	0.05	0.10	0.05	0.09	0.07
CV				9.05	8.24	10.78	11.62	8.08	14.98

**Table 12. Effect of plant bioregulators on colour of callus in 6 varieties of Rose after 2-7 weeks of culture**

Treatment	MS + growth regulator concentration (mg/l)			Pusa Baramasi (V <sub>1</sub> )	Summer Fragrance (V <sub>2</sub> )	Gold Strike (V <sub>3</sub> )	Orange Sensation (V <sub>4</sub> )	Ravel (V <sub>5</sub> )	Sadabahar (V <sub>6</sub> )
	BAP	GA <sub>3</sub>	AdS						
T <sub>1</sub>	-	-	-	Whitish Green	Whitish Green	Whitish Green	Whitish Green	Whitish Green	Whitish Green
T <sub>2</sub>	1.0	0.5	50	Whitish Green	Whitish Green	Whitish Green	Whitish Green	Whitish Green	Whitish Green
T <sub>3</sub>	1.5	0.5	50	Whitish Green	Whitish Green	Whitish Green	Whitish Green	Whitish Green	Whitish Green
T <sub>4</sub>	2.0	0.5	50	Whitish Green	Whitish Green	Whitish Green	Whitish Green	Whitish Green	Whitish Green
T <sub>5</sub>	2.5	0.5	50	Light Green	Whitish Green	Whitish Green	Whitish Green	Whitish Green	Whitish Green
T <sub>6</sub>	3.0	0.5	50	Whitish Green	Whitish Green	Whitish Green	Whitish Green	Whitish Green	Whitish Green
T <sub>7</sub>	3.5	0.5	50	Whitish Green	Whitish Green	Whitish Green	Whitish Green	Whitish Green	Whitish Green
T <sub>8</sub>	4.0	0.5	50	Whitish Green	Whitish Green	Whitish Green	Whitish Green	Whitish Green	Whitish Green



**Fig. 9: Inoculation of Explants**



**Fig 10: Initiation of Pusa Baramasi variety Shoot primordia in treatment T<sub>5</sub>**



**Fig 13: Callus Proliferation in T<sub>5</sub>**



**Fig 12: Shoot Proliferation from seeds of Pusa Baramasi in T<sub>5</sub>**



**Fig. 11: Shoot growth after 7 days of initiation in T<sub>5</sub>**

#### 4. DISCUSSION

##### 4.1 Effect of plant bioregulators on *in vitro* shoot initiation in *Rosa* sp.

Depending on the type and concentration of growth regulators used, the number of days required to shoot initiation varied between 7 to 14 days in *Rosa* sp. Early shoot initiation was observed in Pusa Baramasi (7.15 days) when MS medium was supplemented with BAP (2.5 mg/l), GA<sub>3</sub> (0.5 mg/l) and AdS (50 mg/l) followed by Ravel (11.63 days), Gold Strike (11.89 days), Sadabahar (12.38 days), Summer Fragrance (12.95 days) and Orange

Sensation (13.32 days). Similar findings were observed in *Rosa hybrida* by Rout *et al.*, (1990) and Singh and Syamal (1999) and in *Chrysanthemum morifolium* by Kazeroonian *et al.*, (2018). The present study indicated that inclusion of an adjuvant like AdS in the medium, either with BAP or GA<sub>3</sub> favor multiplication and growth of the micro shoots. The AdS requirement for shoot initiation was essential and two cytokinin (BAP and GA<sub>3</sub>) favored shoot multiplication.

#### **4.2 Effect of plant bioregulators on *in vitro* shoot development of *Rosa* sp.**

The shoot development was observed to be highest in Pusa Baramasi (36.02 days) when MS medium was supplemented with BAP (2.5 mg/l), GA<sub>3</sub> (0.5 mg/l) and AdS (50 mg/l) followed by Ravel (18.17 days), Gold Strike (17.72 days), Sadabahar (17.19 days), Summer Fragrance (16.53 days) and Orange Sensation (15.85 days). Similar results were also observed in Rose by Chaudhary and Prasad (1990), Morya *et al.*, (2004) and in *Chrysanthemum* by Verma and Prasad (2019). The present study indicated that inclusion of an adjuvant AdS in the medium, either with BAP or GA<sub>3</sub> favored shoot development.

#### **4.3 Cessation of growth**

The cessation of growth was delayed in Pusa Baramasi (43.17 days) when MS medium was supplemented with BAP (2.5 mg/l), GA<sub>3</sub> (0.5 mg/l) and AdS (50 mg/l) followed by Ravel (29.79 days), Gold Strike (29.61 days), Sadabahar (29.57 days), Summer Fragrance (29.48 days) and Orange Sensation (29.71 days). The present study indicated that inclusion of an adjuvant AdS in the medium, either with BAP or GA<sub>3</sub> increased the cessation of growth. Adenine sulphate also played an important role for improving the frequency of multiple shoot induction when compared to culture media without adenine sulphate.

#### **4.4 Length of the shoots (cm)**

The shoot length was recorded to be maximum in Pusa Baramasi (2.80 cm) when MS medium was supplemented with BAP (2.5 mg/l), GA<sub>3</sub> (0.5 mg/l) and AdS (50 mg/l) followed by Ravel (1.94 cm), Gold Strike (1.35 cm), Sadabahar (1.17 cm), Summer Fragrance (0.96 cm) and Orange Sensation (0.75 cm). The above findings were corroborated with the research findings in Rose by Podwyszynska (1995), Singh and Syamal (1999) and in Orchids by Indhumati *et al.*, (2004). The present study indicated that inclusion of an adjuvant AdS has synergistic effect when used along with BAP and GA<sub>3</sub> in the medium and increased the length of the shoots. Adjuvant adenine sulphate plays an important role for improving the length of the shoots when compared to culture media without adenine sulphate. However, Verma and Prasad (2019) reported that length of the shoots was maximum on *Chrysanthemum* when MS medium was supplemented with Kinetin (5 mg/l), NAA (0.01 mg/l) and GA<sub>3</sub> (0.2 mg/l).

#### **4.5 Number of leaves/explants**

Adenine sulphate helped in increasing the number of leaves per explant in the treatment T5 where it was used in combination with BAP and GA<sub>3</sub>. The number of leaves per explant was observed to be the highest in Pusa Baramasi (3.40 leaves) when MS medium was supplemented with BAP (2.5 mg/l), GA<sub>3</sub> (0.5 mg/l) and AdS (50 mg/l) followed by Ravel (1.51 leaves), Gold Strike (1.50 leaves), Sadabahar (1.23 leaves), Summer Fragrance (1.07

leaves) and Orange Sensation (0.92 leaves). Similar findings were also reported on Anthurium by Sreelatha et al. (1998) and on Chrysanthemum morifolium by Shatnawi et al. (2010). The present study indicated that maximum number of leaves per explant were produced when MS medium supplemented with an adjuvant adenine sulphate, either with BAP or GA<sub>3</sub>.

#### 4.6 Colour of leaves

The colour of the leaves in Pusa Baramasi was observed to be light green in colour when MS medium was supplemented with BAP (2.0 mg/l), GA<sub>3</sub> (0.5 mg/l) and AdS (50 mg/l) with treatment T<sub>4</sub> and BAP (2.5 mg/l), GA<sub>3</sub> (0.5 mg/l) and AdS (50 mg/l) with treatment T<sub>5</sub>. Other treatments with different concentration of plant growth regulators showed whitish green leaves. In Ravel, Gold Strike and Sadabahar treatment T<sub>5</sub> exhibited light green colour of leaves and rest of the treatments showed whitish green colour leaves. Summer Fragrance and Orange sensation showed whitish green colour leaves in all the treatments. The present study indicated that inclusion of an adjuvant AdS in the medium, either with BAP or GA<sub>3</sub> showed light green leaves when compared to culture medium without adenine sulphate.

#### 4.7 Callus initiation

BAP and GA<sub>3</sub> favored callus initiation among the different hormones tested. Adenine sulphate helped in inducing quick callus initiation in the treatment T<sub>5</sub> in combination with BAP and GA<sub>3</sub>. Early callus initiation was observed in Pusa Baramasi (12.53 days) when MS medium was supplemented with BAP (2.5 mg/l), GA<sub>3</sub> (0.5 mg/l) and AdS (50 mg/l) in the treatment T<sub>5</sub> followed by Ravel (16.59 days), Gold Strike (17.69 days), Sadabahar (18.26 days), Summer Fragrance (18.48 days) and Orange Sensation (19.21 days). Similar findings were also observed in *Jasminum malabathricum* by Gadkar et al., 2011. This was also similar to findings of callus initiation in MS medium + BAP 1.0 mg/l + NAA 0.5 mg/l in *Cordyline terminalis* as reported by Jena et al., 2025. The present study indicated that inclusion of an adjuvant AdS in the medium, either with BAP or GA<sub>3</sub> induced quick callus initiation when compared to culture medium without adenine sulphate.

#### 4.8 Spread of Callus (cm<sup>2</sup>)

The callus spread was observed maximum in Pusa Baramasi (1.14 cm) when MS medium was supplemented with BAP (2.5 mg/l), GA<sub>3</sub> (0.5 mg/l) and AdS (50 mg/l) in treatment T<sub>5</sub> followed by Ravel (0.91 cm), Gold Strike (0.80 cm), Sadabahar (0.68 cm), Summer Fragrance (0.58 cm) and Orange Sensation (0.35 cm). Similar results were also reported in *Jasminum malabathricum* by Gadkar et al., 2011 and in *Jasminum sambac* by Nuralita et al., (2012). The present study indicated that inclusion of an adjuvant AdS in the medium, either with BAP or GA<sub>3</sub> favored the spread of the callus and callus proliferation when compared to the culture medium without adenine sulphate as it acted as a synergist to BAP.

#### 4.9 Effect of Adenine Sulphate

Adenine sulphate, adenosine and adenylic acid show cytokinin activity and therefore are applied to different culture medium in order to improve growth or to reinforce the response normally attributed to cytokinin action as reported by Gatica et al., (2010). Adenine sulphate also stimulates somatic embryogenesis and callogenesis, induces the proliferation of axillary shoots in shoot cultures and promotes adventitious shoot formation indirectly from

calli or directly from explants and the benefits of AdS are often only noticed when it is associated with cytokinins such as BAP as stated Van Staden et al., (2008). Therefore, the addition of AdS to the medium may retard the degradation of cytokinins by competing for the enzyme systems involved in cytokinin metabolism. This helps the explant to accumulate photosynthates which help in better *in vitro* seed germination of rose.

#### 4.10 Environmental Factors

Environmental factors such as light, temperature and humidity were the major requirements for production of multiple shoots as reported by Mroginski et al., (1999). The results of the experiment indicated that culture medium which were kept under light shows better performance when compared to the culture medium which were kept under dark. Incubation temperature was also an important factor for better shoot multiplication. Among the different temperature regimes, 25±2°C was most favorable for the cultures *in vitro*.

The Rose seeds germinated and grew considerably on MS medium supplemented with BAP (2.5 mg/l), GA<sub>3</sub> (0.5 mg/l) and AdS (50 mg/l) under 16-hour photoperiod. Cultures incubated for two weeks in the dark and then transferred to two weeks in the light at 16-hour photoperiod had a higher rate of seed germination.

#### 4.10 Stratification and Scarification

Seed germination within rose taxa is very difficult because of the genetic properties so proper stratification and scarification is an effective method for the seed germination of rose as it helps in breaking the dormancy of rose seeds which is enclosed by a hard seed coat. This method helps in embryo rescue as it helps to grow a plant by breaking the dormancy of rose seeds.

### 5. CONCLUSION

A possible interaction between genotypes and geographic location should be investigated. An optimization of the growing conditions of the plants as well as harvesting and processing of the hips would also help in improving the efficiency of *in vitro* culture of rose achenes. This experiment conclude that higher levels of BAP (more than 2.5 mg/l) affect the seed germination. So optimal concentration of BAP proved to be the best responsive cytokinin for callus formation and shoot initiation.

This experiment also concludes that addition of an adjuvant AdS (50 mg/l) to the culture medium acts as a synergistic effect when used along with BAP and GA<sub>3</sub> and retards the degradation of cytokinin by competing with the enzyme involved in cytokinin metabolism which help for *in vitro* seed germination. High amount of abscisic acid (ABA) is produced in the testa tissues of the seed coat of rose seeds. This abscisic acid inhibits the synthesis of hydrolytic enzymes by GA<sub>3</sub> which help in increasing the availability of storage compounds for seed germination. As ABA interferes with the synthesis of hydrolytic enzymes it should be eliminated for the early embryo development and seed germination. This experiment reports an efficient method of seed germination in different varieties of roses which do not germinate under normal conventional practices. This will help the breeder to develop new varieties by using this novel technique. For future line of work crossing of rose varieties should be done and the seeds can be used for tissue culture to develop new varieties. Seed dormancy is not only complicated but also incompletely understood in Rose, So significant experimentation is necessary to determine treatments that can give maximum germination in rose achenes.

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Commenté [HS3]: update the references

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