ISSN No:-2456-2165

Validation of BAP (6 – Benzyl Amino Purine) Concentration on the Micropropagation of Rose through Shoot Tip Culture

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Abstract:- In this study, the nodal explants collected from the healthy shoots were cultured on the basal MS medium supplemented with 3mg/lit of BAP (6-Benzyl Amino Purine). In the earlier study, several researchers reported that the concentration of 3mg/lit is optimum for the growth and development of shoot tip in rose by the duration of 1month. The different stage of shoot development was observed from the third day to one month interval. It revealed that the callus initiation starts from the 10th day and auxillary shoot initiation from 2 weeks, and subsequent growth and development was observed from the third week and one month interval respectively. Further the developed healthy shoot culture will be maintained and will be treated with NAA at different concentration for root initiation. Finally, the optimum concentration of NAA will be fixed for better root initiation and development.

Keywords:- *Micropropagation of Rose.*

I. INTRODUCTION

Rose belongs to the family Rosaceae, which is the most important crop among the flower crops due to its preference and high aesthetic value. In Rosaceae, a total of 100 genera's and 2000 herbaceous to woody plant species have been identified (Arene et al., 1993). Rose is considered a beautiful flower of immense horticultural importance (Campos and Salome, 1990). It can be used in different fields viz., medicines, rituals, and social; events. Eventually, 20000 cultivars evolved in rose through the crop improvement programs such as selection and hybridization. It is grown worldwide as cut flowers and potted plants in home gardens. Major constraints in rose production are susceptibility to disease viz., bacterial blight, black spot, and powdery mildew (Cline 1994). Various methods of propagation have been practiced in rose viz., seeds, cuttings, layering, and grafting. Where the seed propagation shows variations among the genotypes while the other methods are time-consuming and have slower growth (Dieliman et al., 1995). So there is a need to introduce a new technique that meets the constraints of rose cultivation. The tissue culture system in rose has been established (Hsia and Korban, 1996; Kintzios et al., 1999; Ibrahim and Debergh, 2001; Kim et al., 2003; Rout et al., 2006; Hameed et al., 2006; Drefahl et al., 2007; Previati et al., 2008). To introduce an invitro flowering system, it is essential to develop a novel organogenesis protocol for the rapid multiplication of shoots. The application of the tissue culture technique is advantageous over the existing method in concern of faster multiplication and disease-free production (Bonga 1987). Nowadays, the commercial use of tissue culture in roses is a combination of rapid multiplication and regeneration. In the past, in-vitro propagation enables significant features for the production of disease-free and faster multiplication potential of the rose plant (Dhawan and Bhojwani, 1986). In 1965, Martin demonstrated the invitro technique in roses and cloned around 400,000 plants. In addition tissue culture-derived dwarf rose plants have good features such as earliness, faster multiplication, and more lateral shoots. For which the concentration of growth hormones added in the basal media to be identified properly for the shoot multiplication. Hence our objective is to study the effect of BAP at the differential concentration on the basal MS media. Further, it could be utilized for rapid multiplication of rose through the invitro technique.

II. MATERIALS AND METHODS

- *Plant material*

Five Nodal explants processing a lateral bud is selected from the healthy rose plant and were used as plant material for analyzing the of BAP (6- Benzyl amino purine) at 3mg/lit concentration for shoot initiation through micropropagation.

- *Sterilization technique*

The collected explants were initially washed with tap water for 10-15 minutes. After washing with the tap water the explants were treated with the liquid detergents for 10 minutes and then washed with tap water three to five times to remove the residues of liquid detergents used. Further, the explants

ISSN No:-2456-2165

were treated with 70% ethanol for 15 – 30 seconds and washed with sterile water two to three times. Finally, 0.1 % of mercuric chloride was treated for 2-5 minutes and washed with sterile water two to three times.

➢ *Culture Media preparation*

Culture Media Preparation used in this study is Murashige and Skoog's medium (MS medium) (1962) in semi-solid form. The chemical composition used in the preparation of the MS medium is given in Table 1.

Table 1. Chemical Components and its composition of the MS medium

Components	Volume (mg/lit)	Components	Volume (mg/lit)	Components	Volume (mg/lit)
Macronutrients		**Micronutrients**		**Vitamins**	
NH_4NO_3	1650	KI	0.830	Thiamine	0.100
KNO_3	1900	H_3BO_3	6.20	Pyridoxine	0.500
$CaCl_2.H_2O$	440	$MnSO_4.H_2O$	15.60	Nicotinic Acid	0.500
$MgSO_4.7H_2O$	370	$ZnSO_4.7H_2O$	8.60	Myo-Inositol	100
KH_2PO_4	170	$Na_2MoO_4.2H_2O$	0.250		
		$CuSO_4.5H_2O$	0.250		
		$CuSO_4.6H_2O$	0.025		
		$FeSO_4.7H_2O$	27.80		
		Na_2 EDTA	37.30		

➢ *Final semi-solid media preparation*

All the chemicals were added in double distilled water. To solidify the medium agar-agar was added to the media. Finally, the pH of the media was checked to be 5.8, and boiled the content until it attains homogeneity. In addition, BAP at 3mg/lit was supplemented with the basal MS medium.

➢ *Instruments required*

All the inoculation practices were conducted in a Laminar air flow chamber, which is the most convenient and reliable thing to maintain the aseptic condition throughout inoculation. In this, all the contaminants were blown away by the ultra-clean blower thereby creating the aseptic condition. During media preparation, a Ph meter was used to check and maintain the optimum level of pH of the medium. An autoclave was used to sterilize the media, glass instruments, etc., For drying the washed glass instruments, a Hot air oven is used. Other instruments viz., Pipettes, Test tubes, Scalpels, Cotton plugs, Scissors, Forceps, Conical flask, Beaker, Measuring cylinder, and Petri dish were used for the sterilization and inoculation of the rose explants.

III. RESULTS AND DISCUSSION

In our study, we have taken five rose explants and inoculated the explants in the basal MS media supplemented with the BAP (6 – Benzyl Amino Purine) at the concentration of 3 mg/lit. observations were recorded from the third day on all the five explants under study. On the third day, the number of explants evaluated was five. Among the five explants, two were contaminated and rejected. There is no growth on the third day was recorded. After one week, two explants survived among the three. Also, no growth was found in the two healthy explants. Callus initiation was started after 10 days and was observed on two explants. Further, Auxillary shoot initiation was noticed after two weeks. Subsequently, the healthy well-established explants were maintained carefully and we noticed shoot growth after 20 days intervals. Eventually, shoot growth and shoot development takes place after three weeks and one-month interval respectively (Table 2 and Figure 1). However, there is no elongation and multiplication of shoots were found until one month. Further, the explants are allowed for two months to grow and in the future, the well-established shotted explants will be subjected to rooting media containing (MS + NAA) at different concentrations and will be evaluated for the best concentration on root development. Similar results were recorded by Shabbir et al., 2009, Udom et al., 2009, Kay Thi Oo et al., 2021. The recovery percentage of the five plants revealed that 40% survival from our study (Table 3).

Table 2. Observation of the growth of the explants at various intervals

S. No	Days of observation	No of explants evalauted	No of explants rejected	Growth status
1.	3rd day	Five	Two	No Growth
2.	1 week	Three	One	No Growth
3.	10 days	Two	-	Callus Initiation
4.	2 weeks	Two	-	Auxillary Shoot Initiation
5.	20 days	Two	-	Shoot growth
6.	3 weeks	Two	-	Shoot development
7.	1month	Two	-	Shoot development

Table 3. Recovery percentage of the explants

S. No	No of Explant	Infected	Recovery Percentage (%)

1.	1	Infected	40%
2.	2	Not Infected	
3.	3	Infected	
4.	4	Infected	
5.	5	Not Infected	

IV. CONCLUSION

In this study, we have conducted an experiment to validate the BAP (6- Benzyl Amino Purine) at 3 mg/lit on micropropagation of rose using a shoot tip. From the above results, it is concluded that a 3 mg / lit concentration of BAP is optimum for the proliferation of rose shoot culture. Controversly, higher and lower concentration of BAP diminishes the growth and development of shoot tip. Hence, 3mg/ lit of BAP is optimum for shoot tip growth. Further, we maintain the culture and it will be subjected to NAA for root initiation to evaluate the best concentration of the rooting hormone.

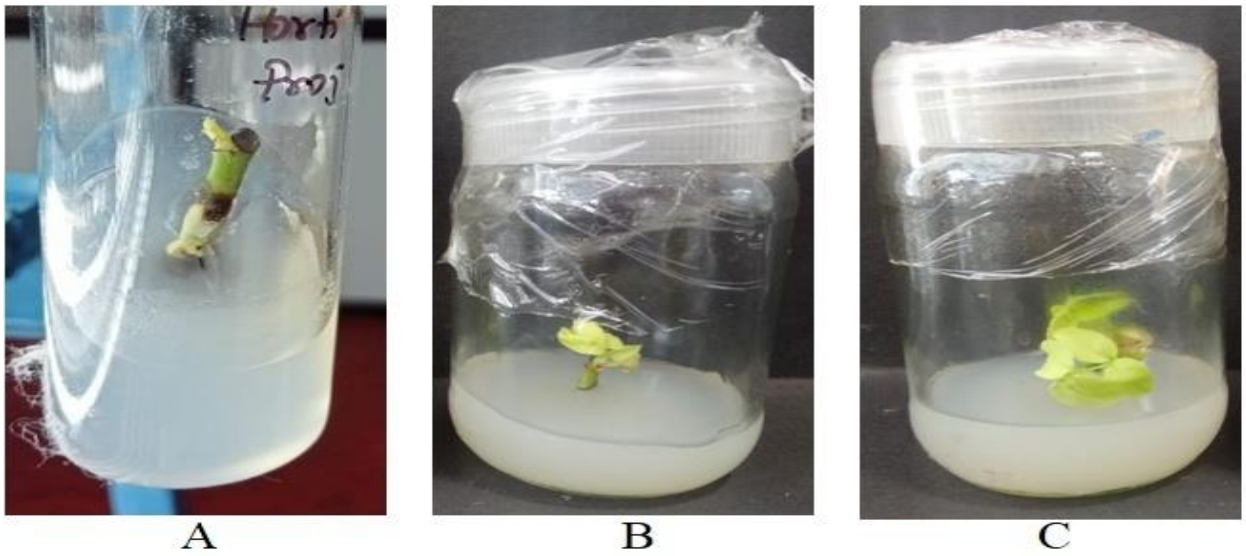

Fig 1. Different growth stages of the Rose explants. (A) Callus Initiation; (B) Shoot I nitiation; (C) Shoot Development.

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