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In Vitro Micropropagation of Rose (*Hybrid Rosa* spp.) through Plant Tissue Culture Technique

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Abstract

Roses are the most important cut flowers in the world. Tissue culture of rose has been improved since last twenty years, and exploited for various purposes from basic anatomical and physiological research to micropropagation from auxiliary buds, shoot tips, leaf explants, etc. Single bud nodal stem segments were surface sterilized and then cultured on MS medium supplemented with 30 g/l sugar, 6 g/l agar and different concentrations of BAP(1, 2, 3, 4 and 5mg/l). Among them, the most suitable concentration for shoot initiation and multiplication was 3mg/l BAP. And it also found that the best for shoot elongation occurred in MS medium supplemented with BAP 3 mg/l and GA_3 1mg/l. The proliferated microshoots were transferred to root inducing media of half strength MS medium supplemented with 15 g/l sugar, 5 g/l agar and NAA (0.5, 1, 1.5, 2 mg/l). The best rooting was observed at NAA 1 mg/l concentration. The least response in root formation was found at NAA 0.5 mg/l supplementation on half strength MS media.

Keywords: BAP, micropropagation, multiplication, NAA, rose.

INTRODUCTION

The rose plant belongs to the plant family Rosaceae is very large, with over 100 genera and 2000 herbaceous to woody species of plants as members [1]. Through out history, no other plants have such wide appeal and been the centre of so much attention than the rose. It is also beautiful flower of immense horticultural importance[2]. Rose is used in religious rituals, medicines and social events. Many species of *Rosa* have been modified through selection and hybridization to give rise to some 20,000 cultivars. One of the primary problems with the rose is their susceptibility to diseases like black spot, powdery mildew, bacterial blight, etc.[3]. Roses can be propagated by seeds, cuttings, layering and grafting. Seed propagation often results in variation while other methods of rose propagation are slow and time consuming. So, there is a need to introduce efficient methods for faster propagation of roses[4].

The application of tissue culture techniques to the regulation and commercial propagation of hybrid roses is more recent developed[5]. The major commercial use of tissue culture techniques in vegetative propagation of hybrid roses is the combination of rapid multiplication and regeneration.The aim of the present investigation was to determine appropriate basal medium and growth regulators for *in vitro* propagation of rosa spp. for micropropagation of disease free plants of roses.

MATERIALS AND METHODS

The rose flower branches in order to use as explants were collected from the local market. The nodal stem segments with auxillary bud were used as explants. Removed all the leaves and thorns from the branches and washed them with detergent and then washed with tap water for several times. Excised single-bud stem segments and dipped them in soap water for a few seconds and then washed them with sterilized distilled water. Afer that the stems were soaked with a fungicide solution, homine for about one hour and then washed with sterilized distilled water. Soaked them again in an antibiotic, rifampicin for 45 minutes and then washed them with sterilized distilled water. Rinsed them with 15% colorax for 20 minutes and then washed with sterilized distilled water. Under laminar flow, sterilized the explants with 70% ethanol for one minute and then thoroughly washed with sterilized distilled water for about three times. Sterilized explant materials were cultured on MS media supplemented with different concentrations of BAP (1,2,3,4 and 5 mg/l) respectively for initiation of shoots. The pH of the media was adjusted to 5.8 before adding agar.

For shoot initiation and multiplication, the gelling agent was 6g/l and for rooting, it was 5.5 g/l. The cultures were incubated at 26±1ºC under continuous fluorescent tube light. Subculting was done every four weeks culture period. The regenerated rose shoots were subcultured on fresh media for every four-week-culture-period. Then, the sufficient shoots were transplanted to half MS media supplemented with different concentrations of auxin (0.5,1 ,1.5, 2 mg/l of NAA).

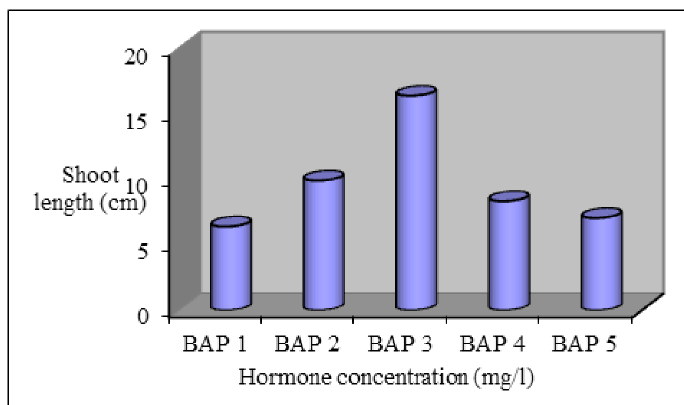

Figure 1: Effect of Different Concentrations of BAP on

Shoot Length in Initiation Stage of Rose after Two Month of Culture Period

Table 1: Effect of Different Concentrations of NAA on Root Formation

Concentration (mg/l)	Rooting response	Time period weeks	Remarks
NAA 0.5	Non	3-5	No root formation
NAA 1	Excellent root	3-5	Long single root formed
NAA 1.5	Little root	3-5	Small root formed
NAA 2	Moderate root	3-5	Small root formed

Plate 1: Shoot Initiation from the Rose Explant on MS Medium Supplemented with 3 mg/l BAP

Plate 2: Multiple Shoot Formation on MS Medium Supplemented with BAP 1 mg/l after Two Months of Culture Period

Plate 3: Multiple Shoot Formation on MS Medium Supplemented with BAP 2 mg/l after Two Months of Culture Period

Plate 4: Multiple Shoot Formation on MS Medium Supplemented with BAP 3 mg/l after Two Months of Culture Period

Plate 5: Multiple Shoot Formation on MS Medium Supplemented with BAP 4 mg/l after Two Months of Culture Period

Plate 6: Multiple Shoot Formation on MS Medium Supplemented with BAP 5 mg/l after Two Months of Culture Period

Plate 7: Shoot Elongation Stage on MS Medium Supplemented with BAP 3 mg/l and GA_3 1 mg/l

Plate 8: Root Formation on MS Medium Supplemented with NAA 1 mg/l after Four Months of Culture Period

RESULTS

A. Shoot Initiation

Full strength MS medium was generally used for the induction of cultured shoots. The excised nodal stem segments with auxiliary buds of roses were induced to form shoots directly on MS medium supplemented with five different concentrations of BAP (1,2,3,4 and 5mg/l).

After about three weeks of culture period, shoot initiated from the explants in all different concentrations of BAP. Among them, BAP 3mg/l concentration was the best performance in shoot initiation (Plate 1) and in this case, most of cultures were found to regenerate shoots, the number of regenerated shoots per explant was 18 and the average number of leaves per explant was 119 and the length of shoot growth was16.4cm (Fig 1).

B. Shoot Multiplication

For investigation of *in vitro* shoot multiplication on rose, five different concentrations of BAP were used in MS media. The effect of BAP concentrations on multiplication rate depicted on MS media and the maximum shoot regeneration and the best regeneration of rose leaves were observed in BAP 3mg/l.

At this concentration, the regenerated shoot length was longer than any other four treatments. It was observed that when 3mg/l concentration of BAP was used in MS medium, average 18 shoots per culture were formed. When the concentration of BAP was observed from 1mg/l to 2mg/l, average number of shoot per culture decreased.

By further increase in concentration of BAP from 4mg/l to 5 mg/l, the average number of shoots per culture formed were also decreased. At 5mg/l BAP, the minimum number of shoots per culture were formed with 8 shoots per culture. Among these five different concentrations used for multiplication stage, the greatest number of multiple shoots was found in 3mg/l BAP concentration.

C. Shoot Elongation

In this experiment, different concentration and combination of BAP and GA_3 were used in MS medium for investigation of shoot elongation. By further increase in concentrations of GA_3 from 1.5mg/l to 2 mg/l, average length of shoots per culture formed was increased. At the combination of BAP 3mg/l and GA_3 1mg/l, 12.8 cm length of shoot per culture were obtained. Whereas at the combination of BAP 3mg/l with GA_3 2mg/l, the shortest of shoots per culture were observed (6.9 cm per culture).

D. Root Formation

Although rose shoots often proliferated readily *in vitro*, rooting those shoots was very difficult. Well established shoots from *in vitro* proliferating culture were placed on half strength MS media supplemented with four different concentrations of NAA viz. 0.5, 1, 1.5, 2 mg/l. Two weeks after transferring to rooting media, roots from regenerated shoots initiated in NAA 1mg/l concentration are shown in Table 4.1.But at NAA 0.5 mg/l concentration, there was no root formation from the inoculated shoots. The other two concentrations of NAA did not give the satisfactory results and at these concentrations, small roots were observed from the proliferated shoots. Among all these treatments, NAA 1mg/l concentration gave the best response in root induction of rose.

DISCUSSIONS

Two concepts such as plasticity and totipotency are central to understanding plant cell culture and regeneration. This plasticity allows plants to alter their metabolism, growth and development to best suit their environment. Particularly important aspects of this adaptation, as far as plant tissue culture and regeneration are concerned and are the abilities to initiate cell division from almost any tissue of the plant and to regenerate lost organs or undergo different developmental pathways in response to particular stimuli. There was a plasticity of rose on MS medium and the regenerated plant expressed true to type explants and showed totipotency which expresses the total genetic potential of the parent plants. There are five main classes of plant growth regulator used in plant cell culture, namely; auxins, cytokinins, gibbrellins, abscisis acid and ethylene.

In the first experiment reported here, different concentrations of BAP (ranging from 1 to 5 mg/l) were used in full strength MS media for shoot regeneration from internodal segments with auxiliary buds of rose. There was significant different among the effects of BAP on the number of regenerated shoots and the number of leaves according to the data. There was also significant difference in shoot length which was detected among all five concentrations tested.

In the shoot multiplication experiment, the effects of the presence of five concentrations of BAP were the same as above experiment were examined during the multiplication stage. The best shoot multiplication was occurred in MS media supplemented with BAP 3 mg/l reported by Hnin Yi Mon in her Master thesis (2007)[6]. According to the results recorded by May Phyo Thu (2007), BAP 3 mg/l gave the best results of shoot initiation at this concentration, shoot length was 9.28 mm and average shoots per bud was 23 after 45 days incubation period . In my study, when using BAP 1, 2, 3, 4 and 5 mg/l, BAP 3mg/l was more favorable for shoot multiplication than the other four treatments. In comparison with the former two candidates who researched on their Master thesis, BAP 3mg/l used in my research gave better result than the same concentration used in their experiments.

In the elongation of shoots, the effects of the combinations of BAP and GA_3 were examined in full strength MS media, the highest shoot elongation was occurred with the combination of BAP 3mg/l and GA_3 1mg/l and at which maximum shoot length 12.8 cm per culture were found. As previously reported in rose cultivars, the longest shoots were observed in MS media supplemented with 5 mg/l BAP.

In root formation, four concentrations of NAA on half strength MS salt were tested on shoots that had been regenerated from internodal segments. The effects of NAA supplementation at 1 mg/l on root initiation gave the best response on root initiation. The microshoots were rooted *in vitro* after eight days on half strength MS media supplemented with 0.5 mg/l NAA alone or in combination with 0.1 mg/l IBA reported by Mahmoud B. Arif and H. Khatamian in the journal of Transactions of the Kansas Academy of Science [8]. So, it can be seen that the best rooting response occurred in using NAA alone or the combination of NAA and IBA or IAA alone supplementation at half strength MS basal media.

CONCLUSION

In shoot regeneration, MS media supplemented with various concentrations of BAP were used. In this experiment, it was observed that the best shoot initiation for rose plant was MS basal medium supplemented with BAP 3 mg/l concentration. At this BAP concentration, the best shoot induction and multiplication the regenerated shoots were observed among all the treatments testing. Thus, it can be concluded that BAP 3mg/l supplementation to full MS media was the best for shoot regeneration and shoot multiplication. The minimum number of shoots was observed in the concentration of BAP 5mg/l. For shoot elongation of rose, the combination of BAP and GA_3 were examined in full strength MS media. In this experiment, the combination of BAP 3mg/l and GA_3 gave the best result. For root formation, using half strength MS media supplemented with different concentrations of NAA (0.5, 1, 1.5 and 2 mg/l), the best response to root induction was observed at the concentration of NAA 1mg/l. In this experiment, the concentration NAA 0.5 mg/l gave no rooting formation and there were little root formation in NAA 1.5 mg/l and 2 mg/l concentration. So, among all these four concentrations, NAA 1mg/l was suitable for rooting.

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