Pak. J. Bot., 41(6): 2877-2882, 2009.

EFFECT OF DIFFERENT CULTURAL CONDITIONS ON MICROPROPAGATION OF ROSE (*ROSA INDICA* L.)

ASAD SHABBIR[1], NOSHEEN HAMEED[1] AMIR ALI[2] AND RUKHSANA BAJWA[1]

[1]*Institute of Mycology & Plant Pathology, University of the Punjab Lahore, Pakistan.*
[2]*Punjab University Seed Centre, Quaid-e-Azam Campus, Lahore, Pakistan.*

Abstract

The aim of the present investigation was to determine a appropriate basal medium and growth regulators for *In vitro* propagation of *Rosa indica* from shoot apical meristems. The shoot meristem was cultured on MS medium supplemented with different concentrations of BAP ranging from 0.5–2.5 mg/l alone and in combination with 0.5 mg/l of kinetin for shoot formation and multiplication. Medium containing 1.5 mg/l BAP proved to be the best medium for *In vitro* shoot formation from apical meristem. At this concentration, rate of shoot formation was 94% obtained in 6.2 days. Maximum 20 shoots per culture were formed on medium having 1.0 mg/l of BAP + Kinetin. This medium proved to be the best for *In vitro* shoot multiplication and proliferation of roses.

Introduction

Among agricultural commodities, floriculture is an important sector with huge market potential worldwide. Unfortunately in Pakistan, floriculture is almost an ignored sector and a very little attention has been given to this direction. Rose "Queen of Flowers" is a beautiful flower of an immense horticultural importance. The genus *Rosa*, member of the family Rosaceae, comprises more than 100 species (Horn, 1992). There are more than 20,000 commercial cultivars, which collectively are based on only 8 wild species (Kim *et al.*, 2003a).

In vitro cultures are now being used as tools for the study of various basic problems in plant sciences. It is now possible to propagate all plants of economic importance in large numbers through tissue culture. 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. Plant tissue culture technique has many advantages over conventional vegetative propagation and the most important one is the propagation of a great number of pathogen-free plants in a short time with high uniformity. The main aim of the present study was to establish protocols for micropropagation of disease free and high quality plants of roses.

Materials and Methods

The explants used for the present investigation were shoot tips (shoot apical meristem) of healthy rose (Var. Cardinal) plants grown in rose garden of Quaid-e-Azam Campus, University of the Punjab, Lahore. The youngest leaves at the tip (that cover the apical dome) were removed from the base with the help of sterilized forceps. After this, the shoot tips were cut into segments of 4-5 mm each in length. Explants were first washed with running tap water and then treated with household detergent for five minutes. This was followed by second washing with tap water to remove all the traces of detergent. The explants were then treated with 10% Sodium hypochlorite for 15 minutes. After discarding Sodium hypochlorite, the explants were washed three times with sterilized distilled water to remove all the traces of Sodium hypochlorite.

Each explant was inoculated to the surface of the MS medium (Murashige & Skoog, 1962) with different concentrations of hormones. After inoculation all the culture tubes were placed in racks and were kept in the culture room. The cultures inoculated with explants were grown under carefully regulated temperature and light conditions. All culture were grown under 16 hours light and 8 hours dark period in air-conditioned culture room, illuminated by 40W (watts) white fluorescent tubes. The intensity of light was regulated between 2500-3000 lux. The temperature of culture room was maintained at 25±2°C (Thorpe, 1981). For shoot initiation and formation from shoot apical meristems, different concentrations of BAP (0.5, 1, 1.5, 2, and 2.5 mg/l) alone or in combination with BAP with kinetin (0.5) were used. For induction of shoot multiplication from *In vitro* grown plantlets of rose, different concentrations of BAP and BAP with kinetin were used. The data recorded was statistically analyzed using Duncan's Multiple Range Test (Steel & Torrie, 1980), to check the level of significance between the treatments.

Results

Effect of different concentrations of BAP on shoot formation from shoot apical meristem: Data on shoot formation revealed that when the concentration of BAP was increased from 0.5 mg/l to 1.0 mg/l, the rate of shoot formation was also increased and number of days was decreased. Maximum shoot formation from apical meristem was obtained at 1.5 mg/l of BAP which was 94 % after 6.2 days of inoculation (Table 1). With further increase in concentration of BAP, not only the rate of shoot formation was decreased but time taken for shoot formation was also increased. At 2.0 mg/l of BAP, 54% shoot formation was obtained after 10.6 days of inoculation while at 2.5 mg/l, it was 46% after 11.4 days of inoculation.

Effect of different concentrations of BAP with kinetin on shoot formation from shoot apical meristem: Data recorded on effect of different concentrations of BAP (ranging from 0.5–2.5 mg/l) with 0.5 mg/l kinetin on *In vitro* shoot formation from shoot apical meristem (Table 2). It was observed that when the concentration of BAP was increased from 0.5 mg/l to 1.0 mg/l with same concentration of kinetin (0.5 mg/l), the rate of shoot formation was also increased and numbers of days were decreased. At this concentration, 72% shoot formation was obtained within 11.2 days of apical meristem inoculation. Maximum shoot formation was obtained at 1.5 mg/ l of BAP with 0.5 mg/ l of kinetin where 92% shoot formation was obtained within 9.2 days of inoculation. It was observed that with further increase in concentration of BAP, not only the rate of shoot formation was decreased but time taken for shoot formation was also increased.

Shoot Multiplication: After a successful shoot formation from apical meristem of rose, they were further subcultured for *In vitro* shoot multiplication. For this purpose MS medium was supplemented with different combinations and concentrations of hormones.

Effect of different concentrations of BAP on *In vitro* shoot multiplication: For *In vitro* shoot multiplication, MS medium was supplemented with different concentrations of BAP ranging from 0.5 mg/l–2.5 mg/l (Table 3). It was noticed that when the concentration of BAP was increased from 0.5 mg/l to 1.0 mg/l, average number of shoots per culture formed was also increased. At this concentration, maximum number (20) of shoots per culture was formed with in 10-15 days (Table 3). By further increase in concentration of BAP, average number of shoots per culture formed decreased.

Table 1. Effect of different concentrations of BAP on shoot formation of rose from apical meristem.

MS+BAP medium conc. (mg/l)	No. of test tubes cultured	No. of test tubes showing shoot formation	Days for shoot formation	Rate of shoot formation (%)
0.5	10	6.6 ± 0.45 b	9.4 ± 0.21	66
1.0	10	7.2 ± 0.33 b	8.2 ± 0.17	72
1.5	10	9.4 ± 0.21 a	6.2 ± 0.17	94
2.0	10	5.4 ± 0.35 c	10.6 ± 0.35	54
2.5	10	4.6 ± 0.21c	11.4 ± 0.35	46

Abbreviation: ± Shows standard error of means of 3 replicates. Values with different letters show significant difference ($p \leq 0.05$) as determined by Duncan's Multiple Test.

Table 2. Effect of different concentrations of BAP with kinetin on shoot formation of rose from apical meristem.

MS+BAP+ kinetin medium conc. (mg/l)	No. of test tubes cultured	No. of test tubes showing shoot formation	Days for shoot formation	Rate of shoot formation (%)
0.5 + 0.5	10	6.4 ± 0.21 bc	12.4 ± 0.35	60
1.0 + 0.5	10	7.2 ± 0.17 b	11.2 ± 0.17	72
1.5 + 0.5	10	9.2 ± 0.17 a	9.2 ± 0.17	92
2.0 + 0.5	10	5.6 ± 0.21 c	12.2 ± 0.33	56
2.5 + 0.5	10	3.8 ± 0.33 d	15.4 ± 0.21	38

± Shows standard error of means of three replicates. Values with different letters show significant difference ($p \leq 0.05$) as determined by Duncan's Multiple Test.

Effect of different concentrations of BAP+Kinetin on *In vitro* shoot multiplication: Different concentrations of BAP ranging from 0.5 mg/l–2.5 mg/l with 0.5 mg/l kinetin in MS medium were also used for *In vitro* shoot multiplication. When the concentration of BAP was increased from 0.5 mg/l to 1.0 mg/l with same concentration of kinetin (0.5 mg/l), the numbers of shoots per culture formed also increased i.e., 18.4 shoots per culture. By further increase in concentration of BAP, average numbers of shoots per culture formed decreased. At 1.5 mg/l of BAP with 0.5 mg/l of kinetin, 13.2 shoots per culture obtained whereas average number of shoots decreased from 13.2 to 8.4 shoots per culture at 2.0 mg/l of BAP with 0.5 mg/l of kinetin (Table 4).

Discussion

The aim of present investigation was to optimize the culture conditions for *In vitro* micropropagation of rose from shoot apical meristems. To achieve this goal, different concentrations of growth regulators were used alone or in combination with one another. It is inferred from the results that shoot formation and multiplication was achieved on MS medium supplemented with various concentrations of BAP alone and in combination of BAP with kinetin. When different concentration of BAP ranging from 0.5 mg/l- 2.5 mg/l were used in MS medium, highest rate of shoot formation from shoot apex was obtained at 1.5 mg/l of BAP at 27°C.

Table 3. Effect of different concentrations of BAP on *In vitro* shoot multiplication of rose.

MS+BAP medium conc. (mg/l)	No. of cultures inoculated	No. of cultures showing shoot multiplication	Average no. of shoots / culture
0.5	10	6.4 ± 0.21 c	6.0 ± 0.63
1.0	10	10 ± 0.28 a	20 ± 0.80
1.5	10	9.8 ± 0.17 a	15.8 ± 0.33
2.0	10	8.2 ± 0.17 b	7.0 ± 0.40
2.5	10	5.6 ± 0.21 d	5.6 ± 0.21

± Shows standard error of means of three replicates. Values with different letters show significant difference ($p \leq 0.05$) as determined by Duncan's Multiple Test.

Table 4. Effect of different concentration of BAP with kinetin on *In vitro* shoot multiplication of rose.

MS+BAP medium conc. (mg/l)	No. of cultures inoculated	No. of cultures showing shoot multiplication	Average no. of shoots / culture
0.5 + 0.5	10	5.6 ± 0.21 bc	4.8 ± 0.33
1.0 + 0.5	10	9.2 ± 0.17 a	18.4 ± 0.21
1.5 + 0.5	10	8.2 ± 0.17 a	13.2 ± 0.52
2.0 + 0.5	10	6.6 ± 0.35 b	8.4 ± 0.35
2.5 + 0.5	10	5.4 ± 0.21 c	5.2 ± 0.17

Abbreviation: ± Shows standard error of means of three replicates. Values with different letters show significant difference ($p \leq 0.05$) as determined by Duncan's Multiple Test.

Many researchers have also reported this positive effect of BAP on shoot multiplication and proliferation. Ara *et al*., (1997) cultured shoot apical and nodal meristem or rose on different media and reported multiple shoot formation on MS medium with 1.0 mg/l of BAP. Similarly, Telgen *et al*., (1992) studied effects of different growth regulators and inhibitors on sprouting and outgrowth of isolated buds of different rose cultivars and reported bud growth stimulation by BAP. Hameed *et al*., (1993) found most prolific growth of *Rosa hybrida* (cultivars Diamond Jubly and Lans France) using 0.5 mg 2, 4-D/l plus 0.5 mg Kinetin/l. Yakimova *et al*., (2000) in a similar study noted the effects of BAP and CPPU on protease and α-amylase activity regarding the *In vitro* break and growth of lateral buds of rose (*Rosa hybrida* L. cvs. Medelon and Motrea) and reported the effectiveness of BAP for growth of lateral shoots.

During this investigation, it was also observed that 1.5 mg/l of BAP was best for shoot development whereas lower (0.5-1.0 mg/l) and higher concentrations (2.0-2.5 mg/l) inhibited it. This was in accordance with the study conducted by Kim *et al*., (2003b) in which they reported that lower concentrations of BAP (1.0–1.5 mg/l) stimulated the bud growth in six rose cultivars (*Rosa hybrida* L. cvs. "4th of July", Graham Thomas", "Tournament of Roses", "Sequoia Ruby", "Play boy") but higher concentrations of BAP (2.0-4.0 mg/l) inhibited shoot proliferation

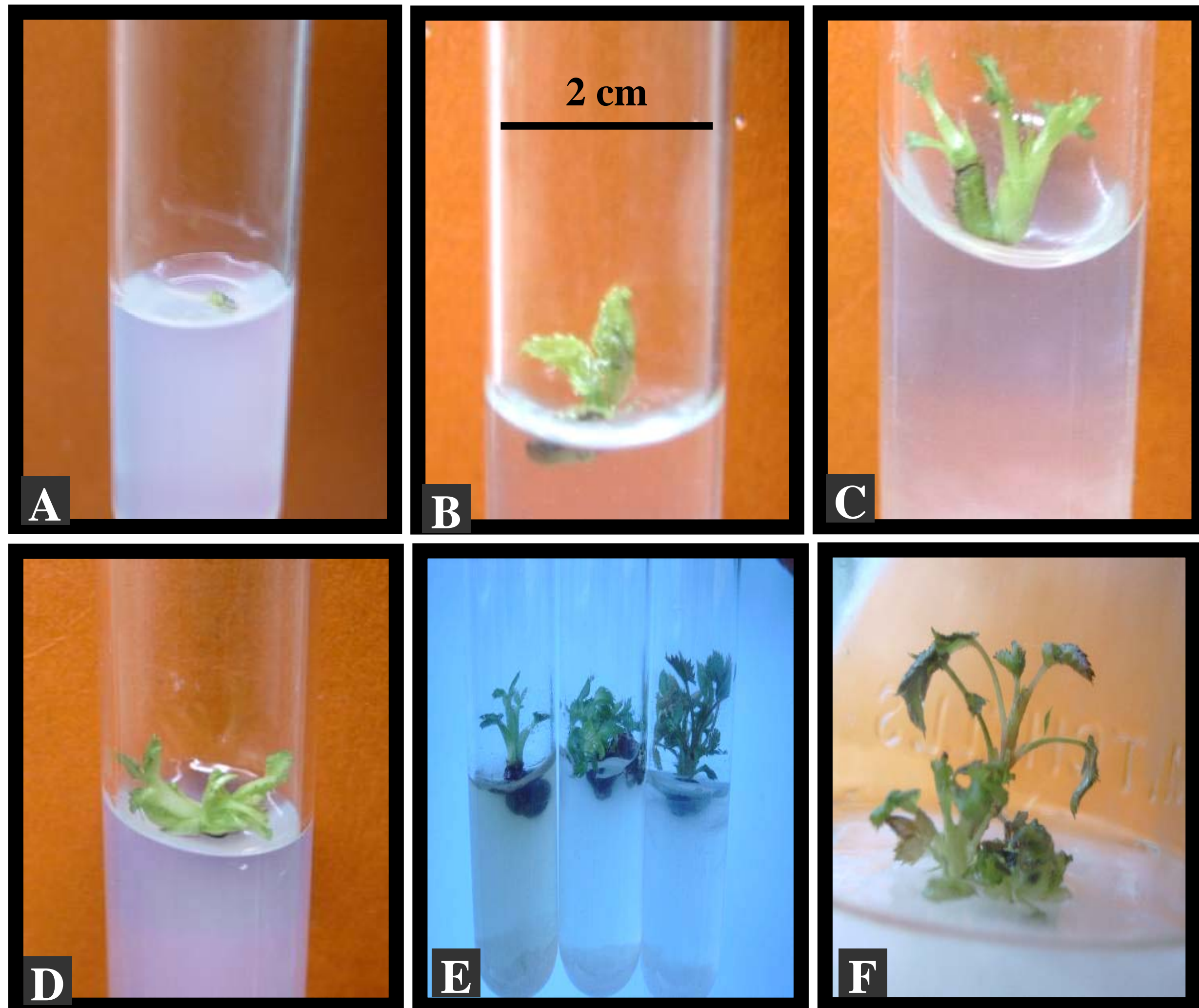

Fig. 1 A-F: Shoot apical meristem inoculated for shoot formation on 1mg/l of BAP+Kinetin (A). Shoot formation from apical meristem after 10 days of inoculation (B). Shoot formation from apical meristem after 12 days of inoculation (C). Shoot growth after 14 days of inoculation (D) Different stages of shoot induction and multiplication (E) Shoot multiplication and proliferation in after 6 weeks of inoculation (F).

When apical shoot was cultured on MS medium having concentration of BAP ranging from 0.5–2.5 mg/l with same concentration of kinetin (0.5 mg/l), maximum 98% shoot formation was obtained at 1.5 mg/l BAP with 0.5 mg/l kinetin. The highest rate of shoot proliferation was obtained on medium having 1.0 mg/l of BAP with 0.5 mg/l kinetin. The shoot proliferation and multiplication were decreased correspondingly with the increase in the concentration of BAP with 0.5 mg/l kinetin. This decrease in shoot multiplication with increasing concentration of BAP followed same pattern as studied by Kim *et al*., (2003b) and Davies (1980). These results however, differed slightly from the results obtained by Carelli & Echeverrigaray (2002). They obtained multiple rates of 30.3 shoots per explant of *Rosa hybrida* after 180 days on medium containing salt formulation of Quoirin and Lepoivre (Carelli & Echeverrigaray, 2002). This basal medium was supplemented with 0.5 mg/l NAA and 3 mg/l BAP. However the difference in results may be due to difference in salt formulation of medium and cultivars tested, the medium used in our studies and those of Davies (1980). Khawer *et al* (2005) achieved mass proliferation from *in vitro* cultured leaf explants of Madonna Lilly (*Lilium candidum* L.) using various concentrations of BAP-IBA.

In summary, results from present investigation manifest that micropropagation in *Rosa indica* can be achieved from different explants under physical and chemical conditions. However the main focus of this study was to actually see the reproducibility of previously reported protocols to roses. To be useful at a commercial level, micropropagation studies need further improvement both in terms of shoots as well as subsequent rooting. The hardening and acclimatization of micropropagated ornamental plants still remain a challenge.

References

Ara, K.A., M.M. Hossain, M.A Quasem, M. Ali and J.U. Ahmed. 1997. Micropropagation of rose: *Rosa sp.* cv. Peace. *Plant Tis Cult.,* 7(2): 135-142.

Carelli, V.P. and S. Echeverrigaray. 2002. An improved system for the *In vitro* propagation of rose cultivars. *Sci Hort.,* 92(1): 69-74.

Khawar K. M., Çöçü S., Parmaksiz I., Sarihan E. O., Sancak C. and S. Özcan 2005. Mass proliferation of Madona Lilly (*Lilium candidum* L.) under *in vitro* conditions. *Pak. J. Bot*, 37 (2): 243-248.

Davies, D.R.1980. Rapid propagation of roses *In vitro. Sci Hort.*, 13(4): 389:107-108.

Hameed S, Ahmad Z, Khan FZ and M. Akram (1993) Callus cultures of *Rosa hybrida* cvs. Diamond Jubly and Lans France. *Pak J. Bot* 25(2):193–198

Horn, W. 1992. Micropropagation of rose (*Rosa* sp. L.). In: *Biotechnology in Agriculture and Forestry*. (Ed.): Y.P.S. Bajaj. 20 – High-Tech and Micropropagation. Berlin: Springer–Verlag: 320-340.

Kim, C.K., J.D. Chung, S.O. Jee and J.Y. Oh. 2003a. Somatic embryogenesis from *in vitro* grown leaf explants of *Rosa hybrida* L. *J. Plant Biotech*., 5(3): 169-172.

Kim, C.K., J.Y. Oh, S.O. Jee and J. D. Chung. 2003b. *In vitro* micropropagation of *Rosa hybrida* L. *J. Plant Biotechnology*, 5(2): 115-119.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant.,* 15(3): 473-497.

Steel, R.G.D. and J.H. Torrie. 1980. *Principles and procedures of statistics*. *McGraw Hill Book Co*., Inc., New York, USA.

Telgen, H., V. Elagoz, A. Mil, A. Paffen and G. Klerk. 1992. Role of plant hormones in lateral bud growth of rose and apple *In vitro. Acta Hort*., 319: 137-142.

Thorpe, T.A. 1981. *Plant Tissue Culture: Methods and Applications in Agriculture*. *Academic Press*, New York. pp 74-81.

Yakimova, E., V. Kapchina-Toteva, I. Groshkoff and D. Ivanova. 2000. Effect of BA and CPPU on protease and a-amylase activity of *In vitro* cultured explants of *Rosa hybrida* L. *Bulg. J. Plant Physiol.,* 26(1-2): 39-47.

(Received for publication 15 July 2007)