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In vitro micropropagation of disease free rose (*Rosa indica* L.)

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Abstract

The rose is the most popular ornamental plant in the world, as well as the most important cut flower. Throughout history no other plant has such wide appeal and been the center of so much attention than the Rose. The aim of present investigation was to determine appropriate basal medium and growth regulators for *in vitro* propagation of *Rosa indica* from nodal meristem explants. Nodal meristem was cultured on MS medium supplemented with different concentrations of BAP ranging from 0.5 – 2.5 mg/l either 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 best medium for *in vitro* shoot formation. At this concentration, 100 % rate of shoot formation was obtained in 7.4 days of inoculation from nodal meristem. . Maximum 18 shoots per culture were formed on medium having 1.0 mg/l of BAP. This medium was proved to be best for *in vitro* shoot multiplication and proliferation of roses.
Key words: Plant tissue culture, micropropagation, rose,

Introduction

Plant tissue culture refers to the *in vitro* culture of plants from plant parts (tissues, organs, embryos, single cells, protoplasts, etc.) on nutrient media under aseptic conditions (Altman, 2000). *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 by tissue culture.

Rose "Queen of Flowers" is beautiful flower of immense horticultural importance. Rose (*Rosa indica* L.) is used in religious rituals, medicines and social events (Krussmann, 1981). One of the primary problems with the roses is their susceptibility to diseases like black spot (*Diplocarpon rosae),* powdery mildew (*Sphaerotheca pannosa),* bacterial blight etc (Horst 1983). 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.

In this context, plant tissue culture is the most efficient and reliable method for rapid and mass scale production of disease free and identical plants of roses through out the year. The main aim of the present study was to establish protocols for micropropagation of disease free plants of rose so as to ensure the year round availability of identical, disease-free and high quality planting material.

Materials and Methods

The explants used for the present investigation were nodal meristem of healthy rose plants grown in fields at the Quaid-e-Azam Campus, University of the Punjab, Lahore. Pre-sterilized shoot cuttings (6-7 mm) were taken. After this, the shoots were cut into segments of 4-5 mm each in length. Explants harvested 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 0.1% 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 MS medium with different concentrations of hormones. Care was taken not to dip explants completely in the medium and also tips of forceps should not touch the agar medium. The culture tubes were sealed immediately. The same procedure was repeated for multiple shoot formation. Forceps, scalpels, needles and other instruments were dipped in alcohol (70%v/v) and were flamed before use. All culture were grown under 16 hours light and 8 hours dark period in air-conditioned culture room, illuminated by 40W (watts) white fluorescent lights. The intensity of light was regulated between 2500-3000 lux. The temperature of culture room was maintained at 25±2°C.

For shoot formation from 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.

Results

Effect of different concentrations of BAP on shoot formation from nodal meristem In present investigation, for *in vitro* shoot formation from nodal meristem of rose, MS medium was supplemented with different concentrations of BAP ranging from 0.5 mg / l – 2.5 mg/l. At 1.0 mg/l concentration, 82 % shoot formation was obtained within 9.6 days of inoculation. Maximum (100 %) shoot formation from nodal meristem was obtained at 1.5 mg/l of BAP within 7.4 days of inoculation (Table 1). By 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, it was 68 % after 11.8 days of inoculation while at 2.5 mg/l, 52 % shoot formation was obtained after 13.4 days of inoculation.

Table 1: - Effect of different concentrations of MS+BAP on shoot formation from nodal meristem

Concentration (mg/l)	No. of test tubes showing shoot formation	Days for shoot formation	Rate of shoot formation (%)
0.5	6.4 ± 0.45	11.6 ± 0.35	64
1.0	8.2 ± 0.33	9.6 ± 0.45	82
1.5	10 ± 0.56	7.4 ± 0.21	100
2.0	6.8 ± 0.33	11.8 ± 0.33	68
2.5	5.2 ± 0.33	13.4 ± 0.45	52

Abbreviation: ± Standard error of means

Effect of different concentrations of BAP with Different concentrations of BAP (ranging from 0.5 – 2.5 mg/l) with 0.5 mg/l kinetin in MS medium were also used for *in vitro* shoot formation from nodal meristem. The effect of different concentrations of BAP with kinetin on shoot formation from nodal meristem is depicted in Table II. The table shows that when 0.5 mg /l of BAP with 0.5 mg / l of kinetin used in MS medium, 66 % shoot formation was obtained after 14.6 days of nodal meristem inoculation. It was observed that by keeping the concentration of kinetin constant and increasing the concentration of BAP from 0.5 mg / l to 1.0 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 13 days of nodal meristem inoculation. Maximum (98 %) shoot formation was obtained at 1.5 mg / l of BAP with 0.5 mg / l of kinetin within 10.4 days of inoculation. By further increase in concentration of BAP, not only the rate of shoot formation was decreased but time taken for shoot formation was also increased. The combination of 2.0 mg / l of BAP with 0.5 mg / l of kinetin showed 68 % shoot formation after 16.6 days of inoculation whereas 2.5 mg / l of BAP with 0.5 mg / l of kinetin showed 48 % shoot formation after 18.4 days of inoculation of nodal meristem (Table 2).

After successful shoot formation from apical 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.

Table 2: - Effect of different concentrations of MS+BAP+Kinetin with kinetin on shoot formation from nodal meristem

Concentration (mg/l)	No. of test tubes showing shoot formation	Days for shoot formation	Rate of shoot formation (%)
.5+0.5	6.6 ± 0.21	14.6 ± 0.45	66
1.0+0.5	7.2 ± 0.33	13.0 ± 0.28	72
1.5+0.5	9.8 ± 0.17	10.4 ± 0.21	98
2.0+0.5	6.8 ± 0.33	16.6 ± 0.21	68
2.5+0.5	4.8 ± 0.33	18.4 ± 0.21	48

Abbreviation: ± Standard error of means

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. It was observed that when 0.5 mg /l concentration of BAP was used in MS medium, average 6 shoots per culture were formed. When the concentration of BAP was increased from 0.5 mg / l to 1.0 mg / l, average numbers of shoots per culture formed were also increased. At this concentration, maximum number of shoots per culture was formed which were 18 shoots per culture. By further increase in concentration of BAP, average number of shoots per culture formed was decreased. At 1.5 mg / l of BAP, 15.8 shoots per culture were formed whereas average number of shoots decreased from 15.8 to 7 shoots per culture at 2.0 mg / l of BAP. At 2.5 mg / l of BAP, minimum number of shoots per culture was formed (6.6 shoots per culture). (Table 3)

Table 3: - Effect of different concentrations of MS+ BAP on *in vitro* shoot multiplication

Concentration (mg/l)	No. of cultures showing shoot multiplication	Average no. of shoots / culture
0.5	6.4 ± 0.21	6.0 ± 0.63
1.0	10 ± 0.28	18 ± 0.80
1.5	9.8 ± 0.17	15.8 ± 0.33
2.0	8.2 ± 0.17	7.0 ± 0.40
2.5	5.6 ± 0.21	6.6 ± 0.21

Abbreviation: ± Standard error of means

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. It was observed that when 0.5 mg /l of BAP with 0.5 mg / l of kinetin was used in MS medium, average 4.8 shoots per culture were formed. 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 were also increased. At this concentration, maximum 17.4 shoots per culture were formed (Table 4). By further increase in concentration of BAP, average numbers of shoots per culture formed were decreased. At 1.5 mg / l of BAP with 0.5 mg / l of kinetin, 13.2 shoots per culture were 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. At 2.5 mg / l of BAP and 0.5 mg / l of kinetin, minimum numbers of shoots per culture were formed (5.2 shoots per culture).

Table 4: - Effect of different concentration of MS + BAP + kinetin on *in vitro* shoot multiplication

Concentration (mg/l)	No. of cultures showing shoot multiplication	Average no. of shoots / culture
0.5+0.5	5.6 ± 0.21	4.8 ± 0.33
1.0+0.5	9.2 ± 0.17	17.4 ± 0.21
1.5+0.5	8.2 ± 0.17	13.2 ± 0.52
2.0+0.5	6.6 ± 0.35	8.4 ± 0.35
2.5+0.5	5.4 ± 0.21	6.2 ± 0.17

± Standard error of means

Discussion

The aim of present investigation was to optimize the culture conditions for *in vitro* micropropagation of rose from nodal meristems. To achieve this goal, different concentrations of growth regulators were used alone or in combination with one another.

It was cleared from the results that shoot formation was achieved on MS media supplemented with various concentrations of BAP alone and in combination 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 nodal meristem was

obtained at 1.5 mg/l of BAP at 27 °C with least duration taken to form shoots, whereas maximum 18 shoots per culture were formed at 1.0 mg/l of BAP.

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. Ara *et al* (1997) cultured apical and nodal meristem or rose on different media and reported multiple shoot formation on MS medium with 1.0 mg/l of BAP Yahkimova *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.

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.* (2003) and Davies (1980). These results however, differed slightly from the results obtained by Carelli and 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. 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).

During investigation, it was also observed that 1.5 mg/l of BAP was best for shoot 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.* (2003) 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. "Sequoia Ruby", "Play boy") but higher concentrations of BAP (2.0-4.0 mg/l) inhibited shoot proliferation.

In summary, results from present investigation manifest that micropropagation in *Rosa indica* can be achieved from nodal 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.

Fig.1 A-C: Nodal meristem inoculated for shoot formation (A). Multiple shoot formation medium after 22 days of inoculation (B). Shoot multiplication and proliferation after 6 weeks of inoculation (C).

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