Effect of 6-benzylaminopurine, naphthaleneacetic acid and activated charcoal on rose micropropagation using nodal explants

Hala M. Abdallah and Mohamed A. Ali

Agricultural Research Corporation, Tissue Culture Unit, Wad Medani, Sudan

ABSTRACT

Rose is one of the most important commercial flower crops. It is used in the floriculture and cut flower industry, perfume, cosmetic and medicinal purposes in many regions of the world. Conventional methods of rose propagation are slow with a low percentage of success. The objective of this study was to develop a protocol for *in vitro* propagation of rose, cv. Sara, using nodal explants. Three experiments were conducted during 2012. In the first experiment, the effects of different concentrations of 6-benzylaminopurine (BAP) (0.0, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/l) were tested on nodal explants of rose, cv. Sara. Different concentrations of naphthaleneacetic acid (NAA) (0.0, 0.1, 0.2 and 0.4 mg/l) combined with 1.0 mg/l BAP were tested in the second experiment. Factorial combinations between BAP at 0.0, 1.0 and 2.0 mg//l and activated charcoal (AC) (0.0 and 3.0 g/l) were tested. Completely randomized design was used in this study with five replicates and four explants per replicate. BAP at 1.0 mg/l was the best concentration for *in vitro* morphogenesis of rose, cv. Sara. The results showed that combinations of NAA with 1.0 mg/l BAP significantly reduced the number of shoots per explant. Addition of AC to MS medium devoid from BAP increased the number of shoots. BAP at 1.0 mg/l without AC was the best for shoot multiplication. AC with 2.0 mg/l BAP resulted in the maximum number of roots per plantlet.

INTRODUCTION

Rose is a woody perennial plant of the genus *Rosa*. It is a shrub which belongs to the family Rosaceae with medicinal values (Jung *et al*., 2005; Yoshizawa *et al*., 2000).Most species are native to Asia and smaller numbers are native to Europe, North America and northwest Africa. Roses are known as ornamental plants grown for their flowers in the garden and indoor. They also have other uses such as cut flowers, perfume, and medicine and into food and drink.

Rose cultivars are traditionally propagated by cuttings or grafting onto seedling or clonal rootstocks. These methods are expensive and they are not efficient to support the increasing demand for healthy plants. It takes a long time to produce enough number of plants for breeding programmes. In contrast to grafting, *in vitro* propagation can yield large numbers of self-rooted plants which are disease- free in a very short time and it has the ability to generate propagules around the year (Dhawan and Bhojwani, 1986). Micropropagation protocol proceeds for initiation of aseptic cultures, shoot multiplication, rooting of microshoots, hardening and field transfer of tissue culture raised plants were developed (Roy *et al*., 2004; Khosh-Khui and de Silva, 2006; Mukhambetzhanov *et al*., 2010). 6-benzylaminopurine (BAP) is a synthetic cytokinin growth regulator. It is a widely used cytokinin supplement to plant growth media. BAP is used in tissue culture media to promote axillary bud growth. Activated charcoal (AC) is pure carbon that has been specially processed to adsorb a wide variety of chemical compounds and

gases (Saenz *et al*., 2010). It has a very fine network of pores with large inner surface area on which many substances can be adsorbed. Activated charcoal is often used in tissue culture to improve cell growth and development. It promotes micropropagation, stem elongation and rooting of plants.

In Sudan, rose cv. Sara is the favourite cultivar for its large and attractive red flowers and for flowering all year around. The conventional propagation by grafting results in spreading of bacterial diseases and the rooting of cuttings is very difficult. Therefore, the objectives of this study were to

determine appropriate basal medium and growth regulators for *in vitro* propagation and multiplication of rose cultivar Sara.

MATERIALS AND METHODS

The research was conducted in the Tissue Culture Laboratory of the Agricultural Research Corporation, Wad Madani, Sudan in 2012. Nodal segments from mature plants were surface sterilized by washing under running tap water and dipping in ethanol (70%) for 10-15 seconds then they were soaked in 10% bleach (0.5% available Clorox) plus 2 drops of Tween- 20 for 10 minutes on a 60 rpm shaker. The explants were washed 3- 4 times with sterilized distilled water. Bleached cut ends of the sterilized nodal cuttings were removed before culturing on Murashige and Skoog (1962) medium containing 3% sucrose and solidified with 0.9% agar. The pH of culture media was adjusted to 5.8 before autoclaving at 121° C under pressure of 1.1 kg cm^{-2} for 15 min. Nodal cuttings obtained from plantlets were used as explants for shoot multiplication and rooting.

Three experiments were conducted. In the first experiment, the effects of 6-benzylaminopurine (BAP) at 0, 1, 2, 3, 4 and 5 mg/l were tested. Combinations of 1 mg/l BAP with different concentrations of NAA (0, 0.1, 0.2 and 0.4 mg/l) were tested in the second experiment. Factorial combinations between BAP at 0, 1 and 2 mg//l and activated charcoal (AC) at 0 and 3g/l were tested in the third experiment. All cultures were maintained in a growth chamber at 25º ± 2ºC under 16/8 h (light/dark) photoperiod. Completely randomized design was used for micropropagation studies. Four explants were inoculated per replicate and each treatment was replicated 5 times. All experiments were repeated twice. The data on percentage of survival rate, number of shoots and number of leaves per explant and plant height were taken after 4 weeks of incubation. Roots were counted and measured after 4 weeks of culture in the third experiment. Duncan's Multiple Range Test was used for means separation.

RESULTS AND DISSCUSSION

Table 1 shows no significant difference between BAP concentrations on the percentage of survival rate of the explants, but there were highly significant differences between the treatments and control on the number of shoots and number of leaves per explant. The results showed that when the

concentration of BAP was increased from 0 to 1 mg/l, the number of shoots and number of leaves per explants significantly increased but not plant height. With further increases in the concentration of BAP, number of leaves was significantly decreased. The number of shoots was comparable on BAP levels from 1 to 5 mg/l. The shoot height was comparable on BAP levels from zero to 3 mg/l then decreased significantly on higher concentration of BAP. It was found that 1 mg/l BAP was the best concentration for maximum number of shoots and number of leaves per explant and for the maximum plant height (Table 1).

Many researchers have reported the positive effect of BAP on shoot multiplication. The results of this study are in line with those reported by Ara *et al*. (1997) who cultured shoot apical and nodal meristems of rose on different media and reported multiple shoot formation on MS medium with 1.0 mg/l BAP. Kim *et al*. (2003) reported lower concentrations of BAP (1.0–1.5 mg/l) stimulated bud growth in six rose cultivars, however, higher concentrations of BAP (2.0-4.0 mg/l) inhibited shoot proliferation. This result was in accordance with the study of Rout *et al*. (1999) who reported that the desirable concentration of BAP mostly ranges between 1-2 mg/l. Kumar *et al*. (2000) reported that the best BAP concentration for micropropagation of Damask rose was 5 µg/l. Inclusion of BAP (1.0-10.0 mg /l) in the culture medium was essential for bud break and shoot multiplication of *R. hybrida* (Hasegawa, 1980). However, the difference in these results may be due to genetical differences between cultivars.

Table 1. Morphogenesis of nodal explants of rose cv. Sara cultured on MS medium containing different concentrations of BAP after 4 weeks of incubation.

BAP conc.(Mg/1)	Explants with shoots(%)	Number of shoots per explant	Number of leaves/exp	Plant height (cm)
0	100a	1.0b	4.7e	1.36ab
1	100a	3.5a	8.8a	1.50a
2	95a	2.3a	8.1b	1.36ab
3	95a	3.2a	7.7c	1.28bc
4	100a	3.2a	7.3d	1.22c
5	100a	3.4a	7.3d	1.22c
Sig. level	NS	***	***	**
SE±	2.89	0.17	0.23	0.02
CV%	6.6	13.2	3.2	7.0

, * and NS. indicate significance at P< 0.01, 0.001 and not significant, respectively. Means in columns followed by different letters are significantly different at p<0.05 according to Duncan's Multiple Range Test.

Table 2 shows that all concentrations of NAA combined with 1 mg/l BAP had 100% survival rate. There were highly significant differences between NAA concentrations on number of shoots and number of leaves per explant. The number of shoots per explant was decreased significantly with the increase in NAA concentrations. Maximum number of shoots per explant (3.0) was obtained on MS medium supplemented with 1mg/l BAP without NAA.

Maximum number of leaves per explant (9.4) was obtained on MS medium supplemented with 0.2 mg/l NAA, but it was comparable with that obtained on MS with 0.4 mg/l NAA (9.2).

When the NAA concentration increased from 0 to 0.4 mg/l, plant height was also increased. Shoot height
was comparable on NAA levels from zero to 0.2 mg/l. MS medium with 0.4 mg/l NAA was the best for plant height.

The results of this experiment are in line with the findings of Nikbakht *et al.* (2005) who reported that NAA had no effect on the proliferation of rose plants. In contrast, Khosh-Khui and Sink (1982) demonstrated that NAA was necessary for micropropagation of damask rose. In the present study, it was clear that 1 mg/l BAP without NAA was the best for shoot multiplication. 1mg/l BAP in combination with high concentrations of NAA is undesirable; it will lead to shoot elongation which is not beneficial for shoot multiplication. The differences in the results might have resulted from the different cultivars of rose tested.

Table 2. Morphogenesis of nodal explants of rose cv. Sara cultured on MS medium supplemented with different concentrations of NAA combined with 1mg/l BAP after 4 weeks of incubation.

NAA conc. (mg/1)	Explants with shoots(%)	Number of shoots per explant	Number of leaves/exp	Plant height (mm)
0	100	3.0a	7.8b	15.2b
0.1	100	2.4b	7.8b	15.6b
0.2	100	1.9c	9.4a	17.2a
0.4	100	9.2c	9.2a	17.2a
Sig level		***	***	*
SE+		0.12	0.19	0.3
CV%		8.7	3.7	7.3

* and *** indicate significance at P< 0.05 and 0.001, respectively. Means in columns followed by deferent letters are significantly different at p<0.05 according to Duncan's Multiple Range Test.

Table 3 shows that BAP alone or with 3 g/1 AC were significantly higher than the control in the survival rate. BAP at 1.0 mg/1 without charcoal had significantly the highest number of shoots and leaves per explant with means of 306 and 8.4, respectively. Addition of AC decreased the number of shoots and leaves per explant on the medium containing BAP and 3 g/1 AC was the best medium for plant height. It was observed thar AC positively affected number of shoots and leaves per explant, leaf expanding,

plant height and plant vigour (Table 3). This agreed with the results reported by Ibrahim (2000) how found that AC increased number of shoots and leaves per explant and growth vigour of plantlets of *A. senegal*.

Table 3. Effect of different concentrations of BAP with and without activated charcoal on shoot morphogenesis of rose cv. Sara after 4 weeks of incubation.

Treatments BAP mg/1	AC 3g/1	Explants with shoots(%)	Number of shoots per explant	Number of leaves/exp	Plant height (cm)
0	-	68b	1.0c	4.9e	1.4cd
0	+	100a	1.2c	6.7cd	2.1b
1	-	100a	3.6a	8.4a	1.5c
1	+	100a	1.1c	6.9bc	2.1b
2	-	100a	3.0b	7.1b	1.3d
2	+	100a	1.2c	6.5d	2.5a
Sig. level		***	***	***	***
SE±		2.34	0.20	0.20	0.09
CV%		4.7	9.3	4.3	9.8

*** indicate significance at P< 0.001. Means in columns followed by different letters are significantly different at p<0.05 according to Duncan's Multiple Range Test.
+ and - are with and without AC, respectively.

Activated charcoal improved root induction and root elongation. BAP at 1mg/l with 3 g/l AC was the best medium which had 100% of rooted plantlets and longest roots. However, MS with 2 mg/l BAP and 3 g/l charcoal produced maximum roots per explant. Roots were not induced on the medium containing 1 or 2 mg/l BAP without activated charcoal

(Table 4). Rumary and Thorpe (1984), who worked on spruce shoots, found that activated charcoal promoted elongation and rooting of *in vitro* produced shoots.

Reduction of the *in vitro* shoot multiplication rate and number of leaves and induction of root number and length on MS medium with 3 g/l AC and BAP, may be due to the partial absorption of BAP by AC. The promotary effects of AC on morphogenesis may be mainly due to its irreversible adsorption of inhibitory compounds in the culture medium and substantially decreasing the toxic metabolites, phenolic exudation and brown exudate accumulation. Tisserat (1979) suggested that addition of charcoal improved growth by reducing browning of the tissues during micropropagation of date palm.

Table 4. Effect of different concentrations of BAP with and without activated charcoal on root induction of rose cv. Sara after 4 weeks of incubation.

Treatments		Root formation	No. of roots	Roots length (cm)
BAP (mg/1	AC 3g/1)	(%)	per explant	
0	-	56 c	4.3 c	4.3 c
0	+	96 ab	6.1 b	4.5 bc
1	-	-	-	-
1	+	100 a	6.4 b	5.2 a
2	-	-	-	-
2	+	90 b	7.2 a	5.1 ab
Sig. level		***	***	*
SE±		4.0	0.27	0.12
CV%		7.4	6.2	8.9

* and *** indicate significance at P<0.05 and 0.001, respectively> Means in columns followed by different letters are significantly different at p<0.05 according to Duncan's Multiple Range Test.
- No response.
+ and -are with and without AC, respectively.

Conclusion

- BAP at 1 mg/l was the best concentration for shoot morphogenesis.
- Activated Charcoal at 3g/l positively affected root induction and root elongation.

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تأثير 6- بنزايل أمينوبيورين, حمض خلات النفثالين و الفحم النباتي على الإكثار الدقيق للورد الإنجليزي باستخدام العقد الساقية

هاله محمد عبدالله و محمد احمد علي
هيئة البحوث الزراعية, وحدة زراعة الأنسجة, واد مدني, السودان

الخلاصة

الورد الإنجليزي هو أحد أهم نباتات الزينة الاقتصادية المستوردة. يستخدم الورد الإنجليزي كنبات مزهر في تنسيق الحدائق و كأزهار للقطف و العطور و ادوات التجميل و الأغراض الطبية في كثير من أنحاء العالم. طرق الإكثار الخضري التقليدية للورد الإنجليزي بطيئة و نسبة نجاحها ضعيفة في مواسم محددة. الهدف من هذه الدراسة هو تطوير تقنية للإكثار الدقيق للورد الإنجليزي الصنف سارا باستخدام العقد الساقية. أجريت ثلاث تجارب في هذه الدراسة في العام 2012. في التجربة الأولي تمت دراسة تأثير ستة تراكيز هي (0.0، 1.0، 2.0، 3.0، 4.0 و 5.0 ملجم| لتر) من 6 - بنزايل أمينوبيورين (BAP) . في التجربة الثانية تم اختبار أربعة تراكيز هي (0.0، 0.1، 0.2، و 0.4 ملجم| لتر) من حمض خلات النفثالين (NAA) بالاتحاد مع 1 ملجم| لتر BAP. في التجربة الثالثة تم اختبار ثلاثة تراكيز هي(0.0, 1.0 و 2.0 ملجم| لتر) BAP مع تركيزين 0.0 و 3.0 جرام| لتر من الفحم النباتي. استخدم التصميم العشوائي الكامل في هذه الدراسة بخمس مكررات و أربعة عقد ساقية في كل مكرر. اوضحت الدراسة أن 1.0 ملجم| لتر من BAP هو أفضل تركيز لمقاييس النمو الخضري للصنف سارا. اتحاد NAA مع 1.0 ملجم| لتر من BAP أدي إلي انخفاض معنوي في عدد الأفرع (النموات الخضرية) لكل عقدة ساقية. إضافة الفحم النباتي الي وسط موراشيجي و سكوق الخالي من BAP أدي إلي ارتفاع عدد النموات الخضرية. كان 1.0 ملجم| لتر BAP من غير فحم نباتي الأفضل لتضاعف النموات الخضرية. كان الفحم النباتي مع 2.0 ملجم/لتر الأفضل للحصول علي أعلي عدد من الجذور لكل عقدة ساقية.