IN VITRO ROOTING OF HYBRID ROSE (*Rosa carolina L.*)SHOOTS AND SUBSEQUENT ESTABLISHMENT

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ABSTRACT

A successful *in vitro* propagation system has been developed for the valuable ornamental plant hybrid rose (*Rosa carolina* L.) by *in vitro* culture of shoot tips from mature plants. First shoot tip explants were excised and cultured on basal medium of Murashige and Skoog (1962) containing several concentrations of BAP and Kinetin. Multiple shoot formation of up to 3 shoots was obtained on MS medium supplemented with 4.0 mg/l BAP. Induced microshoots was then tested for root initiation on full MS medium supplemented with different concentrations of Indolbutyric acid (IBA) and Naphthalene acetic acid (NAA) and the best root formation was observed on a medium containing 1.0 and 2.0 mg/l of IBA and 2.0 mg/l of NAA. The regenerated plants were successfully transplanted to the greenhouse and appeared similar characteristics to the parent plants in morphologic features.

KEYWORDS: In vitro, *Hybrid rose, Rosa carolina*, *Rooting*.

INTRODUCTION

Roses considered as one of the world's most popular ornamental plants for a long time. There are more than 20000 commercial rose cultivars belong to the Rosaceae family and are grown worldwide as cut flowers and potted plants as well as in home gardens (Nak-Udom *et al*. 2009). It is generally propagated by vegetative methods like cuttings, layering, budding and grafting (Horn, 1992). Although propagation by vegetative means is a predominant technique in roses, yet it does not ensure healthy and disease –free plants. Moreover, dependence on season and slow multiplication rates are some of the other major limiting factors in conventional propagation. In the last few years, *in vitro* propagation has revolutionized commercial nursery business (Pierik, 1991). Significant features of *in vitro* propagation procedure are its enormous multiplicative capacity in a relatively short span of time; production of healthy and disease free plants; and its ability to generate propagules around the year (Dhawan and Bhojwani 1986; Martin 1985). Today, rose tissue cultures are exploited for various purposes, from basic anatomical and physiological research and extraction of androgenetic haploids to micropropagation from calli, immature embryos, or protoplasts. The micropropagation of 400,000 plants per year from a single rose bush is possible (Yan et *al*., 1996). The most important technique of micropropagation were reported by various researchers using cultures of apical meristems and axillary buds to regenerate multiple shoots without any intervening callus phase (Davies, 1980; Bressan *et al*. 1982; Barve *et al*.,1984; Douglas *et al*., 1989; Yan *et al*.,1996; Ara *et al*., 1997; Carelli and Echeverrigaray, 2002; Nak-Udom *et al*., 2009; Xing *et al*., 2010 ; Salekjalali *et al*. 2011; Pratheesh and AnilKumar 2012) .The aim of the present study was to describe optimal protocol for rapid clonal multiplication and root system induction from rose cultivated in Kurdistan Region of Iraq through high frequency axillary shoot proliferation from nodal explants.

MATERIALS AND METHODS

Healthy growing shoots measuring 8-10 cm. were collected from Malta Experimental Station, Duhok Province, Kurdistan Region of Iraq in April, 2009. The shoots were thoroughly washed under running tap water to remove dirts and dusts, followed by surface disinfestation by immersing in a mixture of 5% NaOCl commercial bleach solution containing few drops of Tween-20 surfactant. A gentile vacuum was applied for 15 min. to dislodge the air bubbles possibly captured within the tissues. Working within the confines of a laminar-air-flow hood, the disinfectant was discarded and the shoots were rinsed 3 times with autoclaved deinonized water and transferred to sterile Petri dish. The shoot apex, consisting of apical meristeme, leaf primordial and 1-2 expanded leaves were excised and transferred to culture vessel containing nutrient medium.

The nutrient medium employed in this investigation consisted of Murashige and Skoog

(1962) inorganic salts, in addition to the followings (in mg/l): sucrose (20,000), inositol (100), thiamine HCl (1.0), glycine (2.0), pyridoxine HCl (0.5), nicotinic acid (0.5) and BAP (1.0) . The pH of the medium was adjusted to 5.7± 0.1 with 1N NaOH or HCl, prior to the addition of agar (7, 000). The medium was brought up to the final volume, then dispensed at 25 ml rates, into 250 ml Mason jars and capped with colorless PVP covers and fitted with rubber bunds. The medium was then sterilized by autoclaving for 15 min. under 1.04 kg/cm² and allowed to solidify under room temperature.

Following culture initiation, the developed shoots were divided into nodal segments, measuring about 1 cm tall and containing 2 nodes, and cultured on fresh medium of the same composition, but supplemented with specific concentration of the tested cytokinin. The effects of BAP (Benzylamino purine) and Kinetin (N^{-6} furfurylamino purine) were tested for their ability to induce shoot multiplication. Both cytokinins were tested at 0.0, 2.0, 3.0, 4.0 and 5.0 mg/l concentrations. The number and length of the newly initiated shoots were recorded 6 weeks after incubation.

The auxins IBA (indole -1- butyric acid) and NAA (naphthalene -1- acetic acid) were tested for root induction in shoots produced from multiplication stage . The auxins were tested at the 0.0, 0.1 , 0.5 , 1.0 and 2.0 mg/l concentrations. Data, regarding root number and length, were collected following 6 weeks of growth on such media. A total of 24 replicates were initiated for each treatment. The least significant differences were determined and the means were compared according to Duncan's multiple range test ($P \leq 0.05$) using a computerized program of SAS (SAS, 2001).

Finally, for acclimatization stage, a quite number of successfully rooted plantlets were removed from culture vessels and their roots were washed with distilled water and immersed in Benlate fungicide (0.1% for 10 min.). They were transferred to pots containing a steam sterilized soil mix (peatmoss+ loam+ Styrofoam 1:1:0.5, v:v:v) under tightly controlled atmosphere of the greenhouse.

RESULTS AND DISCUSSION

Following 4 weeks of incubation, the explant developed into a shoot, measuring 3-4 cm. tall with several leaves. Figure (1) illustrates a one month old rose culture grown in a mason jar.

Figure (1) axillary shoot development from shoot tips explants of rose on MS medium supplemented with 1mg/l BA after 4 weeks of culture.

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1. Effects of cytokinins on shoot multiplication

Following four weeks of incubation of rose shoot tips on cytokinin - containing medium, new shoots were formed. The effect of BAP on shoot proliferation is shown in Table (1).The highest shoot number was attained at 4.0 mg/l BAP concentration and was significantly higher than other treatments, except the 3.0 mg/l treatment. Although the number of shoots reached its highest level at the 4.0 mg/l treatment, where 3.22 shoots were developed, the next highest number of shoots was attaind at the 3.0 mg/l (3.11 shoots/explant). The difference between these concentration was not significant. On the other hand, inclusion of 5.0 mg/l of BAP in the culture medium reduced the number of shoots, and only 2.67 shoots were developed. Similarly another reduction was achieved by reducing the BAP concentration to 2.0 mg/l; however, this reduction was significant (Table, 1).

Table (1) : Effect of BAP on shoot multiplication of rose.

BAP (mg/l)	Number of branches/ explant	Mean length of branches (cm)
0.0	1.0 d	1.78 a
2.0	2.22c	0.84 b
3.0	3.11a	0.99 b
4.0	3.22 a	0.86 b
5.0	2.67 b	0.90 b

Different letters within columns represent significant differences according to Duncan's multiple range test at 5% level.

The least shoot number was obtained in a medium devoid of BAP and was significantly different from all other treatments. Figure (2) shows representative cultures of rose that received 4.0 mg/l BAP treatment.

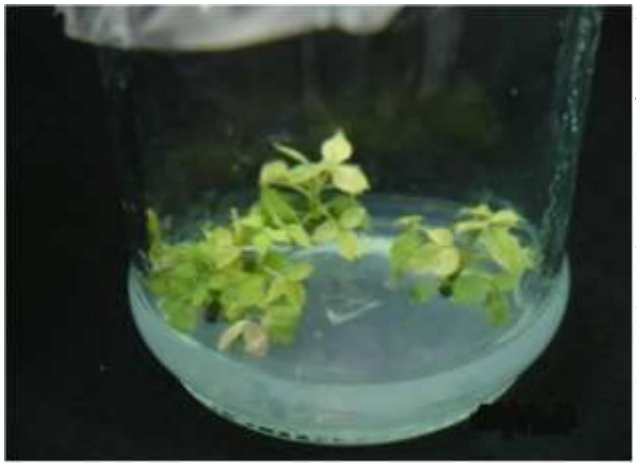

Figure (2) multiple shoots on MS medium supplemented with 4 mg/l BAP after 6 weeks of subculture.

The effect of BAP on shoot length is illustrated in Table (1). In general, the length of the shoot was reduced with different concentrations of BAP. The length of shoot averaged 1.78 cm, as mean, in the control which was significantly higher than shoots developed at different BAP concentrations. However, non significant reduction was observed between BAP treatments. The least length of shoots reached 0.84 cm in the medium supplemented with 2 mg /l BAP.

Inclusion of kinetin in the multiplication medium resulted in slight increase in shoot number; however, this increase was not always significant when compared to the control. The highest shoot number was attained in the presence of 3.0 and 4.0 mg/l of kinetin per liter medium which was not differed significantly from the control (Table, 2). In the presence of kinetin in the culture medium, no significant differences were observed among the treatments. Presence of kinetin in the culture medium caused a significant reduction in shoot length when compared to the control (Table, 2) with the exception of the 2.0 and 5.0 mg/l kinetin treatment.

Table (2): Effect of Kinetin on shoot multiplication of rose.

Kinetin (mg/l)	Number of branches/ explant	Mean length of branches (cm)
0.0	1.0 a	1.62 a
2.0	1.0 a	1.51 a
3.0	1.17 a	1.01 b
4.0	1.17 a	1.12 b
5.0	1.0 a	1.35 a

Different letters within columns represent significant differences according to Duncan's multiple range test at 5% level.

The role of cytokinins in multiplication of rose shoots is well documented.The beneficial effect of BAP was found to be 4mg/l BAP+0.1mg/l NAA (Yan *et a.*, 1996), while Nak-Udom *et al*. (2009) reported a 3mg/l BAP +0.003mg/l NAA level as optimal concentration for shoot multiplication, and an average of 3.2 shoots were developed from each explant at that level. These results are in agreement with those published by Razavizadeh and Ehsanpour (2008) and Xing *et al*. (2010). Similar observations have been reported for the non significance

effects of kinetin (Kanchanapoom *et al*, 2010). Data obtained in this investigation confirmed the forgoing observations. In general, the response to BAP was more pronounced when compared to kinetin in terms of the concentrations needed to achieve a comparable effect. The number of newly initiated shoots reached to 3.22 as an average at 4 mg/l BAP level, while it was necessary to double the concentration of kinetin to reach almost half the number of shoots. This variation in activity may be attributed to the presence of a benzyl ring in BAP structure which provides the cytokinin with more activity than other cytokinins (Economon and Spanodaki, 1985). The variation in cytokinin concentration observed in this investigation may also be attributed to the endogenous level of cytokinin in the mother plant, genetic make up and variety differences (Maheswaran *et al*., 2000).

2. Effects of auxins on rooting

Following shoot multiplication, two experiments were conducted to stimulate root initiation in excised shoots. The results reveled that the two auxins slightly varied in their activity in rooting induction, with NAA as the most active followed by IBA . Table (3) shows that the optimal concentration of NAA to induce rooting is 2.0 mg/l, where 12.57 roots as an average were initiated at each shoot, and it was significantly different than the control. A comparable number of roots were also developed at 1.0 mg/l NAA level; however, significant differences were observed among them. Other NAA concentrations (i.e. 0. 5 and 0.1 mg/l) were also stimulated rooting to a lesser extent; however, significant differences were observed between these two levels and control . As for root length, NAA significantly reduced the mean length of roots as compared to the control in which produced the longest roots estimated at 2.08 cm. Best root number and length were achieved at 2.0 mg/l NAA treatment (Figure,3).

Table (3): Effect of NAA on rooting of rose.

NAA (mg/l)	Number of roots/ explant	Mean length of roots (cm)
0.0	1.67 e	2.08 a
0.1	5.24 d	1.44 b
0.5	8.81 c	1.45 b
1.0	10.53 b	0.78 d
2.0	12.57 a	1.04 c

Different letters within columns represent significant differences according to Duncan's multiple range test at 5% level.

Figure (3) rooting with long roots on MS medium supplement with 2.0 mg/l NNA.

The auxin IBA also stimulated root initiation and development. The highest root number (11.5 root/ explant) was achieved when the medium was supplemented with 2.0 mg/l IBA. This treatment showed significant increase in root number when compared to all other treatments except 1.0 mg/l treatment which showed a significant increase in root number when compared to the control as well as other treatments but not among them (Table, 4). The least root number was achieved when 0.1 mg/l IBA was included in the medium. The highest length was scored at 0.1 and 0.5 mg/l IBA containing medium which significantly differed from all other treatments (Table, 4). Figure (4) illustrates the effects of different IBA concentrations on the rooting stage of rose plant shoots.

Table (4): Effect of IBA on rooting of rose .

IBA (mg/l)	Number of roots/ explant	Mean length of roots (cm)
0.0	1.11 d	1.05 b
0.1	5.13 c	1.67 a
0.5	8.47b	1.48a
1.0	10.67a	1.09b
2.0	11.5 a	0.48 c

Different letters within columns represent significant differences according to Duncan's multiple range test at 5% level.

Figure (4) microshoot of rose rooted on MS medium supplemented with different concentration of IBA (0.0, 2.0, 3.0, 4.0 and 5.0) from left to right

The role of various concentrations of different auxins on root induction is well documented in rose hybrid by Yan *et al*. (1996). In this investigation, it was disclosed that the synthetic auxin IAA was the most favorable auxin for root induction. Salekjalali *et al*. 2011; Xing *et al*. 2010; Senapati and Rout 2008; Razavizadeh and Ehsanpour 2008 ; Soomro *et al*. 2003 reported that the best root formation was achieved in the medium supplemented with IBA. The auxin plays a major role in root induction through their effect on the first cell division which forms root initials (Salman, 1988). In addition, there is a great deal of variation among plant species in their requirements for growth regulators *in vitro* (Murashige *et al*. 1974).The variation observed among various varieties of the same species may be attributable to the genetic make up, endogenous hormonal level, as well as the residual accumulation of auxin from previous culture treatment .

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التجذير خارج الجسم الحي للقمم النامية ونشوئها الأحقة لنبات الروز الهجين (*Rosa carolina* L)

الخلاصة

تم بنجاح تطوير نظام الاكثار الدقيق لنبات الزينة (الروز) خارج الجسم الحي عن طريق زراعة القمم النامية للنباتات الناضجة. تم عزل القمم النامية وزراعتها في وسط موراشيكي وسكوك الحاوي على تراكيز مختلفة من بترايل امينو بيورين والكاينتين حيث بلغ عدد الافرع اكثر من 3 فرع / نبات في الوسط المجهز ب 4 ملغرام/ لتر من البتريل امينو بيورين ، والافرع النابتة من مرحلة التضاعف تم حثها لتكوين الجذور وذلك بنقلها على الاوساط ذات القوة الكاملة للاملاح والحاوية على تراكيز مختلفة من حامض ايندول بيوتريك و نفثالين حامض الخليك ، و كان افضل الاوساط للتجذيرهي المجهزة ب 1 و2 ملغرام / لتر من حامض ايندول بيوتريك و 2 ملغرام / لتر من نفثالين حامض الخليك . النباتات النابتة من الزراعة النسيجية تم نقلها بنجاح الى الظروف الحقلية واظهرت صفات مظهرية مشابهه لنباتات الاصل.

رويهدان وپەروەریا گوپیتکێن شینبوونێ یێن رووەکێ رۆزا (*Rosa carolina* L.) دەرفەی لەشێ زیندی

پوختە

سیستەمەکێ زێدەکرنا هویر هاتە ب دەستفەئینان بو رووەکێ رۆزا دەرفەی لەشێ زیندی ب رێکا چاندنا گوپیتکێن شینبوونێ یێن رووەکێن گەهشتی. گوپیتکێن شینبوونێ هاتنە وەرگرتن وچاندن دبیافێ MS دا ئەوێ هاتیە بهێزکرن ب تیراتیێن ژێك جودا ژبنزیل ئەدەنین وکاینتین وژمارا تایا گەهشتە پتر ژ 3 تایا/ رووەك دبیافێ بهێزکری بـ 4 ملغم/ لتر ژ بنزیل ئەدەنین. تایێن هاتینە بەرهەمئینان دقوناغا دووهێندەبوونێ دا، هاتنە پالدان بو چێکرنا رویها ب رێکا فەگوهاستنا وان بو بیافێن رویهدانێ خودان هێزا تەواو ژخوویا دگەل تیراتیێن ژیك جودا IAA و NAA . باشترین بیافێن رویهدانێ ئەوبوون ئەوێن 1 و 2 ملغم/ لتر ژ IAA و 2ملغم/ لتر ژNAA. رووەکێن هاتینە بەرهەمئینان ب رێکا چاندنا شاناهاتنە فەگوهاستن بو دەرفەی لابورێ بشێوەکێ سەرکەفتیانا وساخلەتێن باش نیشاندان هەروەکی رووەکێن بناغەیی.