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In vitro proliferation for shoot tip and nodal explants of rosa canina

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Abstract

Most previous studies of *in vitro* rose cultures used medium supplemented with a combination of 6-benzylaminopurine (BAP) and Naphthalene acetic acid (NAA), but the medium with the combination of BAP and Gibberellic acid (GA_3) needs to be studied. The present study aims to test the effect of the BAP and GA_3 combination compared to the BAP and NAA combination on *Rosa canina* shoot multiplication. Shoot tip and nodal explants were cultured on MS media containing two concentrations of BAP (0.5 and 0.75 mg/L) combined with two levels (0.25 and 0.5 mg/L) of either NAA or GA_3. Medium content combination of BAP combined with GA_3 showed maximum *in vitro* shoot induction and multiplication for both shoot tip and nodal explants compared with the medium content combination of BAP with NAA. The medium content combination of 0.75 mg/L BAP with 0.5 mg/L GA_3 showed a maximum number of shoots, leaves number, and shoot length for both shoot tip and nodal explant of rose during shoot induction and multiplication period.

Keywords: *Rosa canina*, shoot multiplication, BAP, GA_3, NAA.

الملخص: استخدمت معظم الدراسات السابقة لزراعة الورد في المختبر وسط الزراعة المركب من 6-بنزيل أمينوبورين (BAP) مع حمض النفثالين أسيتيك (NAA)، لكن الوسط المركب من BAP مع حمض الجبر يليك (GA_3) لا يزال بحاجة إلى الدراسة. الهدف من هذه الدراسة هو اختبار تأثير الإضافة المتلازمة لكلٍ من الـ BAP والـ GA_3 مقارنة مع تأثير الإضافة المتلازمة لكلٍ من الـ BAP والـ NAA على تضاعف الأفرع لنبات الورد (*Rosa canina*). تم اكثار كلا من البراعم القمية والعقد الساقية باستخدام وسط الزراعة MS. احتوت بيئات الزراعة على تركيزين (0.5 and 0.75 mg/L) من الـ BAP وتركيزين (0.25 و 0.5 ملجم/لتر) من كل من الـ NAA والـ GA_3. أظهرت النتائج أن الوسط المحتوي على BAP مع GA_3 أعطى أقصى إمكانية لتحريض وتكاثر البراعم في المختبر لكل من البراعم القمية والعقد الساقية المستخدمة مقارنة بالوسط المحتوي على BAP مع NAA. أظهر الوسط المحتوي على 0.75 ملجم/ لتر BAP مع 0.5 ملجم/ لتر GA_3 الحد الأقصى لعدد البراعم وعدد الأوراق وطول الساق لكل من البراعم القمية والعقد الساقية للورد خلال فترة تحريض وتكاثر البراعم.

Introduction

Rosa canina known as dog rose, is one of the most popular and known species of rose [1], which is widely used as a rootstock for the propagation of many rose cultivars [2]. Rose species are typically propagated through seed in nature, but for commercial purposes, cultivars and rootstocks are generally preferred to be propagated vegetatively to obtain true-to-type propagules [3]. Recently,

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plant biotechnology techniques have been broadly used for the micropropagation of many ornamental plants, allowing for the rapid mass production of uniform and pathogen-free plants in a short period [4]. Recently, new challenges to improve the protocol of rose shoot multiplication and development with cost-effective methods have acquired importance [5].

Different reports about the micropropagation of roses have mentioned different types of hormones used for shoot multiplication. Formulation media containing cytokinin were largely used. Among the different types of cytokinin, BAP was the main one used [5].

In some cases, NAA or IAA at low concentrations was combined with BAP [6,7,8,9], and in a few cases, BAP was combined with GA_3 [10,11].
Studies of *in vitro* rose regeneration have mostly focused on the improvement of shoot multiplication of nodal explant using different hormones like BAP, NAA, GA_3, and 2,4-Dichlorophenoxyacetic acid (2,4-D) [12,13]. Each concentration of BAP combined with NAA or GA_3 has a different effect on shoot regeneration. So, this study aims to determine the best concentration of BAP combined with NAA or GA_3 for in vitro shoot multiplication of *Rosa canina*.

Materials and methods

Explants sterilization

Shoot tips (1cm in length) of newly growing branches of *Rosa canina* were collected and used as explants. The study was conducted in 2017 at the Plant Tissue Culture Laboratory, Ministry of Agriculture and Irrigation, Sana'a, Yemen.

To eliminate the superficial dust, the explants were washed using tap water. The explants were sterilized using 20% Clorox (Sodium hypochlorite 5.25%) for 15 min. and then rinsed thrice with sterile distilled water.

Shoot tip initiation and shoot induction.

Explants of shoot tips were cultured on a shoot initiation medium containing 30 g/L sucrose, 7 g/L agar, and 150 mg/L activated charcoal for 4 weeks. Murashige and Skoog (MS) medium [14], supplemented with different concentrations of BAP combined with either NAA or GA3 for shoot initiation as follows:
1. BAP at 0.5 mg/L and NAA at 0.25 mg/L (M1)
2. BAP at 0.75 mg/L and NAA at 0. 5 mg/L (M2)
3. BAP at 0.5 mg/L and GA_3 at 0.25 mg/L (M3)
4. BAP at 0.75 mg/L and GA_3 at 0. 5 mg/L (M4)

To study the effects of four media on shoot initiation, data was collected on a morphological characterization such as leaves number, shoot number, and average shoot length per explant, at weekly intervals, for 4 weeks.

Growth and multiplication of shoot tip stage

Previous studies showed that if small shoots after the first subculture were separated from each other and transferred to the second subculture, they were unsuccessful in growing [9]. Therefore, masses of shoot tips explants were transferred to the multiplication media without being separated from each other. For shoot multiplication and elongation, the mass of shoots produced from the initial culture was considered as an explant that was transferred to newly prepared media of the same four shoot initiation media for another 6 weeks. Data of the mean shoot number and mean shoot length (centimeters) per explant was recorded at the end of the shoot multiplication stage.

Nodal explants establishment and shoot proliferation

After 12 weeks of growing in the shoots' multiplication media, shoots were harvested and cut into nodal segments (1 cm) of newly produced stems cultured on the same four shoot multiplication media and kept for 12 weeks (two subcultures). After the end of the second subculture, data on mean number of shoots and mean length of shoot (cm) per nodal explant were recorded. Finally, well-growing shoots that grew in the two subcultures were separated from each other, before the root induction stage.

Rooting of shoots

For rooting stage, elongated shoots (3 to 4 cm in length) were separated and transferred to a half-strength MS medium, without hormone, supplemented with 30 g/L sucrose, 150 mg/L activated charcoal and 7 g/L agar for a period of 6 weeks (Fig.1). All in vitro cultures were incubated at 26 ± 2°C in the growth room under a 16/8-hour light/dark regime and 3,000 lux light intensity provided by cool-white fluorescent light.

Fig. 1: Rooting plants stage

Statistical analysis

All three experiments were carried out with three replicates. Collected data were analyzed using the SPSS (14 version) Statistical software program. The experimental data were subjected to analysis of variance (ANOVA), followed by the Student–Newman–Keuls (SNK) test at $p = 0.05$ level to compare means [15].

Results

Establishment of shoot tip explant culture and shoot induction

The media containing BAP with GA_3 (M4 and M3) showed maximum shoot growth per explant of shoot tips compared with media containing BAP with NAA (M1 and M2) during four weeks of the induction period. The media containing 0.75 mg/L BAP with 0.5 mg/L GA_3 showed maximum leaves

number, number, and length of shoots, per shoot tip explant during the induction period (Figure 2, Table 1).

From 1st to 4th week of culture incubation, the four media combination media of BAP with NAA and GA_3 showed a gradual increase in the number of leaves among them (Figure 2, Table 1). The highest leaf number per explant was 5.8 on media containing 0.75 mg/L BAP with 0.5 mg/L GA_3 (M4), followed by 4.6 on media containing 0.5 mg/L BAP with 0.25 mg/L GA_3 (M3). However, the least leaf number per explant (3.3) was obtained on media containing 0.75 mg/L BAP with 0.5 mg/L NAA (M2), (Figure 2, Table 1).

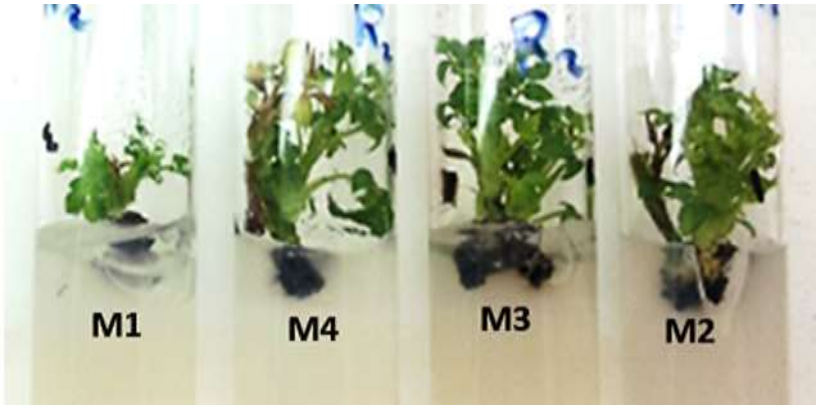

Fig. 2: Growth of shoot tip after three weeks on M1, M2, M3 and M4 medium.

Table 1: Effect of 4 different culture media on shooting characteristics (shoot number, shoot length, and leaves number) of *Rosa canina* shoot tip explant, during four weeks of culture

Media	Hormone combination			Periods (Week)	Mean Leaves number per Explant	Mean shoots number per Explant	Mean shoot length per Explant (cm)
	BAP	NAA	GA3				
M1	0.5	0.25	------	W1	1	1	0
				W2	1.8	1	0.6
				W3	2.5	1.5	0.8
				W4	4.1b	1.7bc	2.2bc
M2	0.75	0.5	------	W1	1	1	0
				W2	1.4	1	0.5
				W3	2.4	1.4	0.8
				W4	3.3c	1.6cd	1.7cd
M3	0.5	--------	0.25	W1	1	1	0
				W2	2.3	1	0.8
				W3	3.5	1.8	1.4
				W4	4.6ab	2.2ab	2.7ab
M4	0.75	-----	0.5	W1	1	1	0
				W2	2.6	1.5	0.8
				W3	3.9	1.8	2.3
				W4	5.8a	2.5a	3.4a

Values followed by the same letters (a–d) for each column are not significantly different at p = 0.05 level according to the SNK test.

A significant difference in shoot length was observed in the 4th week of explant culture on the four tested media combinations (Table 1& Figure 2). The highest shoot length per explant was 3.4 cm on 0.75 mg/L BAP and 0.5 mg/L GA3 (M4) that was on bar with shoot length (2.7 cm) of explant cultured on 0.5 mg/L BAP and 0.25 mg/L GA3 (M3), followed significantly by 2.2 cm on 0.5 mg/L BAP and 0.25 mg/L NAA (M1), and least was 1.7 cm on 0.75 mg/L BAP and 0.5 mg/L NAA (M2) (Figure 2, Table 1).
From week 1 to 4, the four combinations media of BAP with NAA and GA3 showed an increase in the number of shoots per explant. The maximum number of shoots was 2.5 on 0.75 mg/L BAP and 0.5 mg/L GA3 (M4) and 2.2 on 0.5 mg/L BAP and 0.25 mg/L GA3 (M3), followed by 1.7 on 0.5 mg/L BAP and 0.25 mg/L NAA (M1) and least was 1.6 on 0.75 mg/L BAP and 0.25 mg/L NAA (M2) (Figure 2, Table 1).

Growth and multiplication of shoot tip in the second subculture

The media containing combinations of BAP with GA3 (M4 and M3) showed a significantly greater shoot number and shoot length per shoot tip explant compared with media containing combinations of BAP with NAA (M1 and M2) at the end of the second subculture.
The mean of shoot number and shoot length (cm) per shoot tip explant at the end of the second subculture were determined (Table 2).

Table 2: Means of shoot number and shoot length (cm) per shoot tip explant after two subculture phases (12 weeks)

Hormone combination			**Mean shoots number per explant**	**Mean shoot length per explant (cm)**
BAP	NAA	GA3		
0.5	0.25	-----	2.7c	2.5c
0.75	0.5	------	2.4cd	2.2cd
0.5	------	0.25	3.6ab	3.7ab
0.75	------	0.5	4.2a	4.3a

Values followed by the same letters (a–d) for each column are not significantly different at p = 0.05 level according to the SNK test.

The maximum number of shoots was 4.2 when shoot tip explant subcultured on medium containing 0.75 mg/L BAP combined with 0.5 mg/L GA3 (M4), which was on bar with shoots (3.6) produced on medium containing 0.5 mg/L BAP and 0.25 mg/L GA3 (M3), followed by 2.7 on 0.5 mg/L BAP and 0.25 mg/L NAA (M1) and least was 2.4 on 0.75 mg/L BAP and 0.25 mg/L NAA (M2) (Table 2).
The highest shoot length per shoot tip explant was 4.3 cm on 0.75 mg/L BAP and 0.5 mg/L GA3 (M4) and 3.7 cm on 0.5 mg/L BAP and 0.25 mg/L GA3 (M3), followed by 2.5 cm on 0.5 mg/L BAP and 0.1 mg/L NAA (M1) and least was 2.2 cm on 0.75 mg/L BAP and 0.5 mg/L NAA (M2) (Table 2).

Nodal explants establishment and shoot multiplication

Significant differences were observed between the four tested media on each of the shoot number and shoot length characters. The media containing BAP with GA3 (M4 and M3) showed a greater number of shoots and length of shoots per nodal explant than the media containing BAP with NAA (M1 and M2).
The maximum number of shoots per nodal explant was 4.2 on 0.75 mg/L BAP and 0.5 mg/L GA3 (M4) and 3.8 on 0.5 mg/L BAP and 0.25 mg/L GA3 (M3), followed by 2.6 on 0.5 mg/L BAP and 0.25 mg/L NAA (M1) and least was 2.3 on 0.75 mg/L BAP and 0.5 mg/L NAA (M2) (Table 3). The

highest shoot length per nodal explant was 3.4 cm on 0.75 mg/L BAP and 0.5 mg/L GA3 (M4) and 2.8 cm on 0.5 mg/L BAP and 0.25 mg/L GA3 (M3), followed by 2.3 cm on 0.5 mg/L BAP and 0.25 mg/L NAA (M1) and least was 2.1 cm on 0.75 mg/L BAP and 0.5 mg/L NAA (M2) (Table 3).

Table 3: Means of shoot number and shoot length (cm) per nodal explant after two subculture phases (12 weeks)

Hormone combination			**Mean shoots number per nodal explant**	**Mean shoot length per nodal explant (cm)**
BAP	NAA	GA3		
0.5	0.25	-----	2.6c	2.3c
0.75	0.5	------	2.3cd	2.1cd
0.5	------	0.25	3.8ab	2.8ab
0.75	------	0.5	4.2a	3.4a

Values followed by the same letters (a–d) for each column are not significantly different at p = 0.05 level according to the SNK test.

Discussion

In this study, the media containing BAP with GA_3 (M4 and M3) showed a significant number of shoots and shoot lengths per both shoot tip and nodal explant compared to the media containing BAP with NAA. According to previous studies, researchers in the micropropagation of roses have different reports about determining the optimal composition hormones in shoot multiplication media and rose regeneration. Zapata et al, [16] and Noodezh et al, [9] believe that the meristem of the shoot tip has inductive properties, so using a medium hormone-free for the initiation phase led to the best response for rose micropropagation. At the same time, others including Pati et al. [5] believe that BAP is the most important hormone used. In some cases, low concentrations of NAA and IAA combined with BAP were used [6,7,8], in a few cases, BAP combined with GA_3 was used [10,11]. Our results are in line with some recent studies [17,18,19,20] that confirmed the combination of BAP with GA_3 increased the shoot tip growth and the shoot multiplication in rose.

The media containing BAP of 0.5 and 0.75 mg/L combined with GA3 of 0.25 and 0.5 mg/L, showed a maximum shoot number and shoot length for both shoot tip and nodal explant. A similar result and response were obtained by Pahnekolayi, et al. [21], Moallem, et al. [19], and Kavand, et al. [18], they showed that low concentrations of GA3 combined with BAP were most effective in shoots number and shoot length. GA3 participates in the shoot tip induction, multiplication, and overall growth stimulation. The shoot tip started to grow after five days GA3 was added. GA3 is confirmed to increase the shoot's growth, by activating mitotic division and elongation of cells [22, 23]. The GA3 positive influences on breaking the dormancy in buds have been mentioned in the woody plant tissue culture [24, 25].

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