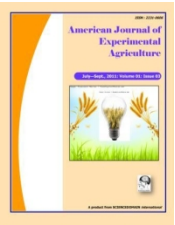

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Rooting Response of *Rosa canina* and *Cotoneaster acuminatus* to Different *in vitro* Factors

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Authors' contributions

This work was carried out in collaboration between all authors. Authors RST and LSMA designed the study. The first author RST performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors LSMA and HSF managed the lab work of the study. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

The current study aimed to test the direct rooting response of rose and cotoneaster explants collected in winter and spring and grown on different culture media including WPM, B5 and MS supplemented with different concentrations of IBA and NAA. The experiments were arranged according to Complete Randomized Design (CRD) and were conducted at the plant tissue culture laboratories of the Faculty of Agriculture and Forestry, University of Duhok, Iraq during the period between December 2012 and August 2013. The results showed that explants collected in winter and spring failed to root in both genotypes tested except cotoneaster explants collected in spring. The best rooting performance was found on those grown on WPM medium containing 0.1 mgl^{-1} IBA by giving 5.62 roots per explant and the longest roots reached to 4.42 cm. This root formation required 22 days from culture date. Concerning shoot multiplication parameters recorded for both plants during this study, rose explants taken in winter and grown on WPM medium containing 0.1 mgl^{-1} IBA gave the maximum number of shoot per explant reached to 2.62. The maximum number of shoots per explant for cotoneaster (3.0 shoots/ explant) was recorded when the explants were collected in spring and grown on WPM medium supplemented with 0.5mgl^{-1} IBA. WPM medium gave the longest shoots (6.98 cm) on rose explants collected in spring and supplemented with 0.5mgl^{-1}IBA. MS medium supplemented with 0.5mgl^{-1}IBA gave the

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longest cotoneaster shoots estimated at 3.37 cm which also recorded the highest number of leaves (13.87 leaves). The highest of leaves per explant of rose reached 21.75 leaves/ explant which were obtained on explants grown on WPM medium supplemented with 0.1mgl^{-1} IBA. It can be concluded that cotoneaster plant can be rooted directly from explants collected in spring season, whereas rose plant need to undergo the developmental stages of usual micropropagation protocols.

Keywords: Rosa canina L.; Cotoneaster acuminatus; direct in vitro rooting; WPM; B5; MS.

1. INTRODUCTION

Rose plant *Rosa canina* L. is a species native to Europe, northwest Africa and western Asia. Itbelongs to Rosaceae family. Rose leaves are pinnate and of 5 to 7 leaflets. Flowers comprise of 5 pink or white petals. Flowers often solitary but sometimes produced in small groups. The plant is high in certain antioxidants. The cultivation of rose plant through unconventional techniques offer many advantages arising from opportunities to improve, conserve and perpetuate a plant with food and pharmaceutical value. Conventionally, seeds are usually used for propagation of rose species, new cultivars and for production of rootstocks [1]. I recent years, in vitro propagation technique has developed marketable nursery business [2]. Significantly feathers of in vitro propagation procedure are its massive multiplicative ability in a relatively short span of time; production of healthy and disease free plants; and its capability to generate support gules around the year [3].

"*Cotoneaster acuminatus* is a species of flowering plant in the Rosaceae family native to the Himalayas. Cotoneaster has dimorphic shoots, with elongated shoots (10-40 cm) producing composition branch growth, and short shoots (0.5-5 cm) carrying the flowers; this pattern often develops a 'winding' from of branching. The leaves are arranged interchangeably, 0.5–15cm long, ovate to lance late, entire; both evergreen and deciduous species occur. The flowers are produced in late spring through early summer, lonely or in corymbs of up to 100 together. The flower is either fully opens or has its five petals half open 5–10 mm in diameter" [4]. Cotoneasters are traditionally propagated by seeds and cuttings. Cotoneaster plant is very difficult to propagate due to double seed dormancy and least rooting of cutting. The difficult correlating with propagation by classical methods can be overcome using tissue culture methods. Plant tissue culture techniques are considered as easy and reliable methods for rapid propagation of plants, especially infrequent and endangered plant species [5,6].

Rooting of shoots remains the most challenged point of the micropropagation process, reducing the possibilities of applying this technique on a large scale. Root initiation is a complex morphogenic process including a major metabolic switch. Despite of that auxins are the main factor involved in inducing rooting; other endogenous and exogenous substances also influence the rhizogenic process. [7] reported other factors like the basal culture medium concentration, the carbohydrate source, light, temperature and the presence of phenolic compounds. However, the rhizogenic process is not limited to root induction, since other two steps, expression and root elongation are recognized [8]. Direct in vitro rooting on explants taken from the field will highly reduce the costs of any micropropagation protocol leading to plant mass production. [9] when trying to get direct regeneration of Rosa canina plants during the tissue culture technique, they achieved the highest root length in 1 mgl^{-1} NAA with 0.25 mg/l BAP (4.167±0.15 cm) while, the minimum root length and the minimum

rooting percentage (73%) is related 1 mgl^{-1} BAP (1.297±0.03 cm). [10] When were *in vitro* propagating Cotoneaster wilsonii reached the maximum rooting on ½ MS medium containing 0.5mgl^{-1} IBA.

The goals of the study were to achieve a high rate of direct rooted plants for a large scale propagation of *Rosa canina* L. and *Cotoeaster acuminatus*, with regard to the effect of culturing on different nutrient media, different growth regulator concentrations and the date of taking explants, A set of conditions that allow for such goals, at least in these plants, are reported in the current study.

2. MATERIALS AND METHODS

This study was conducted at Plant Tissue Culture laboratory of the Horticulture Department, School of Plant Production, Faculty of Agriculture and Forestry, University of Duhok, Iraq. Three different culture media including WPM, B5 and MS each enriched with different concentrations of IBA and NAA (0, 0.1 and 0.5 mgl^{-1}) were prepared for investigating their effect on direct rooting of rose and cotoneaster explants taken from an open field. Rose and cotoneaster cuttings of 10 cm in length were taken directly from the Faculty of Agriculture and Forestry yards on December, 2012 and April, 2013 for two seasons respectively. They were washed thoroughly with tap water and cleanser for 30 minutes, and then they were transferred to the Laminar Air-flow Cabinet for disinfestations. The explants were cut into parts of 1.5 cm in length and then surface sterilized with ethyl alcohol (70%) for 3 minutes followed by immersing in 0.1% mercuric chloride ($HgCl_2$) for 5 minutes and finally the explants were rinsed in sterilized distilled water three times for five minutes each.

Working within the confines of a Laminar-air-flow hood, the explants were removed into Petri dishes and shortened to about 1 cm in length using fine tip scalpels and forceps. Shoot tips of both explants were cultured on WPM, B5 and MS media containing 30gl^{-1} sucrose, 7gl^{-1} agar. Explants were cultured on culture test tubes with their cut base embed in the culture medium and each treatment was replicated ten times. Culture were kept at constant 25±2°C and exposed to 16h daily to 1000 lux lighting. After 6 weeks in culture, number of days required for rooting, number of roots per explants and the mean length of roots produced were recorded as rooting parameters. On the other hand, number of shoots, mean length of shoots and number of leaves per explant were recorded as vegetative growth response parameters. The investigated was designed as Complete Randomized Design (CRD). The comparison between means was carried out according to Duncan's multiple range test ($P < 0.05$.

3. RESULTS AND DISCUSSION

Table 1 shows the effect of the three tested culture media (WPM, B5 and MS) enriched with different concentrations of both auxins IBA and NAA on rose and cotoneaster explants direct rooting response taken in winter and spring. The results clearly show that taking explants during the dormant plant season (December) did not give any rooting response on both plants tested. On the other hand, at the second season (April), cotoneaster explants appeared a good response to the different culture media and auxins different concentration by giving a good root initiation. But rose explants did not show any rooting response even at the spring season (Fig. 1, A). Using WPM medium supplemented with 0.1mgl^{-1}IBA was the best treatment for direct rooting of cotoneaster explants by giving the highest number of roots per explant of cotoneaster (5.62 roots/ explant) and the longest roots reached to 4.42

cm (Fig. 1, B and E). This root formation required 22 days from culture date as compared with 15 days for rooting on MS medium containing 0.1 mgl^{-1} IBA and 32 days for rooting on B5 medium containing 0.5mgl^{-1}NAA.

Table 1. Effect of culture medium and different concentrations of IBA and NAA on rose and cotoneaster rooting stage on explants taken in December and April after six weeks in culture

Culture Medium	IBA and NAA (mgl^{-1})		Rose			Cotoneaster		
			Days for rooting	Number of Roots/ Explant	Mean Length of Roots (cm)	Days for rooting	Number of Roots/ Explant	Mean Length of Roots (cm)
Winter Season								
	0.0		0	0	0	0	0	0
WPM	IBA	0.1	0	0	0	0	0	0
		0.5	0	0	0	0	0	0
	NAA	0.1	0	0	0	0	0	0
		0.5	0	0	0	0	0	0
	0.0		0	0	0	0	0	0
	IBA	0.1	0	0	0	0	0	0
B5		0.5	0	0	0	0	0	0
	NAA	0.1	0	0	0	0	0	0
		0.5	0	0	0	0	0	0
	0.0		0	0	0	0	0	0
	IBA	0.1	0	0	0	0	0	0
MS		0.5	0	0	0	0	0	0
	NAA	0.1	0	0	0	0	0	0
		0.5	0	0	0	0	0	0
Spring Season								
		0.0	0	0	0	0 d	0 e	0
	IBA	0.1	0	0	0	22 b	5.62 a	4.42 a
WPM		0.5	0	0	0	28 a	2.00 cd	2.13 d
	NAA	0.1	0	0	0	24 b	2.87 c	3.01 b
		0.5	0	0	0	32 a	1.62 d	1.46 d
		0.0	0	0	0	0 d	0 e	0 e
	IBA	0.1	0	0	0	28 a	2.00 cd	3.71 b
B5		0.5	0	0	0	30 a	2.00 cd	2.10 d
	NAA	0.1	0	0	0	30 a	1.88 cd	1.66 d
		0.5	0	0	0	32 a	1.63 d	1.22 d
		0.0	0	0	0	0 d	0 e	0 e
	IBA	0.1	0	0	0	15 c	4.75 b	4.41 a
MS		0.5	0	0	0	22 b	2.00 cd	3.41 b
	NAA	0.1	0	0	0	19 b	3.00 c	2.87 c
		0.5	0	0	0	23 c	1.75 d	2.81 c

**Means followed by the same letter within each character (column) do not differ significantly (P≤0.05) according to Duncan's Multiple Range Test (Duncan, 1955).*

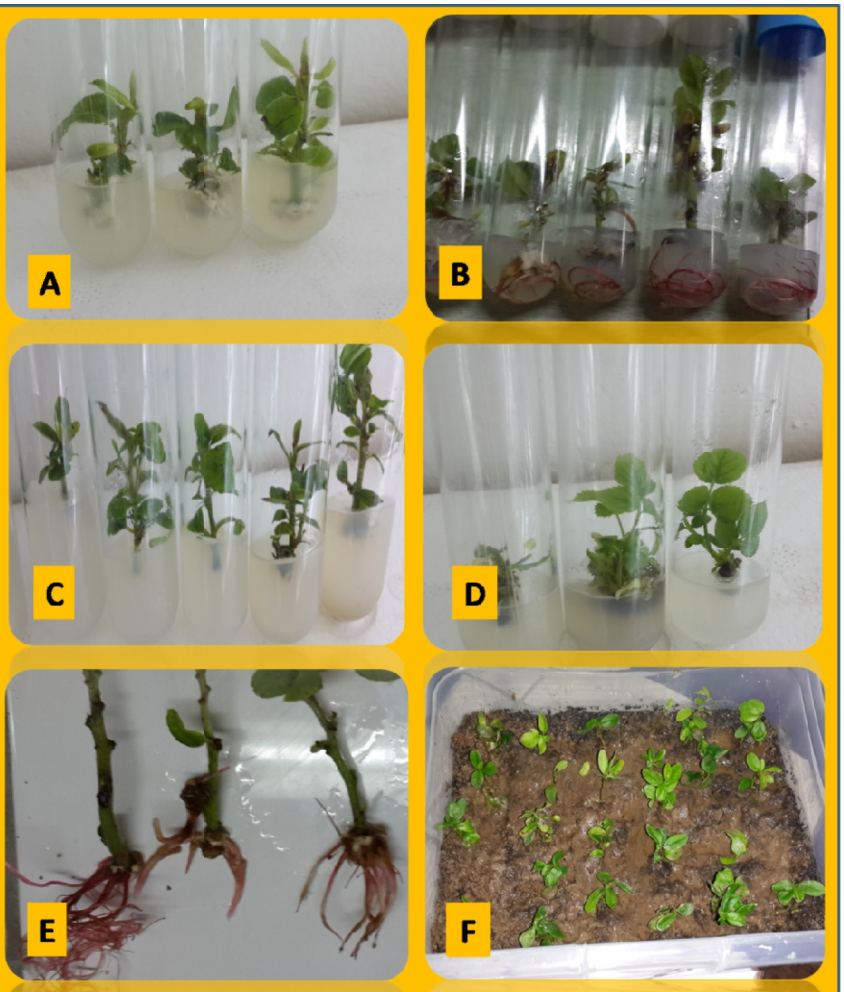

Figure 1: Rose and cotoneaster direct *in vitro* rooting

A. Non rooted rose explants taken in April and grown on WPM medium supplemented with 0.1 mg/l IBA

B. Well rooted cotoneaster explants taken in April

C. Multiple shoots elongation of Cotoneaster on WPM medium supplemented with 0.5mgl^{-1} IBA.

D. Proliferated shoots of Rose on WPM medium supplemented with 0.1mgL^{-1} IBA.

E. The best cotoneaster rooting on WPM medium supplemented with 0.1 mg/l IBA.

F. Cotoneaster plantlets at acclimatization stage.

These results confirm that the best time for collecting explants to be cultured *in vitro* is in spring season when the plant is at active physiological conditions and has a high endogenous level of phytohormones which is associated with changes in rooting co-factor activity [11]. As well as, they confirm that *in vitro* rooting response is highly dependent on plant genotype [12]. In conclusion, direct rooting results obtained in this investigation confirmed the need for auxins for rose and cotoneaster adventitious root formation. These results indicated that the presence of auxins had positive influences on rhizogenesis in rose and cotoneaster *in vitro.* In addition, the most effective auxin in the rooting was IBA followed by NAA. Such differences in the potency of auxin in inducing rooting might attributed to the structure of the auxins under study, the endogenous hormone level, as well as the genetic makeup of species under consideration [13].

Accompanied with direct rooting test, number of shoots per explant, mean length of shoots and number of leaves per explant were recorded as shoot multiplication parameter reflecting the growth response of rose and cotoneaster on different culture media with different concentrations of IBA and NAA (Fig. 1, C and D). Table 2 declares that rose explants taken in winter and grown on WPM medium containing 0.1 mgl^{-1} IBA gave the highest number of shoots per explant reached to 2.62, whereas the lowest number of shoots for rose (1.12 shoots/ explant) was recorded when the explants were taken in spring and cultured on WPM supplemented with 0.1 mgl^{-1} NAA and on auxin-free MS medium. On the other hand the maximum number of shoots per explant for cotoneaster (3.0 shoots/ explant) was recorded when the explants were collected in spring and grown on WPM medium containing 0.5mgl^{-1}. WPM medium gave the longest shoots (6.98 cm) on rose explants collected in spring highest and containing 0.5mgl^{-1} IBA. While concerning cotoneaster plant, MS medium containing 0.5 mgl^{-1}IBA gave the longest shoots estimated at 3.37 cm which also gave the best number of leaves reached to 13.87 leaves on explants collected in spring. Otherwise, the best number of leaves per explant of rose reached 21.75 leaves/ explant which were recorded while culturing the explants on WPM medium containing 0.1 mgl^{-1}IBA. These results confirm that plant genotype plays a great role in shoot multiplication response under *in vitro* culture. As well as, that the different components of culture media greatly influence the growth and development of plants grown under aseptic conditions.

Table 2. Effect of culture medium and different concentrations of IBA and NAA on rose and cotoneaster shoot multiplication parameters on explants taken in December and April after six weeks in culture

Culture Medium	IBA and NAA (mgl^{-1})		Rose			Cotoneaster		
			Number of Shoots/ Explant	Mean Length of Shoots (cm)	Number of Leaves/ Explant	Number of Shoots/ Explant	Mean Length of Shoot (cm)	Number of Leaves/ Explant
Winter Season								
WPM		0.0	1.50 b	1.86 e	4.87 g	1.12 d	1.66 d	4.13 f
	IBA	0.1	2.62 a	2.23 e	7.25 e	3.00 a	1.97 cd	7.13 d
		0.5	2.50 a	2.21 e	6.62 e	2.87 b	2.08 c	5.88 e
	NAA	0.1	2.00 ab	2.10 e	7.13 e	2.25 c	1.81 d	6.75 de
		0.5	2.25 a	1.70 e	6.50 e	2.75 b	1.62 d	5.87 e
B5		0.0	1.37b	1.85 e	5.62 f	1.25 d	1.62 d	4.75 f
	IBA	0.1	2.25 a	2.46 de	8.75 de	2.62 b	1.85 cd	4.50 f
		0.5	2.37 a	2.92 de	9.50 de	2.75 b	1.93 cd	4.87 ef
	NAA	0.1	1.62 b	2.93 de	6.75 e	2.37 b	1.76 d	6.62 de
		0.5	1.87 b	2.31 d	7.87 e	2.37 b	1.80 cd	6.75 de

Table 2 Continued…….

		0.0	1.50 b	1.75 e	6.62 e	1.37 d	1.90 cd	5.25 e
	IBA	0.1	2.50 a	1.98 e	7.38 e	2.00 bc	2.18 c	5.87 e
		0.5	2.75 a	2.15 e	7.13 e	2.62 b	2.32 c	5.62 e
MS	NAA	0.1	1.87 b	1.92 e	6.87 e	1.62 d	2.10 c	5.50 e
		0.5	2.25 a	2.07 e	7.25 e	1.50 d	2.11 c	6.00 e
Spring Season								
		0.0	1.37 b	3.13 d	7.12 e	1.50 bc	2.26 c	6.75 de
	IBA	0.1	1.62 b	5.22 b	21.75 a	2.25 bc	2.90 b	10.5 b
WPM		0.5	2.37 a	6.98 a	7.62 e	3.00 a	3.36 a	8.87 c
	NAA	0.1	1.12 c	5.31 b	16.37 b	1.50 d	2.83 b	6.75 de
		0.5	1.37 b	5.56 b	13.75 d	2.62 b	3.03 ab	6.87 de
		0.0	1.37 b	1.76 e	8.25 de	1.62 d	1.73 d	5.87 e
	IBA	0.1	1.37 b	2.88 de	12.5 d	2.75 b	2.27 c	9.25 bc
B5		0.5	1.62 b	3.17 d	17.87 b	2.37 bc	2.91 b	7.37 d
	NAA	0.1	1.37 b	2.87 de	12.13 d	2.12 c	2.05 b	7.75 d
		0.5	1.50 b	3.12 d	16.63 b	1.62 d	2.50 bc	7.13 d
		0.0	1.12 c	2.05 e	7.37 e	1.62 d	2.06 c	0.0 g
	IBA	0.1	1.75 b	4.53 c	17.75 b	2.50 c	2.95 b	11.5 b
MS		0.5	2.25 a	5.06 b	16.12 b	2.75 b	3.73 a	13.87 a
	NAA	0.1	1.50 b	2.80 de	12.62 d	3.00 a	2.15 c	6.82 de
		0.5	1.62 b	4.35 c	15.87 bc	2.62 b	3.21 ab	8.75 c

4. CONCLUSION

In conclusion, it can be said that cotoneaster plant can be rooted directly from explants collected in spring season, whereas rose plant need to undergo the developmental stages of usual micropropagation protocols. Well-developed cotoneaster plantlets were efficiently transplanted to the field conditions with a high survival rate showing normal features without any morphological variation (Fig. 1, E).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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