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THE EFFECTS OF SOME HORMONES ON THE CALLUS INDUCTION IN *Rosa canina* and *Rosa dumalis* IN VITRO

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SUMMARY: In this study, some factors influencing the induction of callus in *Rosa canina* and *Rosa dumalis* were investigated. From the shoots taken in September, nodal segment of approximately 0.4-0.5 cm were explanted in MS medium. Plant growth regulating substances displayed different effect on the induction process of callus. More callus induction was observed in *Rosa canina* in high concentrations of NAA and BA, however in *Rosa dumalis* low in NAA concentrations but high BA doses led to the same effect. Considering callus weight, in Rosa canina high NAA and BA doses, in *Rosa dumalis* in addition to high NAA, BA concentrations increased callus weight.

Key words: *Rosa canina, Rosa dumalis*, callus induction, hormones

BAZI KUŞBURNU TÜRLERİNDE (*Rosa canina* ve *Rosa dumalis*) İN VİTRO ŞARTLARDA KALLUS OLUŞUMU ÜZERİNE HORMONLARIN ETKİLERİ

ÖZET: Bu araştırmada, *Rosa canina* ve *Rosa dumalis* kuşburnu türlerinde kallus oluşumu üzerine etki eden bazı faktörlerin incelenmesi amaçlanmıştır. Eylül ayında alınan sürgünlerden yaklaşık 0.4-0.5 cm uzunluğundaki boğum araları eksplant olarak alınarak MS ortamına yerleştirilmiştir. Araştırmada, büyümeyi düzenleyici maddelerin türlerde kallus oluşumu üzerine etkilerinin farklı olduğu belirlenmiştir. *Rosa canina* türünde yüksek NAA ve BA konsantrasyonlarında ve *Rosa dumalis* türünde ise düşük NAA ve yüksek BA dozlarında daha fazla oranda kallus oluştuğu tespit edilmiştir. Kallus ağırlığında ise *Rosa canina*'da yüksek NAA ve BA dozlarında, *Rosa dumalis* türünde de yüksek NAA dozlarında ve NAA'te ilave olarak BA konsantrasyonlarının artmasına bağlı olarak kallus ağırlığının arttığı saptanmıştır.

Anahtar kelimeler: *Rosa canina, Rosa dumalis*, kallus oluşumu, hormonlar

INTRODUCTION

Though, plant tissue culture is basically a production method, it is different from well-known conventional methods in that, a small piece (explant) cut from a particular part of the plant is sterilized and planted in a sterile and nutritive medium with suitable environmental conditions. Plant tissue culture has several culture types namely, embryo, meristem, callus and protoplast. These techniques, moreover, are of various metabolites and long-term preservation of plant material (Auge, 1995).

Callus is an unorganized cell mass. Callus culture, however, is callus growing from an explant in a nutritive medium, in other words, it is a sterilized culture of isolated cell mass. Callus culture, has extensive usage in plant improvement studies especially. Plant improvement with classical methods to obtain desired properties takes rather a long time and is hard to attain, owing to such impediments as heterozigoty seen in fruit trees, juvenility, cross incompatibility and self-incompatibility. In recent years, development in plant tissue and cell culture studies have led to the appearance of new methods which can be used in the amelioration of fruit trees. One of such methods is the use of somatic mutations in plant improvement studies. Prolonged callus culture brings about some cytological changes, such as poliploidy, reduction in the number of chromosomes, chromosome fractures (Hartmann et al., 1990). Besides, it has been declared that many secondary metabolites such as caretenoids, steroids, triterpens, tannins and vitamins (especially vitamin C) can be extracted from Rosa species by callus and cell suspension cultures (Short and Roberts, 1991).

In this study, some factors affecting the amount of callus production necessary to make use of the somaclonal variations which arise during callus culture and obtain secondary metabolites are investigated.

In the study with various *Rosa* species, many factors were considered, thereby, the most suitable conditions of callus induction were seeked. By this goal, Khuis-Khui and Sink (1982) who used *Rosa monetti* and *Rosa hybrida* cv. Tropicana, in their study tried to investigate the effects of various media (MS, SH and B5C) and growth regulating materials(casein hydrolyrate and coconut milk on the callus 2.0 mg\l 2,4-D+0.25 mg\l

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kinetin+2.0 mg\l CH or SH medium with 0.5 mg\l 2,4-D+2.0 mg\l p-CPA+0.1 mg\l kinetin).

In Pakistan, explants of *Rosa hybrida* cv. Diamond Jubly and Lans Frances were cultured in MS medium containing various combinations of 2,4-D and kinetin, and it was found out that the best callus growth was observed with 0.5 mg\l 2,4-D+0.1 mg\l kinetin for Diamond Jubly and 0.5 2,4-D+0.5 mg\l kinetin for Lans Frances (Hameed et al., 1993).

Tabaeezadeh and Khosh-Khui (1981), made a study on *Rosa damescana* and *Rosa hybrida*, where they used anters as explants and studied the effects of medium and growth regulating agents on the callus growth. They determined that, the best growths were obtained in MS+2.0 mg\l IAA+0.4 mg\l kinetin and Blaydes+7.5 mg\l IAA+0.8 mg\l kinetin for *Rosa damescana* and *Rosa hybrida*, respectively.

MATERIALS AND METHODS

In this study, one year old shoots taken from *Rosa canina* and *Rosa dumalis* subsp. boissieri var. boissieri (Syn: *Rosa glauca*) species in Erzurum region were used. The shoots were subjected to preliminary selection and their properties were determined by Yamankaradeniz (1982). The shoots were taken in September, and 0.4-0.5 cm segments between the nodes were explanted and transferred to MS medium (Murashige and Skoog, 1962). Before implanting, the explants were kept in 70% ethyl alcohol for 30 sec. and in 30% sodium hypochloride for approximately 20 min. after which washed out by sterile distilled water three times. 24 combinations of NAA (0.0, 1.0, 2.0, 4.0 and 6.0 mg\l) and BA (0.0, 0.4, 0.8, 1.2, 1.6 and 2.0 mg\l) together with 30 g\l sucrose and 7 g\l bacto agar were added to the MS medium. 15 ml volumes of this mixture were then placed into glass pots and sterilized in autoclave under 1.2 atm pressure, at 121 °C temperature for 15 min. The growth regulating agent was filtered through 0.22 μ Millipore, and added into the pots in sterile cabin. Then, the explant was incubated in a dark growth-chamber at 25±1 °C for 4 weeks (Skirvin et al., 1990). Callus percentages and weights were determined at the end of incubation.

RESULTS AND DISCUSSION

The explants from *Rosa canina* and *Rosa dumalis* were placed in the growth-chamber and observed continuously, and the earliest callus induction was experienced 10 days after they had been cultured. The induced calluses in both species were generally white or yellow in color.

The growth regulators were observed to have significant influence over callus induction and weight. In *Rosa canina*, NAA and BA amounts added to the medium have strongly affected callus induction (Table 1). In this species, no callus induction was observed in the control and up to 1.0 mg\l NAA+1.6 mg\l BA as well as at 2.0 mg\l NAA.0.0 mg\l BA doses. Moreover, the highest inductions were observed at high doses of NAA and BA. BA added to almost all of the NAA concentrations increased the percentage of callus induction. In *Rosa canina*, the highest callus induction (100%) was obtained when 2.0 mg\l BA was added to 4.0 mg\l NAA and 1.2, 1.6 and 2.0 mg\l BA was mixed into 6.0 mg\l NAA.

A different situation arised in *Rosa dumalis* (Table 1). In *Rosa dumalis* no induction occurred in the control and 2.0 mg\l NAA+0.0 mg\l BA application, while low NAA+high BA and moderate NAA+low BA concentrations gave high callus induction. The high levels of NAA with low BA doses gave better results. Besides, the best results were obtained for moderate NAA and high BA concentrations. At high NAA doses callus induction generally displayed a reduction.

The effects of growth regulators on callus weight are shown in Table 2. As it is observed the effect of growth regulators on callus weight was in proportional to the percentage of callus induction. In *Rosa canina* heavier callus inductions were observed at high NAA and BA doses. Furthermore, increasing BA doses increased weights. The heaviest callus (0.282 g) was observed for 6.0 mg\l NAA+2.0 mg\l BA combination.

In *Rosa dumalis*, again a different situation existed. In general, with increasing BA doses added to 1.0, 2.0 and 6.0 mg\l NAA concentrations an increase in the weight of callus induced was determined. In 4.0 mg\l NAA concentration no significant difference was found between 0.0 and 0.4 mg/l BA doses, however, the doses 0.8, 1.2, 1.6 and 2.0 mg\l of BA gave rise to form heavier calluses. In *Rosa dumalis*, the heaviest callus was observed for 2.0 mg\l NAA+2.0 mg\l BA concentration, besides, at high NAA concentrations callus increased proportionally.

Table 1. The effect of growth regulators on callus induction (%)

Species	NAA	BA (mg/l)					
	Mg/l	0.0	0.4	0.8	1.2	1.6	2.0
Rosa canina	1.0	0.0	0.0	0.0	0.0	53.33	66.66
	2.0	0.0	20.00	53.33	53.33	33.33	60.00
	4.0	66.67	53.33	53.33	46.67	80.00	100.00
	6.0	53.33	66.67	66.67	100.00	100.00	100.00
Rosa dumalis	1.0	33.33	66.67	80.00	73.33	73.33	100.00
	2.0	0.0	66.67	100.00	66.67	66.67	100.00
	4.0	93.33	100.00	66.67	66.67	53.33	53.33
	6.0	33.33	33.33	53.33	66.67	66.67	33.33

Table 2. The effect of NAA and BA concentration on the callus weight (g)

Species	NAA	BA mg\l					
	Mg\l	0.0	0.4	0.8	1.2	1.6	2.0
Rosa canina	1.0	-	-	-	-	0.037	0.060
	2.0	-	0.051	0.062	0.078	0.11	0.095
	4.0	0.103	0.082	0.093	0.148	0.158	0.263
	6.0	0.098	0.109	0.160	0.214	0.240	0.282
Rosa dumalis	1.0	0.101	0.102	0.077	0.063	0.164	0.194
	2.0	-	0.108	0.185	0.154	0.205	0.309
	4.0	0.119	0.113	0.178	0.175	0.182	0.185
	6.0	0.189	0.215	0.223	0.257	0.241	0.238

It was conducted from our study that the addition of NAA and BA to the culture media was required for the callus induction in *Rosa canina* and *Rosa dumalis*. The experiment results also suggested that as levels of NAA and BA increased, callus induction increased for *Rosa canina* and that in *Rosa dumalis* low and medium levels of NAA and high levels of BA were sufficient to higher callus induction. Furthermore, for the last species, high NAA and BA levels were found to inhibit the callus induction. The differences between *Rosa canina* and *Rosa dumalis* with respect of callus formation were probably due to the genetic variation. Other studies including different *Rosa* species also showed that depending upon the species different combinations of auxin+cytokinin levels were needed for producing best callus. In the studies on variety of *Rosa* species, the growth regulators were found to have varying effects on the cultivars. In a previous study which was carried out with *Rosa damescana* and *Rosa hybrida*, it was determined that the best callus induction in *R. damescana* and *R. hybrida* was obtained when 2.0 mg\l IAA+0.4 mg\l kinetin was added to MS medium, and 7.5 mg\l IAA+0.8 mg\l kinetin to Blaydes medium respectively (Tabaeezadeh and Khosh-Khui, 1981). Another study with *Rosa hybrida* and *Rosa monetti* revealed that the best induction was observed either in MS medium with 2.0 mg\l 2,4-D+0.25 mg\l kinetin and 2.0 g\l CH or in SH medium enriched with 0.5 mg\l 2,4-D+2.0 mg\l p-CPA and 0.1 mg\l kinetin (Khosh-Khui and Sink, 1982). Furthermore, Hameed et al. (1993), in a study with Diamond Jubly and Lans Frances cultivars, determined that 0.5 mg\l 2,4-D+0.1 mg\l kinetin and 0.5 mg\l 2,4-D+0.5 mg\l kinetin combinations were optimum for Diamond Jubly and Lans Frances cultivars respectively.

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