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Original Contribution

DIRECT REGENERATION OF *ROSA CANINA* THROUGH TISSUE CULTURE

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ABSTRACT

Dog rose (*Rosa canina)* is one of the most important flower crops in the world and has a value in ornamental, pharmaceutical and cosmetic trade. Also it was used as a root stock for other cultivars of Rose. Hence this investigation was attempted with the aim to study direct regeneration of *Rosa canina*. The experimental data of the present investigation was analyzed statistically by using completely randomized design. The experimental results showed that the maximum axillaries shoot proliferations were observed in MS medium supplemented with 1.5 mg/l GA_3 + 0.25 mg/l BAP. Among the different hormones, root was optimally derived from shoots in all treatments containing 1mg/l NAA+ 0.25 mg/l BAP.

Key words: *Rosa canina*, direct regeneration, shooting, rooting

INTRODUCTION

Rosa canina belongs to Rosaceae family (1). Rose improvement has depended on crossings followed by selection among large population. However, grafting is expensive and time consuming procedure. Seeds are used for propagation of species, new cultivars and for production of rootstocks (2). In the last few years, in vitro propagation has revolutionized Commercial nursery business (3). 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 prop gules around the year (4). Martin (1985) demonstrated that, using this technology, up to 400,000 plants could be cloned, from a single rose on annual basis. It has investigated many potential and practical uses in tissue culture such as rapid multiplication, cultivar development via somaclonal variation and genetic transformation (5). Tissue culture allows overcome some of the sterility problems through direct regeneration. *In vitro* protocols are ways to shorten growing cycles. Khoshkhuei and Sink (1982) demonstrated Root formation in *R.canina* and *R.damascena* is lower compared to *R.hybrida*. They also studied the effects of concentrations and kind of auxin on *R.hybrida* and mentioned that the combination of 0 to 0.1 mg/l IAA and NAA were more effective (6).

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MATERIALS AND METHODS

Sterilization of leaf explants:

Explants were collected from 3 old mounts seedling. The leaf explants were surfaces sterilized with ethanol 70 % for 40 seconds and then 10 minutes with sodium hypochlorite 2.5%; followed by rinsing three times with sterile distilled water.

Shoot proliferation:

In this experiment, shoot explants were transferred to the MS medium containing 3 mg/l charcoal and hormonal composition of 0.25 mg/l BAP, 1 mg/l GA_3, 0.5 mg/l GA_3+0.250 mg/l BAP, 1 mg/l GA_3+0.250 mg/l BAP, and 1.5 mg/l GA_3+0.250 mg/l BAP. The number, length and percentage of shoot

regeneration and also days of callus induction were noted.

Rooting:
For rooting, shoots were transferred to the MS medium supplemented 3 mg/l active charcoal and of 0.25 mg/l BAP, 1 mg/l NAA, 0.5 mg/l NAA + 0.25BAP, 1 mg/l NAA + 0.25BAP and 1.5 mg/l NAA + 0.25 mg/l BAP. Root length and percentage of roots induction were recorded after 40 days.

Statistical analysis:
The experiments were performed using Complete Randomized Design (CRD) and results were analyzed using MSTAT software.

RESULTS AND DISCUSSION

The effect of hormone and concentrations on shoot proliferation:
For direct regeneration, GA_3 and BAP were used individually that results wasn't considerable. When 2 hormones were used in combination together maximum length of shoot (4.733 ± 0.15 cm), number of shoots per explants (6 ± 1), shooting percentage (100 %) and the shortest required time for shooting (7 days) was observed in medium containing 1.5 mg GA_3 + 0.25 mg/l BAP **(Table 1)**. The minimum length of shoot (0.367 ± 0.15 cm), number of shoots per explants (1 ± 1), shooting percentage (50 %) and the longest required time for shooting (30 days) obtained in medium that contained 0.25 mg/l BAP, respectively. Also by increasing concentration of GA_3 from 0.5 to 1.5 mg/l, length of the shoot and number of shoots increased that this case is due to the evident effect of GA_3 on increasing shoot length by increasing the internal nodes. Of course researchers have different ideas about using hormone in shooting regeneration medium. Zapata *et al*, (1999) believe that meristem has inductive property, so using growth regulators in the medium of shooting is not necessary (7). Whereas others including Agrawal *et al* (1997) believe that concentration of hormone is an effective factor in shooting., in addition they mentioned in their studies that low concentration of cytokinin (less than 1 mg/l) is effective in direct regeneration, therefore low concentration of BAP (less than 1 mg/l) was suggested (8). Canli *et al* (2009) also reported that in proliferation of *R.hybryda,* when combination of two hormones GA_3 and BAP was used; higher shooting percentage was observed compared to BAP was used individually (9). Also Rout *et al* (1990) mentioned that in GA_3 and BAP break dormancy. Of the shoots as well as increasing propagation rate of the shoots will be increased (10).

Table 1. *Effect of BAP and GA_3 hormones on shooting*

Treatment (mgl-1)		Day of shooting	No. of shoots	Shoot length (cm)	Percentage of shooting%
GA_3	BAP				
0	0.25	30	1±1	0.367± 0.15	50
1	0	25	3±1	1.033±0.15	90
0.5	0.25	10	4±1	3.533±0.31	100
1	0.25	9	4±1	4.600±0.20	100
1.5	0.25	7	6±1	4.733±0.15	100

Effect of hormone and its concentrations on root formation:
The effect of basal medium in combination of 2 hormones (NAA and BAP) was studied. As shown in **Table 2**. The highest root length were observed in 1 mg/l NAA with 0.25 mg/l BAP (4.167 ± 0.15 cm) in the other hand, the lowest root length and the lowest rooting percentage (73 %) is related 1 mg/ l BAP (1.297 ± 0.03 cm), growth rate was shown better in all

treatment containing NAA supplied in combination with BAP in comparison with NAA and BAP was used individually **(Table 2)**.These results are similar to Sajid *et al* (2006) that was performed on root formation in tissue culture of grape. They reported that combination of NAA with BAP were more effective for root induction (11). Increasing concentration of NAA from 0.5 to 1 mg/l, increase root length. The addition of NAA up 2 mg/L to the medium leads to the decrease in root length.

Taiz & ziger (2000) also referred to positive effect of NAA on the length increase and number of roots. Low concentrations of auxin cause to increase root growth, but its high concentrations prevent from growth of the root. The reason for reducing growth of the root is producing ethylene in high concentrations of auxin (12).

Table 2. *Effect of BAP and NAA hormones on rooting*

Treatment (mgl-1)		No. of Roots	oot LengthR (cm)	Percentage of rooting%
NAA	BAP			
0	0.25	2	1.297± 0.03	73
0	1	3	2/267 ± 0.29	91
0.5	0	3	2/483 ±00/10	94
1	0.25	6	4/167 ±00/15	100
2	0.25	4	3/700± 0/17	97

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