Trakia Journal of Sciences, No 2, pp158-162, 2013

Available online at:
http://www.uni-sz.bg

ISSN 1313-7050 (print)
ISSN 1313-3551 (online)

Original Contribution

EFFECT OF GAMMA RADIATION ON CALLUS INDUCTION AND REGENERATION OF *ROSA CANINA* THROUGH *INVITRO* CULTURE

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ABSTRACT
This study was conducted to evaluate the effect of media and adding
Hormones on *in vitro* regeneration of Dog rose *(Rosa canina)* by using a completely randomized design and factorial experiment. Dog rose *(Rosa canina)* is an ornamental member of family Rosaceae. It is an important rootstock for other cultivars of Rose. On basal medium B_5 and MS along with different concentrations of IAA and NAA under dark and light condition. Callus was formed and grew well in B_5 medium containing 1 mg/l IAA under the dark condition. In order to indirect regeneration, callus was transferred in B_5 medium supplemented with KIN in combination with NAA, IAA, GA3 or BAP in combination with NAA, IAA, and GA_3. These treatments have no ability to organogenesis.
The effect of γ- irradiation on callus was studied. There was no significant effect of irradiation on organogenesis.

Keywords: *Rosa canina*, Tissue culture, Hormones

INTRODUCTION

Dog Rose (*Rosa Canina*) is a perennial shrub that belongs to Rosaceae family (1). *Rosa canina* is a wild type rose with favorite flowers that is often found in white, red and sometimes yellow colors, and it smells disgraceful (2). In fact, the present red flowers are descents of wild Dog Roses that have been regenerated and breaded. Also this plant is one of valuable pharmaceutical due to tranquility effect as well as improvement of heart diseases. Its flower and seed are full of vitamin C and other vitamins, which could be used for compensating lack of vitamins in body (3). In addition, it was used as ornamentals in land space and in most of the cases as rootstock for other cultivars of Rose. Rose cultivars are traditionally propagated by cuttings or grafting. However, grafting is expensive and conventional breeding is a time consuming procedure. "Tissue Culture" can be used to propagate rootstocks and mass propagation in a very short time (4). The previous investigations indicates that most of the studies of *"Invitro*" propagation has been carried out on ornamental cultivars of roses. A new protocol has been reported for plant regeneration via protocorm like bodies that have been induced from leaf explants of *R. canina* by Tian *et al* (5). Rhizoids were obtained from calli of leaf explants under dark conditions in MS medium containing 1.5 mg/l 2.4-D. Protocorm like bodies were produced in light conditions from root tip in MS medium containing 20 mg/l TDZ. In another research, plant regeneration system through segment culture by embryogenic callus formations in Dog Rose plant was reported. Khoshkhuei and Sink demonstrated Root formation in *R. canina* and *R. damascena* is lower compared to *R. hybrida* (6). They also studied the effects of concentrations and kind of auxin on *R. hybrida* and mentioned that the

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combination of 0 to 0.1 mg/l IAA and NAA were more effective.

MATERIALS AND METHODES

Sterilization of leaf explants: 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.

Provision of medium: Media in this experiment were MS and B_5 which were enriched with 30 g/l sucrose and 7 g/l agar. Hormones related to each test were added to the media. Glass jars contain each 20 ml medium and pH of the medium was adjusted with NAOH 0.1 N or HCL 0.1 N before autoclaving. Autoclaving was performed in 1.05 kg/cm and 121 centigrade degree for 15 minutes.

Callus induction: In order to callus induction callus, leaf explants were cultured in the B_5 and MS medium containing 0.5, 1 and 2 mg/l IAA and 0.5, 1 and 2 mg/l NAA, in light (1200 Lux) and dark condition. Callus fresh weight, percentage of callus induction and the time for callus induction were recorded.

Indirect regeneration: In order to organogenesis callus were transferred to the MS medium that consisted 1.5 mg/l BAP+ 0.5 mg/l NAA, 1.5 mg/l BAP+ 0.5 mg/l IAA, 1.5 mg/l BAP+ 0.5 mg/l GA_3, 1.5 mg/l KIN+0.5 mg/l NAA, 1.5 mg/l KIN+ 0.5 mg/l IAA, and 1.5 mg/l KIN + 0.5 mg/l GA_3. Results were recorded in **table1.**

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

RESULTS AND DISCUSSION

1. The effect of differences hormonal and dark and light treatments on callus induction of leaf explants:

The leaf explants were transferred to medium, produced phenol, Browning was observed around leaf explants. The callus was performed and the younger explants were also used. Rout *et al*, also consider browning of the medium as a result of polyphenols oxidation which is exudates from the cut surface of the explants to the medium (7). The explants did not promote any callus in light conditions. Callus induction was only seemed in dark condition. Greenish yellow callus was friable.

Canh and *et al,* also indicated that the structure of induced callus from leaf explants in *R.multiflora* increased significantly in dark conditions (8). These results also are similar to Simoh *et al* (9). They found that dark conditions are more effective on 3 cultivars Lycopersicon esculentum, Daucus caota and Arabidopsis thaliana. Hangarter and Stasinopoulos mentioned that reduction of callogenes in light condition is due to reduce of nutrient uptakes in the medium (10). Also Thorpe mentioned that growth reduction in the light conditions may be as a result of the differentiation callus in presence of light (11). In other words, differentiation prevents from growth and propagation of callus. These results are in contradiction with Uyong *et al*, that observed increase in callus growth in light conditions at the tissue culture of *Cistach dosertical* (12). Increasing growth of callus could be due to increasing the nutrient uptake in light conditions (13). As shown in **table 1** it is observed that the B5 were better in comparison with the MS, which this affair can be as a result of some factors except hormonal compositions of these two media such as difference in the amount and combination of minerals.

These results showed that the highest callus induction per explants was obtained after 17-23 days in MS-medium in contrast to B_5 within 6-15 days. The time from initiation of culture to production of plants is significantly reduced. These results were in accordance with Ellis and Eknankul that regarded less growth in MS medium may be due to high concentration of ammoniums salts which inhibits tissue culture of some plants (14). Also Omar et al reported that high levels of ammonium may reduce uptake of calcium, magnesium and potassium that causes limited supply of nutrient in medium (15).

Avermis *et al*, also reported that in various hybrid of rose, propagation rate increases with reduction of ammonium in the medium (16). According to table among the plant growth hormones combination used in this study, it showed that the highest calogenesis and fresh weight occurred in B_5 containing 1 mg/l IAA (0.096 g) and also the shortest required time for

callus induction (7 ± 1 days). The lowest fresh weight of callus (0.015 g) and also the most required time for callus induction (22 ± 1 day) was also related to the MS containing 0.5 mg/l NAA. At first by increasing concentration of IAA and NAA (0.5 to 1 mg/l), callus weight increased and then decreased when concentration rinse up 1 mg/l . It may be related to the negative effect of auxins in concentrations higher than optimum.

2. The effect of different hormone on indirect regeneration:

The results obtained from indirect organogenesis were as following:

In the medium containing 1.5 mg/l BAP with 0.5 mg/l NAA and medium 1.5 mg/l BAP with 0.5 mg/l IAA, the size of calluses did not change and no organogenesis was performed during 4 week. In the medium containing 1.5 mg/l KIN with 0.5 mg/l NAA and 1.5 mg/l KIN with 0.5 mg/l IAA, although size of calluses increased gradually from beginning of the first week, but no organogenesis occurred as well. After 1 week of cultures, medium containing 1.5 mg/l BAP in combination with 0.5 mg/l GA_3 and also the medium of 1.5 mg/l KIN with 0.5 mg/l GA_3 necroses was occurred. At the end of the second week, they turned brown. Lloyd *et al* reported that in some types of rose such as *R. laevigata* and *R. Wichuriiana* also organogenesis from callus did not induce (17). These results are in contrast with Rout, who reported regeneration of the callus from leaf explants in the medium containing NAA and BAP and also by IAA and BAP in *R. hybrida cv Landora* (18).

Table 1. *The effect of different concentrations of IAA and NAA on callus induction, fresh weight and callus morphology*

Media	Treatment (mgl-1)	Days of callus induction	fresh weight of callus	Callus Induction (%)	Callus morphology	Darkness-light
B_5	0/5 IAA	1±10	0.055[c]	81	Yellow green –friable	darkness
B_5	1 IAA	1±7	0.096[a]	98	Yellow green/ –friable	darkness
B_5	2 IAA	1±8	0.064[b]	94	Yellow green/ –friable	darkness
B_5	0/5 NAA	1±14	0.25[f]	74	Yellow green/ –friable	darkness
B_5	1 NAA	1±12	0.045[d]	87	Yellow green/ –friable	darkness
B_5	2NAA	1±12	0.034[e]	88	Yellow green/ –friable	darkness
MS	0/5 IAA	1±22	[c]0.035	75	Yellow green/ –friable	darkness
MS	1 IAA	1±18	0.077[a]	80	Yellow green/ –friable	darkness
MS	2 IAA	1±20	0.054[b]	78	Yellow green/ –friable	darkness
MS	0/5 NAA	1±22	[f]0.015	68	Yellow green/ –friable	darkness
MS	1 NAA	1±20	0.035[d]	75	Yellow green/ –friable	darkness
MS	2NAA	1±22	[e]0.024	76	Yellow green/ –friable	darkness

3. The effect of radiation on callus:

It appeared that no callus change in radiation of various Gama ray doses and no organogenesis is occurred, at the end of the 6th week, callus necrosesed. These results were in accordance with Naserian *et al*, they used Gama ray with low doses in order to stimulate response with another culture in several cultivar of spring wheat (19). They reported that Gama raying reduced the ability of callus induction and regeneration. Gahukar and Jambhule also found that on sugarcane plant with increasing dose of Gama ray, callus induction will be decreased (20). Similar result was reported in callus fresh weight by Reddy *et al*, in castor bean and by Singh and Singh, also in sugarcane. Irradiation with high rate of Gama ray dose causes to damage tissue severely that may reduce fresh weight of callus (21, 22).

CONCLUSION

This study indicated callus induction in *Rosa canina* .Different concentration of IAA and NAA under dark and light condition with composition of culture medium were tested. Significant increase obtained in B5 medium containing 1 mg/l IAA under the dark condition. In indirect regeneration no result , observed in medium containing in B_5 medium supplemented with KIN in combination with NAA, IAA, GA3 or BAP in combination with NAA, IAA, and GA_3.when Gama raying used, there was not effective on callus induction positively.

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