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Micropropagation of *Rosa canina* Through Axillary Shoot Proliferation

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Abstract

In vitro propagation of rose has played a very important role in rapid multiplication of species with desirable traits and production of healthy and disease-free plants. Micropropagation using nodal segments of *Rosa canina* under different combinations of BAP, GA_3 and NAA on MS and VS, medium was investigated. The results showed that the highest shoot proliferation was obtained on VS medium containing 2 mg/L BAP without any GA_3 and NAA. Furthermore the highest root regeneration obtained in half strength VS medium. The present investigation recommended a practiciable *in vitro* plant protocol for *R. canina* as an important step for successful implementation of biotechnological techniques for rose improvement in Iran.

Keywords: Micropropagation, Native species, Plant growth regulators, *Rosa canina*.

Abbreviations: MS (Murashige and Skoog), VS (Vander Salm), BAP (Benzyl Amino Purine), NAA (Naphtalene Acetic Acid), GA_3 (Gibberellic Acid).

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INTRODUCTION

Rosa canina is a medicinal plant that its fruits have many important medicinal properties but unknown for many people especially in Iran. However, this crop can be produced commercially and its orchards can be established such as other fruit trees (Sharafi, 2010؛ Luncna, 2005). Rosa canina belongs to Rosaceae family. Dog rose (*Rosa canina*) has been used as a rootstock for ornamental roses (Khosh-Khui and Sink, 1982) while considered as a medicinal plant as well. *Rosa canina* L. (dog rose, rose hip, briar rose) is one of wild roses appreciated for their vitamin C enriched fruits being beneficial to human health (Kazaz *et al*., 2009). More than 200 species are present in the genus Rosa (Wissemann, 2003) from which 14 wild species are present in Iran. Traditionally, most roses are heterozygous and do not breed true to type. Therefore, they are propagated by vegetative methods. Since most rose species are difficult to root, conventional propagating methods are very slow, time consuming, and tiring. Tissue culture on the other hand is becoming increasingly popular as an alternative to the conventional plant propagation methods (Roberts and Schum, 2003). Micropropagation has five major advantages compared to the conventional methods of plant propagation: (i) it is an valuable aid in the multiplication of elite clones of intractable/recalcitrant species؛ (ii) it is important in terms of multiplying plants throughout the year, with control over most facts of production؛ (iii) it is possible to generate pathogen-free plants even from explants of infected mother plants؛ (iv) plant materials such as male sterile, fertility maintainer and restorer lines can be cloned؛ and (v) it enables the production of a large number of plants in a short time from a selected number of genotypes (Jafarkhani Kermani *et al.*, 2011). The objective of the study was to investigate the best hormonal compound and media for micropropagation of *Rosa canina in vitro* condition.

MATERIALS AND METHODS

Sterilization of explants

After removing chilling requirement of buds in February 2012, axillary buds of dog rose (grown at the botanical garden of Ferdowsi University of Mashhad, Iran) were cut and placed under running tap water (for 1 h) and were decontaminated with 70% ethanol (for 30 s), and sodium hypochlorite (2.5%) (for 15 min.). Then all explants were washed three times with sterile distilled water.

Shoot proliferation stage

In this experiment, shoot explants with 3 adventitious buds and 2 cm in size were transferred to 2 different media: MS and VS medium. 20 treatments with 8 replications were considered for each medium in this stage. Different hormonal composition of BAP (0, 0.5, 1, 1.5, 2 mg/L), GA3 (0, 0.5 mg/L) and NAA (0, 0.1 mg/L) were considered for both media. The shoot number, shoot length, percentage of shoot regeneration, leaf number and percentage of green leaves were recorded after 60 days. The pH of all media was adjusted to 5.8 using 1.0 N potassium hydroxide (KOH) or 1.0 N hydrochloric acid (HCl), before adding 8 g/L plant agar. Media were autoclaved for 15 min. at 121℃ and 1.2 kgf/cm pressure. All the *in vitro* cultures were placed under 16/8 h light/dark cycle in a culture room maintained at ±24℃.

Rooting stage

For rooting, new shoots (1-2 cm height) were transferred to 2 media of VS and ½ VS with different hormonal composition of IBA (0, 0.3, 0.6, 0.9 mg/L) and NAA (0, 0.3, 0.6, 0.9 mg/L). Root length, root number and percentage of root induction were recorded after 30 days.

Acclimatization of plantlets

The plantlets were cultivated for 5 weeks in plastic glasses containing steril mixture of peat: perlite (3:1). The plantlets were put in culture room under a 16-hour photoperiod, at a tempreture

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of 23/25℃ (night/day) and 80% relative humidity. When a growth cycle was over, the shoot survival percentage was evaluated.

Experimental design and statistical analysis

The shoot proliferation experiment was performed in a factorial based completely random design with 40 treatments and 8 replications. Rooting experiment was carried out in a factorial based completely random design with 16 treatments and 15 replications. Analysis of variance was performed and comparisons of means were conducted using LSD test. All analysis were regarded as significant if p value was less than 0.05. The percentage data was transformed using angular transformation (Arc Sin√%) before carrying out ANOVA.

RESULTS

Shoot proliferation

The results showed that there was significant difference of effect of type and concentration of PGRs on vegetative traits of R. canina in proliferation stage ($p \leq 0.05$). Maximum number of new leaves (19.96) was produced on the medium containing 2 mg/L BAP (Fig. 1A), whereas the maximum percentage of green leaves were obtained on the media containing 1.5 mg/L BAP

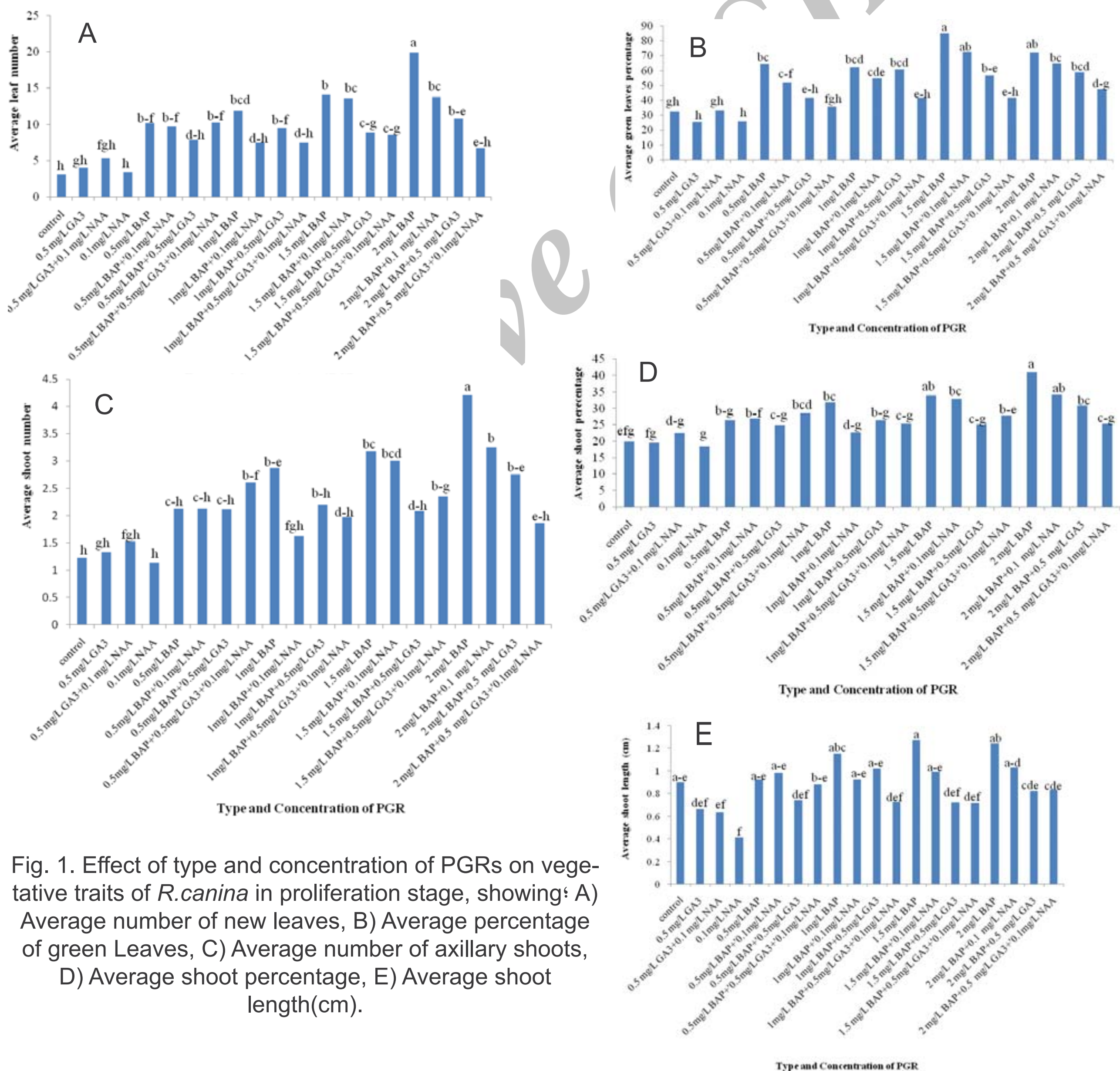

Fig. 1. Effect of type and concentration of PGRs on vegetative traits of *R.canina* in proliferation stage, showing: A) Average number of new leaves, B) Average percentage of green Leaves, C) Average number of axillary shoots, D) Average shoot percentage, E) Average shoot length(cm).

Table 1. Effect of different media on some vegetative traits of *R. canina* in proliferation stage.

Medium Culture	Shoot percentage	Grean leaves percentage	Shoot length (cm)	Leaf number	Soot number
Murashige & Skoog (MS)	26.25a	46.29b	0.768 b	8.37b	2.15a
Van der Salm (VS)	28.21a	56.49a	0.993 a	10.37a	2.4a

Table 2. Analysis of variance (ANOVA): Effect of type and concentrations of different plant growth regulators (PGRs) and medium on some traits of *R. canina* in rooting stage ($p \leq 0.05$).

Source of Variation	Degree of Freedom	Root number	Root length	Rooting percentage (%)
PGRs type	1	13.43 *	0.74 ns	882.25 *
PGRs concentration	3	14.46 *	0.48 ns	871.28 *
Medium Culture	1	85.12 **	45.88 **	6036.00 **
PGRs type * Concentration	3	11.69 *	0.85 ns	704.061 *
PGRs type * Medium culture	1	36.85 **	18.07 **	2523.17 **
Concentration * Medium culture	3	19.03 **	9.15 *	1312.36 **
PGRs type * concentration * Medium Culture	3	5.23 ns	3.33 ns	324.209 ns
Error	224	4.54	3.44	295.96

Table 3. Effect of different media on some traits of *R. canina* in rooting stage.

Medium Culture	Root number	Root length (cm)	Rooting percentage (%)
VS	1.01 b	0.922 b	14.03 b
½ VS	2.28 a	1.85 a	24.75 a

Table 4. Effect of type and concentrations of PGRs on rooting traits of *R.canina.*

Concentration (mg/L)	Root number	Root length (cm)	Rooting percentage (%)
0	0.77 b	1.42 a	12.29 b
0.3	1.4 ab	1.27 a	17.78 ab
0.6	2.14 a	1.38 a	23.08 a
0.9	1.83 a	1.48 a	20.86 a

Different letters show significant differenced according LSD Test ($p \leq 0.05$).

(84.84%) (Fig. 1B). The maximum number of axillary shoots (4.21) and shoot percentage (41.10%) were observed in 2 mg/L BAP (Fig. 1C, D) and the highest increase in plant height (1.27 cm) was observed in the medium containing 1.5 mg/L BAP (Fig. 1E). Also, there was significant difference of type of medium on vegetative traits. The lowest shoot multiplication was observed on medium MS while the highest shoots were formed on medium VS (Table 1).

Kim *et al*. (2003) indicated that *in vitro* shoot proliferation and multiplication are largely based on media formulations containing cytokinins as major plant growth regulators, although low concentrations of auxins or GA_3 are also essential. The results of the present study demonstrated that inclustion of 0.5 mg/L GA_3 to the culture media didn't increase the number of axillary shoots and stem height in all of the BAP concentrations. The results showed that in some of the traits the differences between the treatments were not significant, however, the maximum number of axillary shoots were significantly higher in the VS medium containing 2 mg/L BAP. AS the concentration of BAP was lower than 2 mg/L, a reduced growth rate was noted. Also, in this experiment 2 types of MS and VS media were used and the results showed that the best plantlets (in proliferation and rooting stage) were in VS medium because of the alleviating Fe deficiency in leaves.

Root initiation and acclimatization

Results of ANOVA on effect of different media on root number, root length and rooting percentage revealed significant difference ($p \leq 0.01$) (Table 2). The results showed that there was

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significant difference between PGRs concentrations and types in root number, rooting percentage but there was no difference in root length ($p \leq 0.05$). The highest root length, root number and rooting percentage was recorded in ½ VS medium (Table 3). Also, maximom average of root number (2.14) and maximum average of rooting percentage (23.08%) were observed in 0.6 and 0.9 mg/L concentration (Table 4). The results showed that there was no difference between IBA and NAA in root length with control, but they had significance difference in root number and rooting percentage. Therefore, the PGRs concentrations had more importance than the type in rooting stage.

The resulting shoots cultured on VS medium containing ½ and full strength of VS macro, micro elements and vitamins showed variable response to rooting afters 4 weeks of culture. The best results were obtained on VS medium containing ½ strength of VS macro, micro salts and vitamins. The rooted plants were not difficult to acclimatize at ±24℃ and relative humidity of 80% during initial stages of development gradually reduced to 40% after 4 weeks of culture and was transferred to the greenhouse for flowering.

DISSCUSION

Tissue culture techniques are used extensively for growing plants commercially. This process involves growth of new plants from small pieces of plant tissue in a nutrient medium in sterile conditions. Under sterile conditions, plants can be induced to rapidly produce new shoots and these can be subdivided to produce more plants. (Dixon and Gonzales, 1993). In this study, according to the responses of explants cultured on different medium, VS and VS/2 medium was relatively the best and the most appropriate treatment for shoot and root formation. A successful micropropagation protocol proceeds through a series of stages, each with a specific set of requirements. These are (i) initiation of aseptic cultures, (ii) shoot multiplication, (iii) rooting of macro shoots and (iv) hardening and field transfer of tissue culture raised plants (Kumar Pati *et al.*, 2006). For initiation of aseptic cultures, a thorough knowledge of the physiological status and the susceptibility of the plant species to different pathological contaminants are required. A review of literature showed that surface sterilization of rose is problematic and different approaches have been adopted to achieve sterilization. Other researchers have reported a high percentage of bud break on hormone-free medium within 10-12 days, but the growth rate was very low in roses (Rout and Jain, 2004). In our study the effects of different concentrations of BAP (0 to 2 mg/L) and GA_3 (0, 0.5 mg/L) and NAA (0, 0.1 mg/L) were obvious on bud break and growth but on MS and VS medium without growth regulators the growth rate was lower.

In vitro shoot multiplication relies largely on medium formulations containing BAP as the major PGRs in combination with a low concentration of NAA (Xing *et al*., 2010). In the present study, 2 mg/L BAP was the optimum treatment for *in vitro* multiplication of Rosa canina. This result is similar to the findings on optimal BAP concentration (4.4 – 13.2 µM) (Carelli and Echeverrigaray, 2002). BAP is needed for proliferation of canina rose plants, but a high concentration is undesirable: at a concentration higher than 2.2 µM it will lead to multiplication of shoots, which is not beneficial to shoot elongation which is not in line with our results. When the culture medium contains NAA at higher concentration (0.1 mg/L), the bud can form more callus from the base selection, which will greatly affect the young seedlings absorption of water and nutrition, and thus inhibit its growth. The inhibition is especially obvious when NAA concentration is increased to 0.1 mg/L. Auxin is a rooting hormone and application of synthetic auxin i.e. IBA might have increased the biosynthesis of indole acetic acid (IAA) or could act as synergistic to IAA. The another possible reason for higher rooting and early root initiation by IBA might be involvement of IBA in ethylene biosynthesis (Arteca, 1990) and it has been suggested that auxin induced ethylene may induce adventitious root formation instead of action of auxin itself (Mudge, 1989). A high concentration of GA_3 (0.5 mg/L) always finally caused the nigrescence and death of young shoots, which appeared waterlogged that is in line with Xing *et al*. (2010). The best results in rooting stage

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achieved with 0.6 or 0.9 mg/L IBA/NAA in ½ VS medium. Hyndman *et al*. (1982) recommended decreasing medium salt concentration from MS medium generally increase rooting for rose propagation. The same results were reported in *R. hybrida* cv. Peace (Kirichenko *et al.,* 1991) and R. rugosa (Xing *et al*., 2010). Our study yielded a practical protocol for efficient axillary bud multiplication from *Rosa canina* explants. Here we demonstrate high-efficiency micropropagation of *R. canina* for the first time. Micropropagation of *R. canina* provides an opportunity to harvest virus-free material and conserve important germplasm resources *in vitro*. It furnishes material for genetic transformation and foe employing molecular techniques in breeding.

CONCLUSION

In conclusion, the present investigation recommended a practicable, *in vitro* propagation protocol for *R.canina*. At the proliferation stage, maximum number of axillary shoots was achieved in the medium containing 2 mg/L BAP. At the rooting stage, 0.6 and 0.9 mg/L IBA/NAA were effective. Also the VS medium with additive Fe was better than MS medium in all stages of micropropagation of this plant.

Fig. 2. Different stages of micropropagation of Rosa canina: A) Proliferation stage B) Rooting stage C) Acclimatization stage.

Fig. 2. Different stages of micropropagation of *Rosa canina*: A) Proliferation stage B) Rooting stage C) Acclimatization stage.

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