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In Vitro Establishment Procedures of Dog Rose (Rosa canina)

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Abstract

Dog rose (Rosa canina) is one of the medicinal plants with important role on vitamin C and active ingredients but a few researches has been conducted on the in vitro efficient establishment of this plant. Therefore, for optimization of establishment stage of R. canina, this study was carried out by 8 decontamination procedures included T1: 5% Sodium Hypochlorite, T2: 10% Povidone Iodine, T3: commercial Dettol, T4: commercial Banzalkonium Chloride, T5: Mercury (II) Chloride for 5 min, T6: Mercury (II) Chloride for 8 min, T7: 2.5% Sodium Hypochlorite with Cefotaxime and Tetracycline, T8: Mercury (II) Chloride for 2 min with Cefotaxime and Tetracycline. for elimination of bacteria and fungi contamination. In this study minimum browning percentage was observed in T7 and T8 (6 and 8% respectively), Bacterial contamination was completely controlled in T7 and T8, Fungi contamination percentage was completely controlled (100%) by T2, T3, T4, T6 and partially controlled by T8 and non-controlled by T7.

Maximum bud break percentage was observed in T8, T7 and T2. The highest shoot length in T2 and T7 but the lowest shoot lengths were observed in T6. In T2, T3 and T7, node numbers were more than other decontamination treatments. T8 on the base of browning percentage, bacteria and fungi contamination percentage, bud break percentage was better than other procedures. T7 and T2 on the base of shoot length and node numbers were decontamination procedure better than other procedures. In this investigation decontamination procedures are considered as one the most important steps for successful implementation of biotechnological techniques for R. canina

Keywords: Decontamination procedures bacterial contamination, fungi contamination, In vitro, Rosa canina

INTRODUCTION

Dog rose (Rosa canina) is one of the old roses, which has been used as a medicinal plant from ancient times. Preparations of R. canina are especially given preventively for chill, flu, infectious diseases, vitamin C shortage and fever. The plant is a laxative and tonic to increase the body's immune defences against gastric, disorder of the urinary tract. Seed oil of R. canina is used for health and cosmetics industry. Also, the leaves of R. canina are used for washing wounds and burns [1, 2].

Rosa sp. is commonly propagated by vegetative (asexual) methods such as cutting, layering, grafting and tissue culture. Micropropagation of Rosa sp. could be done through the meristem tip, shoot tip and axillary buds. [3, 4, 5]. Several factors in investigations about In vitro culture of R. canina, Several factors were affected on micropropagation, such as the contamination of explants on establishment stage [6, 7, 8].

Also, the effects of plant growth regulators [9, 10, 11], inorganic nitrogen source [12] and basal medium influences [13, 14] good physical conditions (i.e., optimum light and temperature) and optimum basal medium, different decontamination procedures were important for explants establishment, which are chief factors on tissue culture. Generally, Ethanol, Sodium Hypochlorite and Calcium Hypochlorite, Mercury (II) chloride were used for surface decontamination. In tissue culture for elimination of bacterial and fungal contamination various antibiotics such as Gentamycin, Ampicillin and Tetracycline were applied. Decontamination of Rosa sp. explants were used by Sodium Hypochlorite, Ethanol, Mercury (II) Chloride and Gentamycin, Ampicillin, Tetracycline, Ornamoxicillin for elimination of systemic contamination especially for systemic bacterial contamination [6, 15]. Few reports exits about decontamination procedures in establishment stage of R. canina micropropagation. Purpose of this study was to determine the best procedure for elimination of fungi and bacterial contamination.

MATERIALS and METHODS

After removing chilling requirement of buds in February 2010, axillary buds of R. canina (grown in botanical garden of University of Tabriz) were chosen and then were washed with tap water (for 1 h) and Tween 80 (0.1%) (15 min), first with 70% Ethanol (5 min) and then were decontaminated with different decontamination components (Table 1). Then, all explants were washed with sterile water. Organic decontamination components (including 10% Povidone Iodine, commercial Dettol, commercial Benzalkonium Chloride) and inorganic decontamination components (including Mercury (II) Chloride and Sodium Hypochlorite) for surface disinfection and Cefotaxime and Tetracycline antibiotics for control the systemic infection were used.

MS [16] basal medium was used for explants establishment. The establishment medium was contained 30 g L-1 sucrose, 0.8% Agar, 1 mg L-1 GA3 and 1 mg L-1 BAP. The pH of media was adjusted to 5.8 then culture medium was autoclaved at 121°C at 105 kPa for 20 min. Also, explants were transferred to establishment medium after sterilization.

They were incubated in the culture room at a temperature of 25±2°C and with a photoperiod of 16 h light and 8 h dark cycle. The 20 test tubes were used for culturing explants in each treatment (one explant/per test tube). After 4 weeks the percentage of browning, fungi and bacterial contamination, break of buds, shoot length and node number were recorded. Data were analyzed with SPSS Ver. 16 and mean comparison was performed using by Duncan's New Multiple Range Test ($p \leq 0.05$).

Table 1. Types of different decontamination procedures

Treatments	Decontamination procedures
T1	5% Sodium hypochlorite for 20 min
T2	10% Povidone iodine for 10 min
T3	Commercial dettol for 10 min
T4	Commercial benzalkonium chloride for 10 min
T5	0.1% (w/v) solution of mercury (ii) chloride for 5 min
T6	0.1% (w/v) solution of mercury (ii) chloride for 8 min
T7	2.5% Sodium hypochlorite for 20 min with cefotaxime 250 mg L-1 and tetracycline 100 mg l-1 through direct applying on media
T8	0.1% (w/v) solution of mercury (ii) chloride for 2 min and cefotaxime 250 mg L-1 plus tetraccline 100 mg L-1 through direct appling on media

RESULTS

Browning percentage: Analysis of variance showed significant differences among the various decontamination procedures in the case of browning percentage ($p \leq 0.01$) (Table 2). Browning rangeof explants was 6-68% in T6 and T7. Minimum browning percentage observed in T7 and T8 (6 and 8% respectively). Browning percentage showed no significant difference among these decontamination procedures, but these treatments were significant differences with other treatments. In addition, except T7 and T8, significant differences observed between other treatments (Fig. 1). Browning of explants can be due to high concentrations or long-term use of decontamination components. T7 had weak decontaminant component (2.5% Sodium Hypochlorite) and browning percentage of explants in T7 was lower than T1 (5% Sodium hypochlorite). In decontamination procedures containing Mercuric (II) Chloride the browning percentage in T8 was lower (Short time treatment) than T5 (Medium time treatments) and T6 (Long time treatments).

Bacterial contamination percentage: Bacterial contamination percentage observed significantly different among decontamination procedures ($p \leq 0.05$) (Table 2). Range of bacterial contamination percentage of explants was ranged 0% in T7 and T8 - 86% in T4. It was completely controlled in T7 and T8. There is non significant difference among decotamination procedures, but significant differences shown with other treatments in the bacterial contamination percentage.

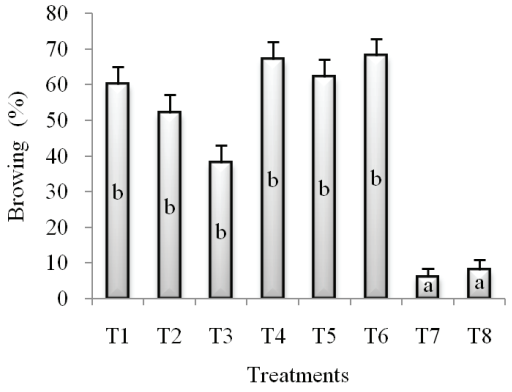

Figure 1. Effect of different decontamination procedures on browning of R. canina explants (30 days)

Table 2. Analysis of variance table

Source	DF	Browning (%)	Bacterial contamination (%)	Fungi contamination (%)	Bud Sbreak (%)	Hoot length (mm)	Node number
Decontamination procedures	7	1.523**	2.453*	0.705*	2.620**	5.614**	35.582**
Error	229	0.212	0.182	0.096	0.354	1.237	7.954

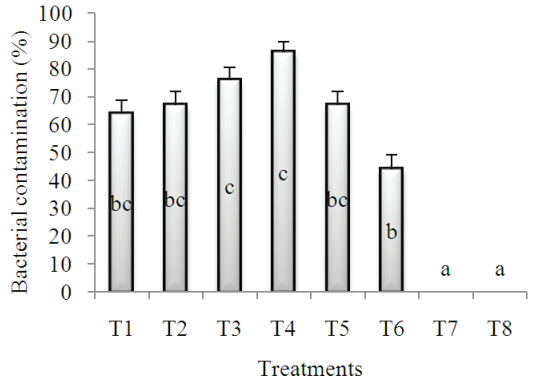

Figure 2. Effect of different decontamination procedures on bacterial contamination of R. canina explants (30 days)

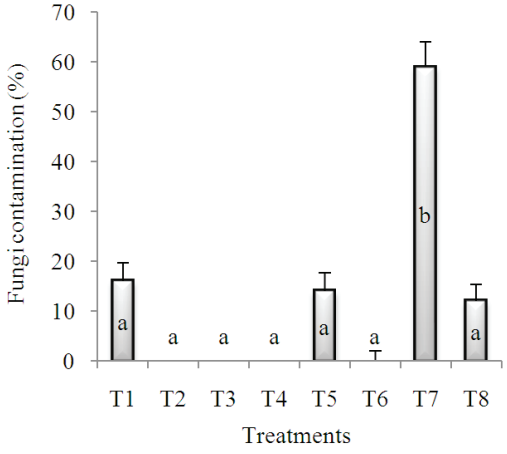

Figure 3. Effect of different decontamination procedures on fungi contamination of R. canina explants (30 days)

There were significant differences among T1, T2, T3, T4, T5, T6 for bacterial contamination (Fig. 2). Bacterial contamination percentage in T8 was significantly lower than T6 and T5 because in T8 addition Mercury (II) Chloride, two anti-bacterial (Cefotaxime and Tetracycline), also used for surface sterilization.

In this study, the effects of 10% Povidone iodine, commercial Dettol, commercial Benzalkonium Chloride were fewer than anti-bacterial properties, so that bacterial contamination has not a significant decrease.

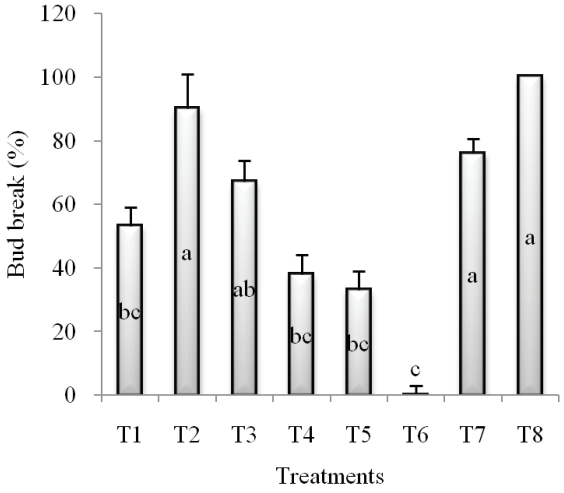

Figure 4. Effect of different decontamination procedures on bud break of R. canina explants (30 days)

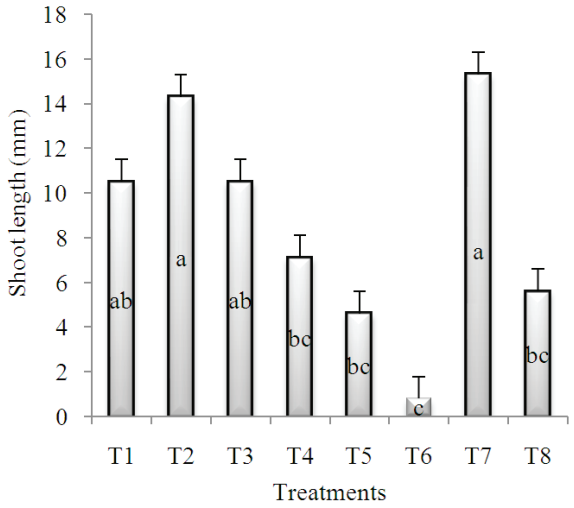

Figure 5. Effect of different decontamination procedures on shoot length of R .canina explants (30 days)

Fungi contamination percentage: The effect of different contamination procedures on fungi contamination percentage were significant. ($p \leq 0.05$) (Table 2). Fungi contamination percentage was from 59% in T7 - 0% in T2, T3 and T4. Fungi contamination percentage among other procedures were 12-18%. It controlled completely (100%) by T2, T3, T4, T6 and partially controlled by T8 and non controlled by T7 (because weak decotamination components were used in T7) (Fig. 3) Also,fungi contaminations percentage in T1 was less (5% Sodium Hypochlorite) than T7 (2.5% Sodium Hypochlorite).

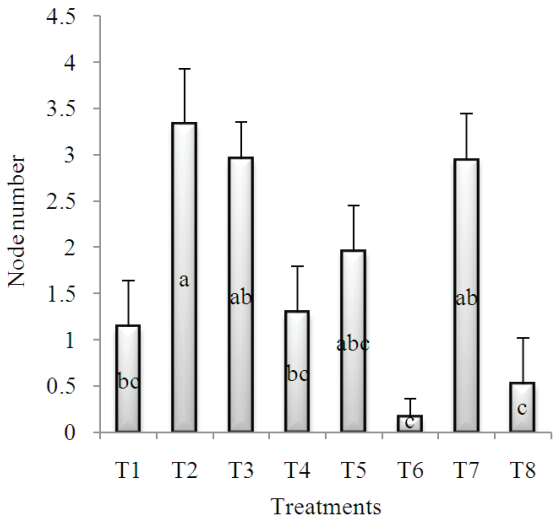

Figure 6. Effect of different decontamination procedures on node number of R. canina explants (30 days)

In this investigation, long-term using of Mercury (II) Chloride affected on fungi contamination percentage. In T6, using long-term treatment of this material, fungi contamination percentage was less compared to T5 (Medium time treatment) and T8 (Short time treatment). Povidone Iodine 10% and commercial Dettol and commercial Benzalkonium Chloride were property of decontamination components and powerful in reducing of fungi contamination, so that fungi contamination percentage was 0%.

Bud break percentage: Bud break percentage was significantly different among decontamination procedures ($p \leq 0.01$) (Table 2). Bud break observed in T8 completely (100%) but in T6was not observed. In other procedures, bud break percentage ranged from 33-90%. Maximum bud break percentage observed in T8, T7 and T2. There was non-significant difference between them but significant differences among these procedures with other decontamination procedures were observed, also there was non-significant difference between treatments T1, T4 and T5 for bud break (Fig. 4). Bud break percentage in T7 was more than T1 because there used weak decontamination component (2.5% Sodium Hypochlorite) in T7. Long time using of Mercury (II) Chloride caused to bud break by toxicity of Hg+ ions. Therefore, bud break percentage in T8 (short time treatment) was higher than T5 (medium time treatment) and T6 (long time treatment). In T2 bud break percentage was higher than T3 and T4 because among treatments chlorine was less in T2 (10% Povidone Iodine) according to T3 (commercial Dettol) and T4 (commercial Benzalkonium Chloride).

Shoot length: The effect of various decontamination procedures on shoot length was significant. ($p \leq 0.01$) (Table 2). Variation of shoot length observed from 0.08-15.3 mm. The highest shoot length in T2 and T7 and the lowest shoot length in T6 observed. Shoot length in T1, T2, T3 and T7 was non-significant difference but with other treatments showed a significant difference. Among the treatments, T4, T5 and T8 shoot length was non significant difference (Fig. 5). Also, shoot length in T7 was higher than T1 because there was used weak decontamination components (2.5% Sodium Hypochlorite) in the T7. Long time using of Mercury (II) Chloride showed lower shoot length by toxicity of Hg+ ions. Therefore, shoot length percentage in T8 (short time treatment) was higher than T6 (long time treatment) and T5

(medium time treatment). Shoot length of T2 was higher than T3 and T4 because less of chlorine was in the Povidone Iodine.

Node numbers: The effect of treatments on node numbers was significant. ($p \leq 0.01$) (Table 2). Node numbers ranged from 0.16-3.33.

Also, in T2, T3 and T7, node numbers were more than other decontamination treatments. Node numbers between T6, T8 and T4, T1 was not significant (Fig. 6). Node numbers in T7 was more than T1 because of weak decontamination components (2.5% Sodium Hypochlorite) used in T7 according to T1. In decontamination procedures that contain Mercury (II) Chloride, node numbers in T6 was less (Long time treatment) than T5 (Medium time treatment) and T8 (Short time treatment) by long time use of mercury (II) Chloride node numbers in T2 were more than T3 and T4 because of chlorine was less in T2.

DISCUSSION

Explant browning can be due to high concentrations or long-term use of decontamination components [6, 8].

In this study, minimum browning percentage was in T7 and T8 treatment. But weak decontaminant component observed in T7 and shortest time for establishment procedures and sterilisation obtained in T8 treatment.Organic decontamination components used to decontamination but the effects of components on plant tissues were harmful and most of samples were destroyed. Bacterial contamination controled completely by T7 and T8 (Mercury (II) Chloride, Cefotaxime and Tetracycline) treatments. Bacterial contamination in R. damascena was controlled by Cefteriaxon and Ofloxacin [6]. In this study, Povidone iodine, commercial Dettol, commercial Benzalkonium Chloride were fewer anti-bacterial properties so that the effect of this treatments on reduce of bacterial contamination was not significant. d. Fungi contamination completely controlled (100%) by T8 and some treatments such as T2, T3, T4, T6 as well as Povidone Iodine and commercial Dettol and Benzalkonium Chloride. Maximum bud break observed in T8 and T7 and T2. Long time using of Mercury (II) Chloride caused to bud break by toxicity of Hg+ ions. Therefore, bud break percentage in T8 (short time treatment) was higher than T5 (medium time treatment) and T6 (long time treatment) [15]. Long time using of Mercury (II) Chloride showed lower shoot length by toxicity of Hg+ ions. Therefore, shoot length percentage in T8 (short time treatment) was higher than T6 (long time treatment) and T5 (medium time treatment). Also, node numbers was high on weak decontamination components (2.5% Sodium Hypochlorite) used in T7.

CONCLUSION

In this study contamination percentage reduced and survival and growth of explants increased generally. Among different decontamination treatments, T8 could be controlled of fungi and bacterial contamination because it was containing Cefotaxime and Tetracycline for surface and internal sterilization as well as Mercury (II) Chloride for surface sterilization. Through of Mercury (II) Chloride that was used in low time treatment, Highest bud break and lowest browning percentage observed in T8 , however in T8 chlorophyll destroyed by Tetracycline and Cefotaxime. Shoot length and node numbers were less in these two treatments because photosynthesis reduced due to chlorosis and necrosis of leaves.

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