EFFECT OF CULTURE MEDIA ON MICROPROPAGATION AND *IN VITRO* FLOWERING OF RED EDEN ROSE (*Rosa* 'Red Eden')

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Abstract

Roses are one of the most popular flowers in the world because of their beauty, color, aroma as well as their various uses and Red Eden rose is one of the most popular hybrid roses. Results of the study showed the most optimal medium for shoot multiplication, which was MS + 30 g/l of sucrose + 8 g/l of agar supplemented with 0.5 mg/l of BA. The optimal medium for growth and flowering was ½MS + 30 g/l of sucrose + 8 g/l of agar supplemented with 30 µM of $AgNO_3$. However, NAA at all tested concentrations did not show any effect on in vitro plant growth.

Keywords: *In vitro, MS medium, Red Eden rose, Rosa 'Red Eden'.*

ẢNH HƯỞNG CỦA MÔI TRƯỜNG NUÔI CẤY ĐẾN VI NHÂN GIỐNG VÀ RA HOA ỐNG NGHIỆM CỦA HOA HỒNG LEO MỘNG VY (*Rosa* 'Red Eden')

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Tóm tắt

Hoa hồng là một trong những loài hoa được ưa chuộng nhất thế giới bởi nét đẹp, màu sắc và hương thơm cũng như những công dụng khác nhau của nó. Trong đó, giống hoa hồng leo Mộng vy (Rosa 'Red Eden') là một trong những giống hoa hồng lai được ưa chuộng. Kết quả nghiên cứu cho thấy môi trường phù hợp nhất cho sự nhân chồi của cây hoa hồng leo Mộng vy là môi trường MS + 30 g/L sucrose + 8 g/L agar có bổ sung 0,5 mg/L BA và môi trường tối ưu nhất cho sự phát triển và ra hoa của cây là môi trường ½MS + 30 g/L sucrose + 8 g/L agar có bổ sung 30 µM $AgNO_3$. Kết quả nghiên cứu chưa ghi nhận được tác động tích cực của NAA đến sự sinh trưởng và phát triển của cây in vitro.

Từ khóa: *In vitro, môi trường MS, hoa hồng leo Mộng vy, Rosa 'Red Eden'.*

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1. Introduction

Roses are one of the most popular flowers in the world because of their beauty, color and aroma. Currently, roses have been sold in forms of cut flowers and potted plants. They are used for different purposes such as decoration, cosmetics, medicine and so on. There are many varieties of bred and hybridized rose, and Red Eden rose is one of the most attractive hybrid roses. With high economic values, maintaining and propagating rose cultivars are essential issues. In traditional breeding methods, seedlings may be infected with different plant pathogens, the productivity is low while the process is time-and-labour-consuming. In contrast, tissue culture technique can overcome those limitations and produce high-quality plants. Hence, the research, entitled "Effect of culture media on micropropagation and in vitro flowering of Red Eden rose (*Rosa* 'Red Eden'), was carried out .

2. Materials and methods

2.1. Materials

Stem nodal segments of Red Eden rose.

Figure 1. Stem nodal segments and Red Eden rose (*Rosa* 'Red Eden')

Chemicals: MS medium (Murashige and Skoog, 1962), 6-benzyladenine (BA), 1-naphthaleneacetic acid (NAA), silver nitrate ($AgNO_3$), sucrose, agar, activated charcoal…

In tissue culture technique, $AgNO_3$ is supplemented into media for inhibition of ethylene activities, a flower-inhibited compound in plants (Beyer, 1976).

Experiments were carried out in plant tissue culture laboratory condition (26°C±2°C of temperature, 1500 lux of illumination power, 16h/day of illumination time). The culture media were adjusted to 5.8 of pH, autoclaved at 121°C and 1 atm for 20 minutes.

2.2. Methods

Figure 2. A shoot of Red Eden rose after 20 culture days

Experiment 1: Effects of cytokinin (BA) on shoot multiplication of Red Eden rose

Materials disinfection: stem nodal segments (about 3 – 4 cm in length) were immersed in a mixture of water and dishwashing detergent (7:3), stirred for 5 minutes and rinsed under tap

water. Then, they were disinfected by ethanol 70° for 1 minute and household detergent for 15 minutes in a laminar flow cabinet. Stem nodal segments were rinsed with sterilized- distilled-water four times before inoculation. Disinfected explants were inoculated into proliferation media including MS medium, 30 g/l of sucrose, 8 g/l of agar, 2 mg/l of BA and 0.1 mg/l of NAA. Then, shoots were formed (Figure 2) and used for next experiments.

Materials: *in vitro* shoots with 1 cm in length.

Experimental design: the experiment was designed in CRD (completely randomized design) with four replications. The experiment consisted of 1 factor and 5 treatments (NT) with different BA concentrations (0 – 2.0 mg/l).

Observed norms: multiplication coefficient, number of leaves/shoot, shoot morphologic feature (after 10, 20 and 30 days).

Experiment 2: Effects of auxin (NAA) combined with cytokinin (BA) on shoot multiplication of Red Eden rose

Materials: *in vitro* shoots with 1.5 cm in length (from Experiment 1).

Experimental design: The experiment was designed in CRD with four replications. The experiment consisted of 1 factor and 5 treatments (NT) with different NAA concentrations (0 – 0.4 mg/l), which were combined with the surveyed concentration of BA from Experiment 1.

Observed norms were similar to Experiment 1.

Experiment 3: Effects of medium content and $AgNO_3$ on growth and flowering ability of Red Eden rose

Materials: *in vitro* 3-month-shoots with 2 cm in length (from previous experiments).

Experimental design: the experiment was designed in CRD with seven replications. The experiment consisted of 2 factors and 12 treatments (NT) with different MS medium contents and $AgNO_3$ concentrations.

Medium formulas were the combination of 3 different medium contents (MS, ½MS and ¼MS) and 4 different concentrations of $AgNO_3$ (10, 20, 30 and 40 µM).

Observed norms: root formation rate (%), flower-bud formation rate (%), flowering rate (%), flower color (after 60 days).

Experimental data was processed by Microsoft Excel 2013 software. ANOVA (Analysis of Variance) and mean values were compared by Duncan test 5% of SAS (Statistical Analysis Systems) 9.1 program.

3. Results and discussion

3.1. Effects of cytokinin (BA) on shoot multiplication of Red Eden rose

Results of the experiment are presented in Table 1. The numbers of leaves per shoot were significantly different at 1% probability level among treatments after 10 days. The concentration of BA giving the highest number of leaves was 1 mg/l (NT_3) (6 leaves/shoot) and it statistically had no significant difference compared to other treatments except 2 mg/l of BA concentration in NT_5 (3.75 leaves/shoot). After 30 days, NT_2 (medium with 0.5 mg/l of BA) gave the highest shoot-multiplication coefficient (3.00), however, the difference was statistically not significant as compared to the other treatments. Observing shoot morphologic feature is also an essiential factor for plant growth research. Figure 3 shows that shoots were almost not multiplicative and tended to become brown, withered, which were inoculated in media without BA supplement. The study demonstrated that Red Eden rose adapted well to low BA concentration (0.5 mg/l) with the healthiest shoots.

Nguyen (2005) indicated that red roses' shoots gave the highest multiplication coefficient (3.47) when using 2 mg/l of BA while 5.94 was recorded for white roses when using 1.5 mg/l of BA after 4 weeks of culture. Hameed et al. (2006) suggested that the best bud growth of *Rosa indica* L. was achieved when the medium

contained 1 mg/l of BA supplement. Nguyen, Ngo et al. (2015) recorded that the medium supplemented with 1.5 mg/l of BA resulted in the fastest growth of *Rosa sericea* Lindl which the shoot multiplication coefficient was 2.73 after 6 weeks. Multiplication coefficient of shoot in this experiment yielded lower values than above mentioned studies. It proved that different varieties of rose had different requirements for cytokinin as well as culture conditions.

Table 1. Effects of BA on shoots of Red Eden rose after 10, 20 and 30 culture days

Treatment	BA concentration (mg/l)	Multiplication coefficient of shoot			Number of leaves/shoot			Shoot morphologic feature
		10 days	20 days	30 days	10 days	20 days	30 days	
NT_1	0	1.25	1.25	1.25	5.25[a]	4.25	5.00	withered, brown
NT_2	0.5	1.75	2.75	3.00	5.00[a]	5.50	6.75	healthy, green
NT_3	1.0	1.50	1.50	1.75	6.00[a]	6.00	6.50	healthy, green
NT_4	1.5	1.50	1.50	1.50	5.75[a]	5.00	6.25	healthy, green
NT_5	2.0	1.50	1.75	1.50	3.75[b]	4.25	6.00	healthy, green
F		ns	ns	ns	**	ns	ns	
CV (%)		54.09	49.21	49.43	12.90	21.06	16.86	

*Note: In a column, values with the same letter are significantly not different in statistical meaning. With Duncan 5% test, **: difference at 1%; ns: no significant difference in statistics.*

Figure 3. Cultured shoots in media of different BA concentrations after 30 days

3.2. Effects of auxin (NAA) combined with cytokinin (BA) on shoot multiplication of Red Eden rose

Achieved results of the experiment are displayed in Table 2. After 30 days, shoot multiplication of NT_1 (without NAA supplement) was significantly different at 1% probability level compared to other treatments (with NAA supplement). The number of leaves per shoot was significantly different at 5% probability

level among treatments after 10 days, however, the difference was no longer apparent after 20 and 30 days of culture. Figure 4 shows that shoots were almost not multiplicative, brown and withered in culture media supplemented NAA at different concentrations. In NT1 (with 0.5 mg/l of BA and no NAA supplement), shoots grew and multiplied stronger than NAA-supplemented treatments. This consequence indicated that NAA had no effect on Red Eden rose growth. It was against plenty of reports, for instance, Arif and Khatamian (1991) showed that the medium supplemented with 3 mg/l of BA and 0.05 mg/l of NAA achieved the highest multiplication coefficient (4.00) on 'Petite Folie' rose shoot after 30 days. Salekjalali (2012) also recorded that the combination of 0.1 mg/l of NAA and 2 mg/l of BA obtained a high multiplication coefficient (6.00) on shoots of *Rosa damascena* Mill.

Table 2. Effects of NAA combined with BA (0.5 mg/l) on shoot of Red Eden rose after 10, 20 and 30 culture days

Treatment	NAA concentration (mg/l)	Multiplication coefficient of shoot			Number of leaves/shoot			Shoot morphologic feature
		10 days	20 days	30 days	10 days	20 days	30 days	
NT_1	0	1.00	1.50	3.25^{a}	3.00^{ab}	5.75	5.50	healthy, green
NT_2	0.1	1.00	1.00	1.00^{b}	2.75^{b}	4.00	4.50	withered, brown
NT_3	0.2	1.00	1.00	1.00^{b}	3.25^{ab}	3.50	3.75	withered, brown
NT_4	0.3	1.00	1.00	1.00^{b}	4.00^{a}	4.50	4.25	withered, brown
NT_5	0.4	1.00	1.00	1.00^{b}	4.00^{a}	5.25	5.50	withered, brown
F			ns	**	*	ns	ns	
CV (%)			23.47	38.81	18.41	24.39	24.18	

*Note: In a column, values with the same letter are significantly not different in statistical meaning. With Duncan 5% test, **: difference at 1%; *: difference at 5%; ns: no significant difference in statistics.*

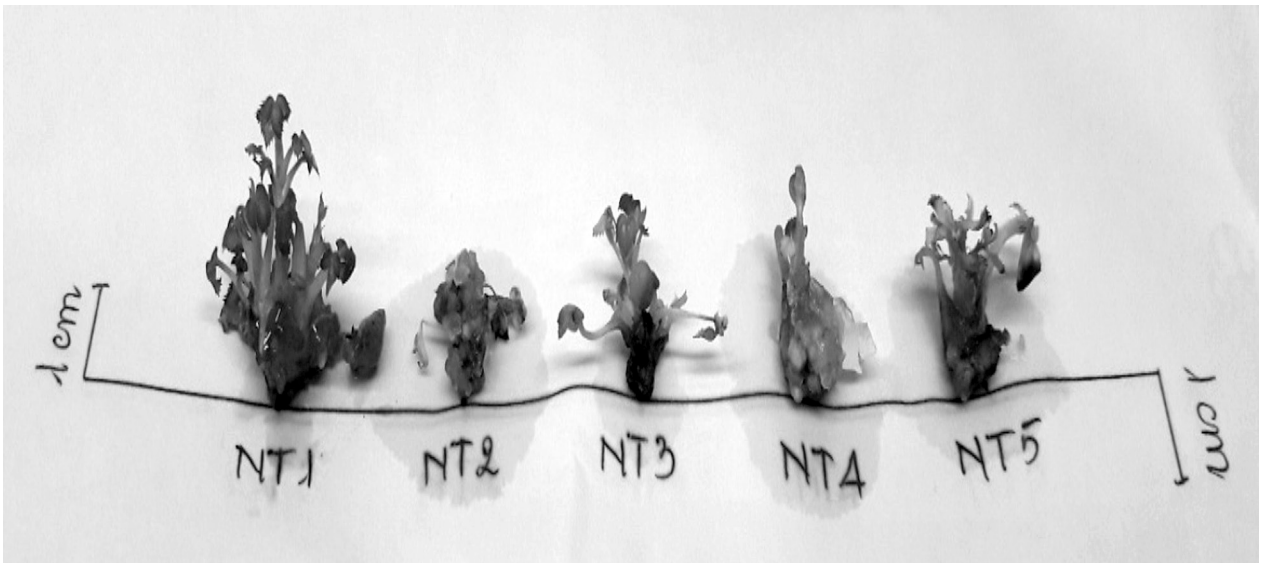

Figure 4. Cultured shoots in different NAA-concentration media combined with 0.5 mg/l of BA after 30 days

3.3. Effects of medium content and $AgNO_3$ on growth and flowering ability of Red Eden rose

Effects of medium content and $AgNO_3$ on rose's growth and flowering ability are showed in Table 3. After 60 days of culture, root formation rate and flower-bud formation rate were significantly different at 1% probability level among not only treatments, but also experimental conditions. The medium containing ½MS gave better results than ones with MS and ¼MS. While ½MS medium supplemented with 30 µM of $AgNO_3$ (X_7) resulted in the highest rate of root formation (71.43%) and flower-bud formation (71.43%), there was no root formation in ¼MS medium supplemented with 30 µM of $AgNO_3$ (X_{11}) which caused the lowest flower-bud formation rate (14.29%). Moreover, X_7 also got the highest flowering rate (42.86%). Results of flowering rate were significantly different at 1% probability level among treatments, conditions of $AgNO_3$, and at 5% probability level among different medium contents. Besides, ½MS medium supplemented with 20 µM of $AgNO_3$ (X_6) showed the closest color compared to the characteristic color of Red Eden rose (Figure 5). As a result, the supplementary medium of ½MS and 30 µM of $AgNO_3$ was the most optimal medium for growth and flowering of Red Eden rose. It almost matched with study results of Nguyen, Ngo et al. (2015) who indicated that the flowering rate of *Rosa sericea* Lindl was 50% in MS medium supplemented 30 µM of $AgNO_3$. Nguyen, Dang et al. (2015) recorded that MS medium supplemented with 30 µM of $AgNO_3$ resulted in 30%, 40% and 50% of flowering rates respectively for white roses, yellow roses and red roses. Furthermore, Arif and Khatamian (1991) showed that 'Petite Folie' rose was *in vitro* rooted when being cultured in a ½MS medium supplemented with 0.5 mg/l of NAA or 0.1 mg/l of IBA after 8 days.

Table 3. Effects different MS contents and $AgNO_3$ concentrations on Red Eden rose growth after 60 culture days

Treatment	MS content (M)	$AgNO_3$ concentration (µM) (A)	Root formation rate (%)	Flower-bud formation rate (%)	Flowering rate (%)	Flower color
X_1	MS	10	42.86^{bc}	57.14^{ab}	14.29^{bc}	dark pink
X_2	MS	20	28.57^{c}	57.14^{ab}	28.57^{ab}	dark pink
X_3	MS	30	0^{d}	42.86^{bc}	0^{c}	-
X_4	MS	40	42.86^{bc}	28.57^{cd}	0^{c}	-
X_5	½MS	10	57.14^{ab}	71.43^{a}	14.29^{bc}	dark pink
X_6	½MS	20	57.14^{ab}	71.43^{a}	28.57^{ab}	dark red
X_7	½MS	30	71.43^{a}	71.43^{a}	42.86^{a}	cerise
X_8	½MS	40	42.86^{bc}	42.86^{bc}	0^{c}	-
X_9	¼MS	10	42.86^{bc}	42.86^{bc}	14.29^{bc}	dark pink
X_{10}	¼MS	20	71.43^{a}	28.57^{cd}	0^{c}	-
X_{11}	¼MS	30	0d	14.29^{d}	14.29^{bc}	dark pink
X_{12}	¼MS	40	0d	57.14^{ab}	14.29^{bc}	dark pink
F_M			**	**	*	
F_A			**	**	**	
F_{M*A}			**	**	**	
CV (%)			39.73	32.21	105.11	

*Note: In a column, values with the same letter are significantly not different in statistical meaning. With Duncan 5% test, **: difference at 1%; *: difference at 5% in statistics; -: flowering rate (%) =0.*

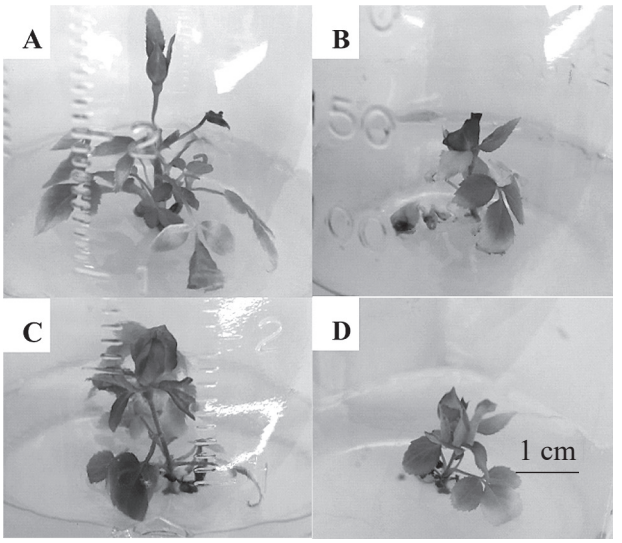

Figure 5. *In vitro* flowering of Red Eden roses in different MS content and $AgNO_3$ concentration media after 60 culture days: (A) MS + 20 µM of $AgNO_3$, (B) ½MS + 20 µM of $AgNO_3$, (C) ½MS + 30 µM of $AgNO_3$, (D) ¼MS + 30 µM of $AgNO_3$

4. Conclusions

The most optimal medium for Red Eden rose multiplication was MS medium supplemented with 0.5 mg/l of BA, which multiplication coefficient result was 3.00 after 30 days.

The combination of 0.5 mg/l of BA and different concentrations of NAA did not record any positive result for the growth of Red Eden rose after 30 days.

½MS medium combined with 30 µM of $AgNO_3$ was the most optimal medium for the growth and flowering of Red Eden rose. It resulted in high root formation rate, flower-bud formation rate and flowering rate after 60 days, which were 71.43%, 71.43% and 42.86% respectively.

The study results can be applied to disinfected Red Eden rose micropropagation. Besides, the success of *in vitro* flowering is the premiss for researching flowering mechanism, a beneficial aspect for "*in vitro* bonsai" and theirs commercial values.

References

Arif, M. B., & Khatamian, H. (1991). In vitro propagation of 'Petite Folie' miniature rose. *Transactions of the Kansas Academy of Science,* (94), 116-124.

Beyer, E. M. (1976). A potent inhibitor of ethylene action in plants. *Plant Physiol*., (58), 268-271.

Hameed, N., Shabbir, A., Ali, A., & Bajwa, R. (2006), In vitro micropropagation of disease free rose (Rosa indica L.). *Mycopath*, (4), 35-38.

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum,* (15), 473-497.

Nguyễn, T. K. T. (2005). Nhân giống cây hoa hồng bằng kỹ thuật nuôi cấy *in vitro. Tạp chí Nông nghiệp và Phát triển nông thôn*, (1), 39-41.

Nguyen, T. P. T., Dang, Q. B., Nguyen, T. T., Nguyen, T. T. L., Pham, T. T. H., Dang, T. T. T., Ninh, T. T., Nguyen, T. L. H., & Nguyen, T. H. (2015). In Vitro Propagation and Flower Induction of Rosa sericea Lindl. *J. Sci. & Devel., (13)*, 606-613.

Nguyen, T. T., Ngo, T. V., Nguyen, T. P. T., Nguyen, T. T. L., Ninh, T. T., Nguyen, T. L. H., & Nguyen, T. H. (2015). Effects of Plant Growth Regulators and Micronutrients on In vitro Growth and Flowering in Roses. *J. Sci. & Devel., (13)*, 1488-1496.

Salekjalali, M. (2012). Phloroglucinol, BAP and NAA enhance axillary shoot proliferation and other growth indicators in vitro culture of Damask Rose (Rosa damascena Mill). *American-Eurasian J. Agric. & Environ. Sci.,* (12), 960-966.