INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 6, No. 6, p. 10-16, 2015

RESEARCH PAPER OPEN ACCESS

Effects of different hormonal concentrations on damask rose (*Rosa damascena* Mill.) micro-propagation in liquid tissue culture medium

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Key words: *Rosa damascena* Mill., Micropropagation, AgNO3.

http://dx.doi.org/10.12692/ijb/6.6.10-5 Article published on March 22, 2015

Abstract

Damask rose (*Rosa damascena* Mill.) from Rosaceae family is one of the most important medicinal plants in Iran which its secondary metabolites are extensively used in pharmaceutical, flavors and fragrance industries. It is usually propagated by cutting and transplanting. Since this method is time-consuming and has usually some problems therefore, in the current study the effects of different hormonal concentrations were studied on micro-propagation of superior genotype Khuzestan in liquid tissue culture medium. The lateral buds were cultured on modified Murashige and Skoog (MS) culture environments treated with different concentrations of hormones Benzyl Adenine (BA) and Indole-3-butyric acid (IBA). Results of Means comparison showed that the BA concentration of 0.75 mg.l^{-1}and IBA concentration of 0.1 mg.l^{-1}had the highest effect on both shoot number with mean number of 4 and height with mean 2 cm. Also, the effects of NAA and IBA hormones on root number were significant. The best culture medium for increasing root number and percentage was found to be ½ MS with a BA concentration of 0.01 mg.l^{-1} and IBA concentration of 1 mg/l in combination with AgNO3 concentration of 58.85 µmol.l^{-1}. The top proliferation treatment was determined at a combination of 0.75 mg.l^{-1} BA and 0.1 mg.l^{-1} IBA. The best treatment of rooting percent (55%) was determined to be a combination of 0.01 mg.l^{-1} BA and 1 mg.l^{-1} IBA in presence of 58.85 µmol.l^{-1} AgNO3.

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Introduction

Rose is the king of flowers and Damask rose (*Rosa damascena* Mill.) is classified in old garden roses (Peter Bealis, 1990). The origin of Damask rose is Iran. It's an important species among the scented roses, yields a highly fragrant commercially valuable essential oil. The products of Damask rose are rose water, rose oil and dried petals that are used in medicine, food and perfume industry, as well as make-up and health products. Damask rose is also used as an ornamental plant in parks, gardens and houses.

Traditionally, rose plants have been propagated on rootstocks and through cutting method (Roberts and Schum, 2003). The conventional propagation of this species was associated with various problems such as limitation of stock plants and prolonged production time (Skirvin *et al*., 1990) and low adventitious root formation on cutting. In-vitro propagation methods have been used by many rose growers because of enormous potential for mass multiplication of elite colons and production of healthy and disease-free planting materials (Pati *et al*, 2005). The success of these methods for Damask rose cultivars is dependant to the cultivar and genetic background of the plant (Kornova and Michailova, 1994). A liquid culture system using nodal segments was used for shoot proliferation and root induction in *Rosa damascena* Mill. and *Rosa. burboniana*. For efficient and larg-scale induction of roots in micro shoots, a rooting vessel was designed and developed to facilitate the micro propagation protocol (Pati *et al*., 2005). Their work highlights the significance of osmotic potential in relation to enhanced growth and development in liquid cultures, vis-à-vis agar-gelled cultivars, especially in relation to root induction during micro propagation. Khosh Kui and Sink (1982) concluded that the best hormonal compound for in vitro propagation of Damask rose is BA (2 $mg.l^{-1}$) and NAA (0.1 $mg.l^{-1}$). However, it's necessary to work more in micropropagation of Damask rose in liquid culture.

The superior genotype Khuzestan cultivate in Iran and it is tolerant cold and disease. The objective of the study was to investigate the effects of PGRs on micro-propagation of superior genotype Khuzestan in liquid MS medium.

Materials and methods

Plant material

Nodal segments containing lateral buds were collected from mature rootstocks of genotype Khuzestan planted in the Damask rose Research Center of Agriculture and Natural Resources of Central province of Iran. First, the explants were thoroughly washed with dishwashing liquid and running tap water for 15 min before putting them in 2 $g.l^{-1}$ carbendazim for 45 minutes.

Surface sterilization was done under a laminar hood and sterile conditions using 70% (v/v) ethanol for 30 seconds followed by a 22 min soak in 3% (v/v) sodium hypochlorite solution. The samples were then rinsed three times with one minute intervals with sterile distilled water.

Culture Medium, establishment and shoot proliferation stage

The medium was autoclaved at 121°C for 20 min. For proliferation, explants were established in an MS medium containing 30 $g.L^{-1}$ (w/v) sucrose and 3.5 $g.L^{-1}$ plant agar (as solidifying agent) supplemented with some PRGs such as BA (0, 0.5, 0.75, 1, 1.25 $mg.l^{-1}$) and IBA concentrations (0.1, 0.3, 0.5 $mg.l^{-1}$). Micro-shoots were sub-cultured triweekly. Number and length of axillary shoots and size of leaves were recorded.

Rooting stage

After proliferation and in rooting stage, explants were sub-cultured on a ½ MS liquid medium supplemented with IBA (0.1, 0.3, 0.5 $mg.l^{-1}$) and NAA (0.1, 0.3, 0.5 $mg.l^{-1}$). In order to improve the quality of leaves, the concentration level of NH_4NO_3 was lowered down to 1500 $mg.l^{-1}$ and 58.85 $\mu mol.l^{-1}$ $AgNO_3$ was also added to the liquid medium. In order to add heat sensitive materials (such as $AgNO_3$) to the medium, a cold filter was used under sterile conditions when the medium's temperature reached 40°c. The medium pH was adjusted between 5.8±0.1. Samples were grown in

growth chamber at 25°c under a photoperiod of 16h light and 8h dark cycle. Percent of rooting, number and the length of the roots were recorded.

Experimental design and statistical analysis

The experimental design used was a randomized complete block with three replications. For proliferation stage, analysis of variance was performed by using SAS program and comparison of means was conducted using Duncan Test. Rooting stage data was analyzed using non-parametric Mann Whitney (U) test since the distribution of the data did not follow the normal distribution.

Results

Analysis of variance of data for proliferation stage showed that BA and IBA together with their different combinations had significant effects on shoot and leaf size (Table 1). However, shoot number did not show significant differences among hormonal treatments.

Mean comparisons showed that combination of BA (0.75 or 1 $mg.l^{-1}$) plus IBA (0.1 $mg.l^{-1}$) increased shoot length with a mean 2 cm (Table 2). Maximum leaf size was observed in the medium containing 1-BA (0.75 $mg.l^{-1}$) + IBA (0.1 $mg.l^{-1}$) + $AgNo_3$ (58.85 $\mu mol.l^{-1}$) + NH_4NO_3 (1500 $mg.l^{-1}$) and 2-BA (1.25 $mg.l^{-1}$) + IBA (0.1 $mg.l^{-1}$).

Table 1. Analysis of variance for different concentrations and combinations of BA+IBA on measured traits.

Source of variance	DF	Mean Squares		
		Shoot number	Shoot length	Leaf size
Different Hormonal Combinations	17	0.00374 n.s	0.0418**	1.656**
Replication	2	0.00757 *	0.0612 *	0.296 n.s
Error	34	0.00224	0.0148	0.375
Coefficient of variation%		4.57	13.20	13.66

* and ** are significant at 5% and 1% respectively and n.s is not significant.

Table 2. Analysis of the mean concentration of different combinations of treatment BA+IBA by method Duncan (α=0.05).

	Hormonal combinations	Shoot number	Shoot length	Leaf size
1	BA(0.75)IBA(0.1)	4 a	2 a	2.33 ab
2	BA(0.75)IBA(0.1)AgNo3(58.85)NH4NO3(1500)	0 b	0.5 dc	3 a
3	BA(0.75)IBA(0.3)	2 ab	0.5 dc	1 c
4	BA(0.75)IBA(0.3)AgNo3(58.85)NH4NO3(1500)	1 b	1.5 abc	1 c
5	BA(0.75)IBA(0.5)	2 ab	0.67 bcd	1 c
6	BA(0.75)IBA(0.5)AgNo3(58.85)NH4NO3(1500)	0 b	1 abcd	1 c
7	BA(1.00)IBA(0.1)	4 a	2 a	2.33 ab
8	BA(1.00)IBA(0.1)AgNo3(58.85)NH4NO3(1500)	2 ab	1 abcd	1.67 bc
9	BA(1.00)IBA(0.3)	2 ab	0.67 bcd	1 c
10	BA(1.00)IBA(0.3)AgNo3(58.85)NH4NO3(1500)	2 ab	1 abcd	1 c
11	BA(1.00)IBA(0.5)	2 ab	0.5 dc	1 c
12	BA(1.00)IBA(0.5)AgNo3(58.85)NH4NO3(1500)	0 b	0.5 dc	1 c
13	BA(1.25)IBA(0.1)	2 ab	0.83 bcd	3 a
14	BA(1.25)IBA(0.1)AgNo3(58.85)NH4NO3(1500)	2 ab	1 abcd	1.67 bc
15	BA(1.25)IBA(0.3)	2 ab	0.33 d	1 c
16	BA(1.25)IBA(0.3)AgNO3(58.85)NH4NO3(1500)	1 b	1 abcd	1 c
17	BA(1.25)IBA(0.5)	4 a	1.67 ab	2.33 ab
18	BA(1.25)IBA(0.5)AgNO3(58.85)NH4NO3(1500)	1 b	0.83 bcd	1 c

In each column, means followed by the same letters are not significantly different using Multiple Duncan Test.

At rooting stage, the liquid medium consisting of BA (0.01 mg.l^{-1}) + IBA (1 mg.l^{-1}) in presence of $AgNo_3$ (58.85 µmol.l^{-1}) resulted in the highest percentage of rooting (55%), root length (5 cm) and root number (7) (Table 3 and Fig. 1C). No rooted plantlet was observed in treatments 6, 8, 9 and 12 (Table 3).

Discussion

A single node containing lateral buds in the establishment stage in solid MS medium containing BA and IBA started growing after one week and produced one full branch containing few leaves after three weeks (Fig. 1A). After sub-culturing plantlets and transferring to the new liquid medium, new branches were formed indicating that the formation of new primordial branches were affected by hormones added to the medium (Fig. 1B). Combination of BA (0.75 mg.l^{-1}) and IBA (0.1 mg.l^{-1}) promoted lateral bud emergence and plant reproduction in the later stages of proliferation. According to our tests, 0.75 mg.l^{-1} BAP with 0.1 mg.l-^{1} IBA was the best concentration for propagation of Damask rose but Jabbarzadeh and Khosh-Khui (2005) concluded that 2.5 to 3 mg.l-^{1} BAP with 0.1 mg.l-^{1} IBA is the best treatment for propagation of Damask rose (Jabbarzadeh and Khosh – Khui, 2005). On the other hand, our results showed that higher concentration of BA in the medium reduced number of branches and shoots became abnormal (Fig. 1D). Carelli and Echeuerrigaray showed that number of shoots increased with addition of BAP concentration in the medium (Carelli and Echeuerrigaray, 2002).

Table 3. Comparison among treatments (Average ranking) using Mann Whitney method (U test).

Row	Hormonal combinations	Root number		Root length		Rooting percentage	
		Mean	Rank	Mean	Rank	Mean	Rank
1	IBA(1)AgNO3(58.85)	2.67 ab	19.67	2.67 ab	20.00	22 ab	19.83
2	IBA(2)AgNO3(58.85)	0.33 b	17.67	1 ab	18.83	11 ab	18.33
3	IBA(3)AgNO3(58.85)	5.67 ab	20.67	0.83 ab	18.33	11 ab	18.33
4	NAA(1)AgNO3(58.85)	4 ab	20.17	3 ab	20.33	33 ab	20.50
5	NAA(2)AgNO3(58.85)	0.33 b	17.67	0.67 ab	17.67	11 ab	18.33
6	NAA(3)AgNO3(58.85)	0 b	13.00	0 b	13.00	0 b	13.00
7	BA(0.01)IBA(1)AgNO3(58.85)	7 a	31.83	5 a	31.17	55 a	31.17
8	BA(0.01)IBA(2)AgNO3(58.85)	0 b	13.00	0 b	13.00	0 b	13.00
9	BA(0.01)IBA(3)AgNO3(58.85)	0 b	13.00	0 b	13.00	0 b	13.00
10	BA(0.01)NAA(1)AgNO3(58.85)	1.67 ab	23.33	2.33 ab	24.33	33 ab	25.17
11	BA(0.01)NAA(2)AgNO3(58.85)	1.67 ab	19.00	1.33 ab	19.33	11 ab	18.33
12	BA(0.01)NAA(3)AgNO3(58.85)	0 b	13.00	0 b	13.00	0 b	13.00

In each column, means followed by the same letters are not significantly different using Mann Whitney Test.

our results showed that IBA as compared to NAA hormone was a better rooting hormone for this genotype. it has been suggested that differences in rooting varieties are caused due to differences in genotypes (Hasegawa, 1980). Arnold *et al.*, (1992) showed that the concentration of medium required for roses is dependent on cultivar response to appropriate rooting (Arnold. Pratapkumar *et al.* (2001) were able to obatin a suitable rooting for Damask rose using half-strength MS liquid medium and IBA.

Rooting of older rose such as Damask rose is more difficult than modern rose such as Rosa hybrida. In this research, Khuzestan genotype was rooted by IBA hormone.

Aside from the manipulation of hormone levels to promote shoot, root and proliferation, current studies indicate that there are genes responsible for increased number of bud initials and shoot proliferation. Moreover, the possible involvement of the gene in modulating hormone levels has also been

reported (Tantikanjana *et al*., 2001).

Conclusions and Recommendations

Elimination of agar in the liquid medium reduces the cost (Sandal *et al*., 2001). Therefore, our effort in the complete elimination of agar in the multiplication medium, the use of lower volume of liquid medium, prolong the culture period and increased rate of multiplication of shoots in liquid medium and all accounted for substantial cost reduction (Pati *et al*., in press). Moreover, better response in static liquid cultures could be ascribed to (1) a better contact between explants and the liquid medium which increases the availability of cytokines and other nutrients in liquid state (Debergh, 1983), (2) dilution of any exudates from the explants in liquid medium (Ziv and Halevy, 1983), and (3) adequate aeration in liquid media which ultimately enhances growth and multiplication (Ibrahim, 1994).

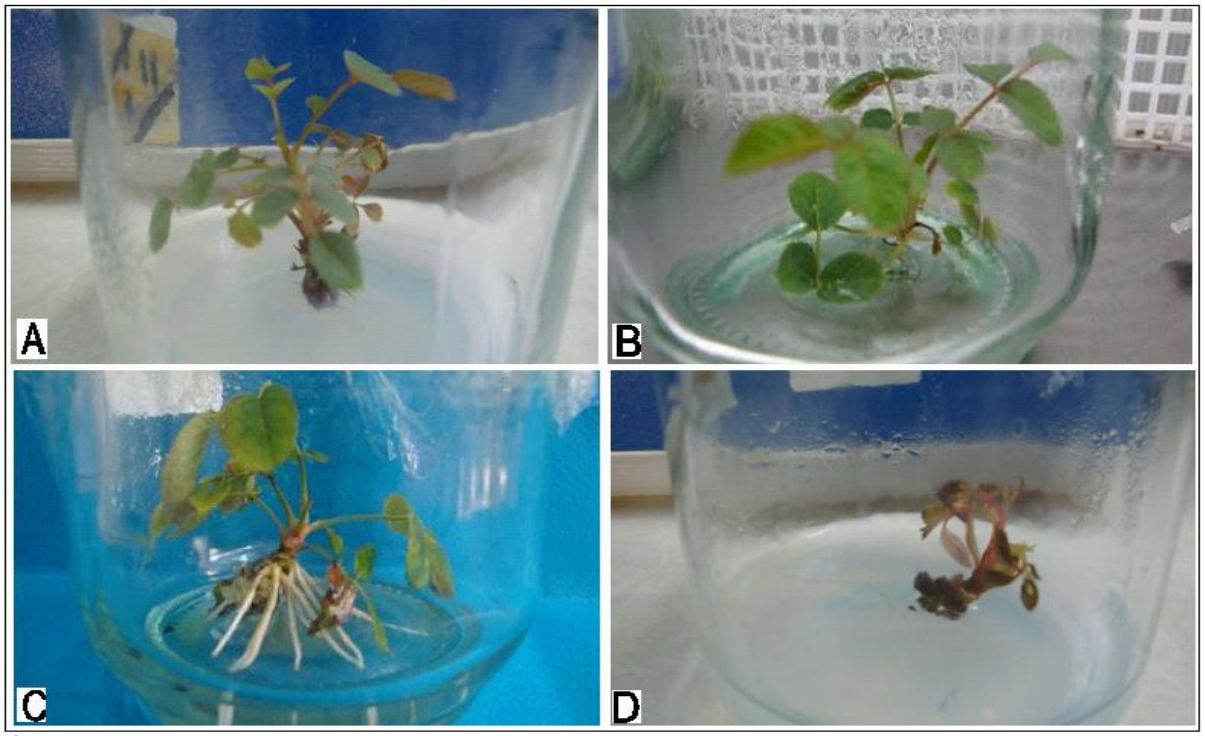

Fig. 1. A: Establishment medium after 3 weeks with concentration 0.75 mg.l-1 BA and 0.1 mg.l-1 IBA, B: Proliferation medium after adding AgNO3, C: Rooting in treatment 0.01 mg.l-1 BA, 1 mg.l-1 IBA and 58.85 µmol L^{-1} AgNO3, D: Abnormal plantlet at high concentration of BA in the medium.

Results of this study showed that micro propagation of *Rosa damascena* Mill. influenced by hormones BA and IBA in liquid culture didn't have a good effect on shoot length and leaf size but the highest number of shoots were observed when IBA hormone was used at a concentration of 0.1 mg.l-1. Although reducing the concentration of ammonium nitrate in micro propagation of Damask rose had no effect on increasing the number of branches and stem length but appeard to have improved the greenness of the leaves. AgNO3 acts as a direct inhibitor of Ethylene (Beyer, 1976), thus able to delay aging during proliferation of branches. Since Damask rose is sensitive to high salt levels at rooting stage in the culture media, in this study a half-strength liquid MS medium was used. Kafi *et al*. (2005) indicated that rooting of Damask rose is not easy and as such were not so successful in obtaining good rooting. In this research, Damask rose was rooted using BA and IBA hormones but similar to Kafi *et al*. (2005) rooting occurred with much difficulty.

Abbreviations

MS-Murashige & Skoog Medium, BA- Benzyl

Adenine, IBA- Indole-3-butyric acid, NAA- α-Naphthaleneacetic acid.

Acknowledgements

I would like to thank the Dr. Saidi in department of Biotechnology of Shahid Beheshti University for his support, valuable guidance, providing materials and laboratory facilities, Dr. Kalate jari in Department of Horticulture of Islamic Azad University for guiding me about Damask rose and tissue culture, Dr. Goodarzi in Research Center of Agriculture and Natural Resources for giving plant materials. This work was funded by department of Biotechnology of Shahid Beheshti University and Olum Tahghighat University in Tehran.

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