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Micropropagation of Damask Rose (*Rosa damascena* Mill.) cvs Azaran and Ghamsar.

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ABSTRACT

This research was performed to investigate the regeneration of two Iranian cultivars of Damask rose (*Rosa damascena* Mill.), "Azaran" and "Ghamsar" *in vitro*. The shoot single node segments included lateral buds were taken from bushes. After external disinfections for internal (bacterial) disinfections, appropriate antibiotics for each cultivar were used. Research findings showed that among 12 different media, a liquid modified MS (Murashige & Skoog) medium (with eliminated Cl^- and reduced NH_4^+ ions) caused the best growth of newly proliferated shoots and no senescence occurred. BA (Benzyl Adenine) in four levels (0, 1, 2 and 3 mg L^{-1}), GA_3 (Gibberellic Acid) in four levels (0, 0.1, 0.25 and 0.5 mg L^{-1}) and NAA (Naphthalene Acetic Acid) in two levels (0 and 1 mg L^{-1}) were used and 32 different combinations of them were tested to investigate the most suitable hormonal treatment for establishment and multiplication stages as a factorial test on the base of a completely randomized design. Finally it was shown BA (1-2 mg L^{-1}), GA_3 (0.1 mg L^{-1}) and NAA (0-0.1 mg L^{-1}) for "Azaran" and the same concentrations of BA and GA_3 but with 0 mg L^{-1} NAA for "Ghamsar" had the best proliferation and multiplication rate, appearance and leaf color. For *in vitro* rooting, quick deep treatment of microshoots' bottom in 2000 ppm IBA (Indole Butyric Acid) solution and then rooting in liquid half strength of the same MS medium showed the best result compared with 1000 ppm.

Key Words: Damask rose (*Rosa damascena* Mill.); Micropropagation; Optimizing the media; *In vitro* culture; *In vitro* rooting

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 and the Middle East region and it is the national flower of Iran. The major use of Damask rose is distillation of petals in order to extract its essential oil and producing rosewater. The common ways in propagating Damask rose are cutting and using suckers. But there is the risk of spreading viral and bacterial diseases which result in low yield. Similarly, these methods are not efficient to support the increasing demand for healthy shrubs in Iran. The establishment of tissue culture system for various rose species has been described (Arnold *et al*., 1992; Telgan *et al*., 1992; Rout *et al*., 1999; Carelli & Echeverrigary, 2002) but it is not much specifically in the case of Damask rose. Carelli and Echeverigary (2002) showed that BA may cause producing more shoots in comparison with Kinetin and 2iP 6-[Ÿ,Ÿ dimethylallyl amino]-purine in rose micropropagation system. Kumar *et al.* (2000) stated supplementing the media with GA_3 can improve multiplication of Damask rose explants. They also reported the senescence symptoms of proliferated shoots and try to cope with the problem by modifying the media. Khosh-Khui 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}). They also found *in vitro* rooting of old roses including Damask rose is much more difficult than modern roses. Pratpakumar *et al.* (2001) found 2 mg L^{-1} BA is the most appropriate concentration for *in vitro* propagation of Damask rose. The objective of the study was to investigate the best hormonal compound and media for micropropagation of two important Iranian Damask rose cultivars.

MATERIALS AND METHODS

Two local Iranian cultivars of Damask rose, "Azaran" and "Ghamsar" were used in this study. Single-nodes two-cm long explants were disinfected by surface sterilizing in 80% ethanol for 3-4 seconds and then in a 10% household bleach (containing 5.25% sodium hypochlorite) for 15-20 min followed by three rinses in sterilized distilled water each time 5 min. Finally after an antibiogram test, disinfection performed by applying the aquatic solution of ofloxacin (80 mg L^{-1}) for "Azaran" and cefteriaxon (640 mg L^{-1}) for "Ghamsar" through direct applying on explants. In establishment stage all explants cultured on a solid modified MS medium (modified MS1) and standard vitamins plus 30 mg L^{-1} sucrose, supplemented with 0, 1, 0.25 or 0.5 mg L^{-1} BA; 0, 0.1, 0.25 or 0.5 mg L^{-1} GA_3 and 0 or 0.1 mg L^{-1} NAA for shoot proliferation in culture tubes containing about 7.5 mL of the modified MS1 medium. Because of senescence problem 12 different reported media for roses micropropagation (Table I) were tested in multiplication

stage. Subculturing was performed every 4 weeks to fresh medium. In rooting experiment, the bottom of newly proliferated microshoots were treated through quick deep method in 1000 or 2000 ppm IBA for 10 sec and then cultured in liquid modified MS1 medium. Cultures were kept under a 16-hrs photoperiodic of 2000 lux light intensity at 23±3°C. The proliferation of shoots and qualitative characteristics of newly proliferated microshoots were recorded every 4 weeks. In the later case, appearance (on the base of leaf area, normal or abnormal feature, vitrification or healthy shoots) and leaf color were recorded visually. It was on the base of 1 to 10 scoring which indicate the poorest and the best conditions respectively.

To assay rooting, microshoots which had produced at least 2 roots with more than 5mm length recorded as rooted. All experiments were conducted twice as a completely randomized design with 10 replications. Qualitative data were transformed to quantitative ones with scoring method and after statical analysis, means were compared using Duncan's multiple range test (DMRT).

RESULTS

In establishment stage, solid modified MS medium 1 was used and the effects of different hormones were observed. Statistical analysis of collected data showed a significant effect of BA on proliferation and leaf color index at 5% level, GA_3 on proliferation at 1% level and NAA on appearance at 1% level. Their interaction had also significant effects on some traits. (Table II). Fig. 1 shows proliferated shoots in establishment stage. After reviewing and analyzing the effect of hormonal combinations on the characteristics of proliferated shoots in establishment stage, the most suitable combinations with the highest effect on proliferation rate and qualitative traits were selected for next stage (Table III) (data are not presented). In this stage some treatments showed a high proliferation rate but failed in qualitative characteristics especially appearance index i.e. they had abnormal shoots with too low leaf area and pale leaves. These abnormal shoots couldn't grow normally on multiplication stage. Table III shows the effect of hormonal compounds on both cultivars separately in multiplication stage and through 4 subcultures. It is obvious that along with increasing BA concentration, the indices of appearance and leaf color decrease. In treatment 8 although the multiplication rate is the same as treatments 6 and 7 but the qualitative indices failed dramatically in comparison with treatments 6 and 7. Its proliferated shoots couldn't survive in the subsequent subcultures. The problem of newly subcultured microshoots senescence in multiplication stage solved just in liquid modified MS1. In other media we observed senescence after one week and shoots declined gradually. Statistical analysis of rooting experiment data showed that 2000 ppm IBA solution with 21% rooting was more efficient than 1000 ppm with 13.5% rooting but there was no significant difference between two cultivars.

DISCUSSION

The effects of plant growth regulators were completely significant in establishment and multiplication stages. Malformation and abnormal shoots induced by high BA concentration has been reported by some researchers (Carelli & Echeverrigary, 2002). Kumar *et al.* (2000)

Table I. The composition and concentration (mg/L) of media used for overcoming senescence problem

Constituents	MS	Mod. MS1	Mod. MS2	Mod. MS3	Mod. MS4	Mod. MS5	Mod. MS6	Mod. MS7	Mod. MS8	Mod. MS9	Mod. MS10	WPM[x]
NH_4NO_3	1650	1410	1410	1410	1350	705	1410	1410	1410	1410	-	400
$Ca(NO_3)_2.4H_2O$	-	706.77	706.77	706.77	350	353.8	706.77	706.77	706.77	706.77	706.77	556
KNO_3	1900	1900	1900	1900	1900	950	1900	1900	1900	1900	950	-
$MgSO_4.7H_2O$	370	370	370	370	370	185	370	370	370	370	370	370
$MnSO_4.H_2O$	16.9	16.9	16.9	16.9	33.8	8.45	16.9	16.9	16.9	16.9	16.9	22.3
$ZnSO_4.7H_2O$	8.6	8.6	8.6	8.6	8.6	4.3	8.6	8.6	8.6	8.6	8.6	8.6
$CuSO_4.5H_2O$	0.025	0.025	0.025	0.025	0.025	0.0125	0.025	0.025	0.025	0.025	0.025	0.025
KI	0.83	0.83	0.83	0.83	0.83	0.415	0.83	0.83	0.83	0.83	0.83	-
$CoCl_2.6H_2O$	0.025	0.025	0.1	0.025	0.025	0.0125	0.025	0.025	0.025	0.025	0.075	-
$CaCl_2.2H_2O$	440	-	-	-	-	-	-	-	-	-	-	96
KH_2PO_4	170	170	170	170	170	85	170	170	170	170	170	170
H_3BO_3	6.2	6.2	6.2	6.2	6.2	3.1	6.2	6.2	6.2	6.2	6.2	6.2
$Na_2MoO_4.2H_2O$	0.25	0.25	0.25	0.25	0.25	0.125	0.25	0.25	0.25	0.25	0.25	0.25
$FeSO_4.7H_2O$	27.8	27.8	27.8	27.8	80.37	13.9	27.8	27.8	27.8	27.8	27.8	27.8
NaEDTA	37.3	37.3	37.3	37.3	37.3	18.65	37.3	37.3	37.3	37.3	37.3	37.3
$AgNO_3$	-	-	-	3.4	-	-	-	-	-	-	-	-
$Mg(NO_3)_2$	-	-	-	-	580	-	-	-	-	-	-	-
$(NH_4)_2SO_4$	-	-	-	-	-	-	-	-	-	-	1350	-
K_2SO_4												990
Calcium pantothenate	-	-	-	-	-	-	-	5	-	5	-	-
Adenine hemisulfate	-	-	-	-	-	-	-	-	5	5	-	-
Agar	6500	6500&0	6500	6500	6500	6500	4500	6500	6500	6500	6500	6500

[x]Woody Plant Medium

Fig. 1. Proliferated shoots in establishment media

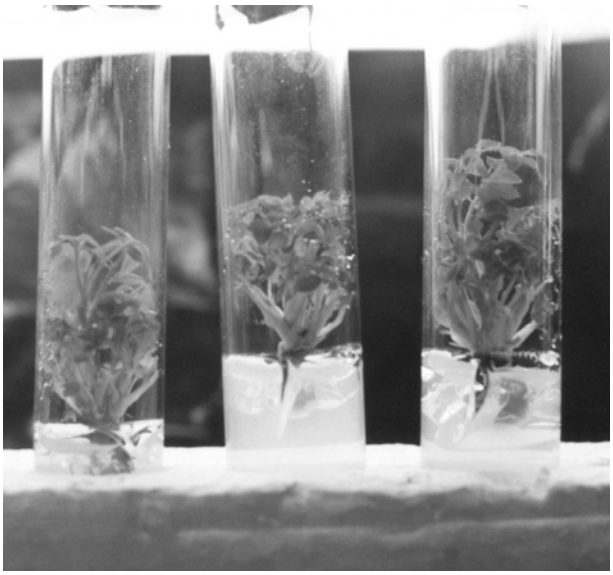

Table II. The main effects of plant growth regulators on proliferation, appearance and leaf color in establishment stage

		Proliferation	Appearance index[y]	Leaf color index
BA	0	1.87*ab[x]	6.83[ns]a	6.52*ab
(mg L^{-1})	1	1.94a	6.95a	6.81a
	2	1.77b	6.79a	6.51b
	3	1.78b	6.80a	6.50b
GA_3	0	1*b	5.6**c	6**c
(mg L^{-1})	0.1	2.3a	7.2a	7.2a
	0.25	2.2a	6.1b	6.7b
	0.5	2.2a	6.5b	6.6b
NAA	0	1.81**a	6.73a	6.55[ns]a
(mg L^{-1})	0.1	1.71b	6.82a	6.57a

[x]In each column (for each individual growth regulator), means followed by the same letters are not significantly different using DMRT. * and ** are significant at 5% and 1% respectively and [ns] is not significant

[y]Appearance and leaf color indices are on the base of 1 to 10 which indicate the poorest and the best conditions respectively.

reported that the best BA concentration for micropropagation of Damask rose was 5 µg L^{-1} (1.25 mg L^{-1}). Some rose species are relatively more resistant to too BA concentration in media (e.g. some hybrid tea roses up to 5.8 mg L^{-1} (Damiano *et al.*, 1987)). But the desirable concentration mostly ranges between 1-2 mg L^{-1} (Rout *et al*., 1999). Adding GA_3 to media for increasing the response of rose explants to culture has been reported by some researchers (Rogers *et al.*, 1992; Rosu *et al.*, 1995). We observed in all GA_3 concentrations (0.1-0.5 mg L^{-1}) positive effects on proliferation in comparison with media without GA_3. Kumar *et al.* (2000) have also reported the same results for Damask rose micropropagation. They used 0.1-0.35 mg L^{-1} of GA_3. In the case of "Azaran", NAA had no significant effect on multiplication rate but adding NAA to the media of "Ghamsar" resulted in decreasing multiplication rate. It is possibly due to genetical differences between cultivars. Cai *et al.* (1984) observed no significant result with NAA but Khosh-Khui and Sink (1982) reported that NAA (0.1 mg L^{-1}) and BA (1 mg L^{-1}) were necessary for Damask rose. On the other hand Kumar *et al.* (2000) concluded that just BA (1.25 mg L^{-1}) and GA_3 (0.1-0.35 mg L^{-1}) were necessary for Damask rose micropropagation.

Using liquid media for *in vitro* propagation of some roses such as hybrid tea and minator roses has been reported before (Chu *et al.*, 1993; Ramesh *et al.*, 1993). Pratpakumar *et al.* (2001) applied liquid media successfully for micropropagation of Damask rose. They reported that not only the overall results improved but also multiplication rate increased in liquid media. Some researchers have used different anti-ethylene compounds in culture media to overcome senescence problem (Rout *et al.*, 1999; Kumar *et al.*, 2000), but we didn't find them helpful. We tried different modified MS media and woody plant medium (WPM), but only liquid modified MS medium 1 (with eliminated Cl^- and reduced NH_4^+ ions) could overcome the senescence problem. Rout *et al.* (1999) had reported that roses are sensitive to chloride and high amount of ammonium ions in media.

Quick deep treatment of microshoots in Auxin compounds have been reported frequently (Rogers & Smith, 1992; Vardja & Vardja, 1995; Kumar *et al.*, 2000). Pratpakumar *et al.* (2001) could get proper rooting in liquid half MS medium by means of IBA. There are some reports which state *in vitro* rooting of old roses (including Damask rose) are more difficult than modern roses (Khosh-Khui & Sink, 1982).

Our investigations showed that liquid modified MS1 medium with BA (1-2 mg L^{-1}) and GA_3 (0.1 mg L^{-1}) plus NAA (0.1 mg L^{-1}) for "Azaran" and the same medium but without NAA are suitable for "Ghamsar" micropropagation. It is advisable other rooting methods to be tested for rooting Damask rose cultivars.

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Table III. The effect of plant growth regulator compounds on multiplication rate, appearance and leaf color of "Ghamsar" and "Azaran" in 4 sub-cultures

	Multiplication rate		Appearance index[z]		Leaf color index	
	"Ghamsar"	"Azaran"	"Ghamsar"	"Azaran"	"Ghamsar"	"Azaran"
Treatment-6 $BA(1^{x}),GA_3(0.1),NAA(0)$	2.56a[y]	2.50a	7.20a	7.00a	7.40a	7.48a
Treatment-7 $BA(2),GA_3(0.1),NAA(0)$	2.59a	2.52a	6.85a	7.00a	7.20a	7.33a
Treatment-8 $BA(3),GA_3(0.1),NAA(0)$	2.85a	2.63a	5.10b	4.70b	6.20b	6.47b
Treatment-22 $BA(1),GA_3(0.1),NAA(0.1)$	-	2.57a	-	6.81a	-	7.65a
Treatment-23 $BA(2),GA_3(0.1),NAA(0.1)$	2.16b	-	6.85a	-	7.12a	-

[x] All concentrations are mg L^{-1}

[y] In each column, means followed by the same letters are not significantly different using DMRT at 1%.

[z] Appearance and leaf color indices are on the base of 1 to 10 which indicate the poorest and the best conditions, respectively

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