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Micro-propagation of Damask Rose (*Rosa damascena* Mill.) cv. Almarah

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ABSTRACT

Almarah is the most important cultivar of Damask rose in Syria. This study aimed to determine the best protocol for its *in vitro* micro-propagation. The experiments were conducted at Damascus University in Syria. Effects of different concentrations of benzyladenine BA (0, 0.5, 1 and 2 mg L^{-1}) and gibberellic acid GA3 (0, 1 and 2 mg L^{-1}) were evaluated on proliferation of the explants. To evaluate *in vitro* rooting, proliferated micro-shoots were cultured on a MS/2 medium supplemented with 3 g L^{-1} activated charcoal and (0, 1, 2 and 3 mg L^{-1}) concentrations of Indol-3-Butyric Acid IBA. The experimental design of the all stages was a Complete Randomized Design. Means were analyzes with Duncan's test at $p \leq 0.05$ using SPSS. BA increased the proliferation of the explants up to 2 mg L^{-1}. The interaction of BA with GA3 significantly increased proliferation rate of the explants. The highest number of proliferated explants was obtained in the presence of 2 and 2 mg L^{-1} of GA3 and BA, respectively. The highest percentage of rooted explants was obtained in the presence of 3 mg L^{-1} IBA in the rooting medium but the number and length of roots significantly increased in response to increasing IBA concentration in the medium up to 2 mg L^{-1}. Only 8% of the plantlets lost during hardening. For the first time in Syria a mass *in vitro* production protocol of Syrian Damask rose has been achieved. The interaction of BA (2 mg L^{-1}) with (GA3 2 mg L^{-1}) is highly recommended for proliferation rate of the explants while adding IBA (2 mg L^{-1}) to the MS/2 rooting medium is highly recommended for high quality *in vitro* rooting.

Key words: Damask rose, *in-vitro*, micropropagation, tissue culture protocol

INTRODUCTION

Damask rose (*Rosa damascena*) is a species of old roses. It is a salt and drought tolerant plant and may be grown on poor soils. It is considered as a rose oil-bearing species (Gunes, 2005). In Syria, Almarah is the most important cultivar of Damask rose cultivated in Kalamoon Mountains where the village of Damask rose is located (Alsemaan *et al*., 2011). *In-vitro* propagation techniques have been established for fast cloning of many species of aromatic plants in recent years (Gantait *et al*., 2011). Successful micro-propagation of some rose cultivars have been reported previously (Pati *et al*., 2010). However, the success of these methods for damask rose cultivars is dependant to the cultivar and genetic background of the plant. Some cultivars do not response to *in vitro* conditions, their proliferation rate is slow (Kornova and Michailova, 1994), rooting of explants is also limited and many plantlets die in the acclimatization stage (Pati *et al*., 2006). The aim of this study was to investigate an efficient method for micro-propagation of the Syrian cultivar of damask rose called Almarah. It is tolerant to cold, salinity and disease. Its shrubs are hard to root and their propagation efficiency is low.

MATERIALS AND METHODS

This study was conducted at the plant tissue culture laboratory of Damascus University in Syria. Eight-years-old mother plants of damask rose were used to get the plant material. Shoot explants with a nodule section, after removing of leaves and thorns were exposed to running tap water (1 h) and dipped in alcohol (70%, 30 sec). Then, the explants were surface sterilized by immersion in 0.1% $HgCl_2$ for 5 min and rinsed three times in sterile distilled water (Soundararajan and Karrunakaran, 2011). Each explant was inoculated to MS medium supplemented with 30 g L^{-1} sucrose, 8 g L^{-1} agar and 3 g L^{-1} activated charcoal. In order to control of phenolic compounds production and necrosis of explants (Fig. 1). Care was taken not to dip explants completely in the medium and also tips of forceps should not touch the agar medium. The culture tubes were sealed immediately (Antony Ceasar *et al*., 2013). The same procedure was repeated for multiple shoot formation but without adding charcoal (Fig. 2). Effects of different

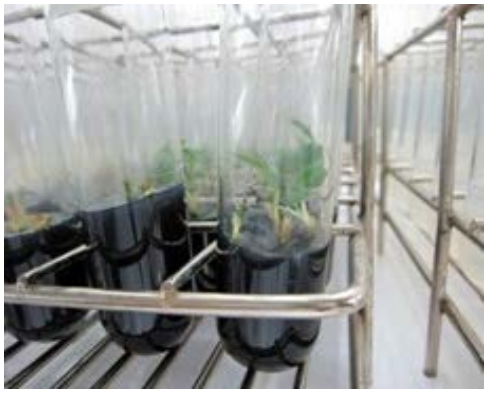

Fig. 1: Initial stage: Control of phenolic compounds production by adding activated charcoal to the medium

Fig. 2: Multiplication stage: Multiple shoot formation of Damask rose

Fig. 3: Rooting stage: Micro-shoots were rooted on a MS/2 medium supplemented with activated charcoal

concentrations of growth regulators 6-benzyladenine (BA) and gibberellic acid (GA3) were evaluated on proliferation of the explants. BA (0, 0.5, 1 and 2 mg L^{-1}) were added to the medium prior to autoclaving at 121°C for 15 min. Different GA3 concentrations namely (0, 1 and 2 mg L^{-1}) were added into the proliferation medium directly after autoclaving. Observations were made one month after incubation and the percentage of sprouted buds and shoot length were recorded. To evaluate *in vitro* rooting of damask rose explants, proliferated micro-shoots were cultured on a MS/2 medium supplemented with 3 g L^{-1} activated charcoal and (0, 1, 2 and 3 mg L^{-1}) concentrations of Indol-3-Butyric Acid (IBA) (Fig. 3). Rooting percentage, root number and root length were recorded after a month. The plantlets were transplanted to plastic pots at the end of rooting stage. Pots were placed in a greenhouse.

Methods:

- **Experimental design:** The experimental design of the all stages was a Complete Randomized Design with 12 tubes per treatment in 3 replications
- **Statistical analysis:** Means were analyzes with Duncan's test at $p \leq 0.05$ using SPSS

RESULTS AND DISCUSSION

Micropropagation of woody plants is a challenging work. Damask rose is not only a woody plant but also an aromatic one. So, in the current study, another problem was faced because of the dark phenolic substances production after wounding. Accumulation of such compounds in medium adversely affects the growth and survival of *in vitro* explants. Adding activated charcoal to medium has been reported to be effective in controlling phenolic compounds production (Arumugam and Gopinath, 2012). In the current study, adding it has significantly reduced the production of phenolic compounds.

Different hormonal mixtures are suggested for proliferation of rose cultivars (Kornova and Michailova, 1994). Cytokinins are essential for *in vitro* proliferation of damask rose explants. In most cases, using synthetic cytokinins such as kinetin and BAP in medium did not show a proper

Table 1: Effects of BA and GA3 on proliferation (%) of Damask rose explants

	GA3 (mg L^{-1})			
BA (mg L^{-1})	0	1	2	Mean
0	0.8^{cd}	0.7^{d}	0.7^{d}	0.7^{C}
0.5	1.2^{bcd}	1.2^{bcd}	1.2^{bcd}	1.2^{B}
1	1.2^{bcd}	1.4^{abc}	1.6^{ab}	1.4^{AB}
2	1.3^{bcd}	1.4^{abc}	2.0^{a}	1.6^{A}
Mean	1.1^{A}	1.2^{A}	1.4^{A}	

Means with the same letters did not show a significant difference in accordance to Duncan's multiple range test, at $p \leq 0.05$

Table 2: Effects of BA and GA3 on shoot length (cm) of Damask rose shoots

	GA3 (mg L^{-1})			
BA (mg L^{-1})	0	1	2	Mean
0	2.1^{e}	4.7^{be}	6.8^{bcd}	4.5^{B}
0.5	3.7^{cde}	5.1^{be}	7.1^{bcd}	5.3^{B}
1	5.1^{cde}	5.7^{cde}	8.0^{b}	6.3^{AB}
2	3.5^{de}	7.7^{bc}	9.2^{a}	6.8^{A}
Mean	3.6^{C}	5.8^{B}	7.7^{A}	

Means with the same letters did not show a significant difference in accordance to Duncan's multiple range test, at $p \leq 0.05$

performance in proliferation of rose explants. However, Pati *et al.* (2006) reported that BA can be used in proliferation of damask rose. Table 1 shows the effects of different concentrations of GA3 and BA on proliferation of damask rose explants. Adding BA to the medium significantly increased the proliferation of the explants. Although GA3 did not affect it, the interaction of BA with GA3 significantly increased proliferation rate of the explants. The highest number of proliferated explants was obtained in the presence of 2 and 2 mg L^{-1} of GA3 and BA, respectively. While, Table 2 shows the effects of different concentrations of GA3 and BA on shoot length of damask rose shoots. Increasing BA and GA3 concentrations in the proliferation medium significantly increased length of damask rose shoots. The highest length of explants (9.2 cm) was obtained in the presence of 2 and 2 mg L^{-1} of GA3 and BA, respectively. So that, increasing BA concentration up to 2 mg L^{-1} in medium increased proliferation rate of damask rose explants significantly. However, there was some evidence of shoot growth limitation following increasing BA concentration in the proliferation medium. No signs of vitrification were observed. The rate of proliferation was lower in the lower concentration of BA. The results showed that GA3 prevented the effects of high concentrations of BA in the proliferation medium. Adding GA3 to medium improved the growth of explants. Bhoomsiri and Masomboon (2003) suggested, using GA3 in addition to BA in proliferation medium of rose cultivars. *In vitro* growth of the explants may be improved by GA3 (Elavazhagan and Arunachalam, 2010).

The results in Table 3 show, that the highest percentage of rooted explants (95%) was obtained in the presence of 3 mg L^{-1} IBA in the rooting medium and the lowest (0%) was in the control treatment. Number and length of roots significantly increased in response to increasing IBA concentration in the medium up to 2 mg L^{-1}. IBA has been suggested as the best plant growth regulator to induce rooting of rose explants (Pati *et al.*, 2006). The results showed that presence of IBA in the rooting medium is necessary for rooting of damask rose explants. The best results of rooting of the explants obtained following adding 2 mg L^{-1} IBA to the rooting medium. Increasing

Table 3: Effects of IBA on root formation (%) of Damask rose explants

IBA (mg L^{-1})	Rooting (%)	No. of roots	Length of roots
0	0^{c}	0.0^{c}	0.0^{c}
1	81^{ab}	2.4^{a}	1.6^{ab}
2	83^{ab}	2.6^{a}	1.9^{a}
3	95^{a}	1.9^{b}	1.4^{ab}

Means with the same letters did not show a significant difference in accordance to Duncan's multiple range test, at $p \leq 0.05$

Fig. 4: Acclimatization stage of Damask rose *in vitro* plants

IBA concentration up to 3 mg L^{-1} in the medium resulted in maximum number of rooted explants but with lower roots quality. These findings are in accordance to (Pati *et al*., 2010). Plantlets acclimated in a greenhouse and transferred to outdoor after a month successfully. About 8% of the plantlets lost during hardening. The results showed that damask rose explants may be acclimatized to outdoor without intensive care or treatments (Fig. 4). The percentage of survival was 92%. This method is highly advised for mass production of damask rose.

CONCLUSION

This study achieved for the first time a mass production protocol of Syrian Damask rose *in vitro*. The interaction of BA (2 mg L^{-1}) with (GA3 2 mg L^{-1}) is highly recommended for proliferation rate of the explants while adding IBA (2 mg L^{-1}) to the MS/2 rooting medium is highly recommended for high quality *in vitro* rooting.

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