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Factors affecting tissue culture of Damask rose (*Rosa damascena* Mill.)

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Abstract

The present study was conducted to evaluate the regeneration ability of Damask rose. Single-node explants were surface sterilised with 10% chlorox for 15 min and cultured on Murashige and Skoog (MS) medium supplemented with various concentrations of N^6-benzyladenine (BA) or kinetin (Kin) separately or in combination with different concentrations of indole-3-butyric acid (IBA). Combination of 2.5–3 mg/l BA and 0.1 mg/l IBA was the most suitable treatment for proliferation. In vitro derived shoots were subcultured four times on the fresh medium within a 4-week period. Other treatments such as explant orientation (horizontal, vertical and oblique) and explant wounding were also examined but did not affect shoot multiplication rate significantly. Several experiments were carried out to stimulate in vitro rooting of Damask rose. Application of different media such as MS, 1/2 MS, 1/3 MS and 1/4 MS with different concentrations of indole-3-acetic acid (IAA), IBA and naphthaleneacetic acid (NAA) did not produce satisfactory results. Quick-dip method using sterilised 0–2000 mg/l IAA, IBA and NAA solutions was also studied. Other treatments such as using various concentrations of abscisic acid (ABA) in combination with various concentrations of IAA, IBA and NAA, and using various concentrations of sucrose and agar did not produce any roots. The best treatment for rooting of shoots was 2.5 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) for 2 weeks in MS medium and then transferring the explants to MS medium without any growth regulator. Plantlets were acclimatised in a soil mixture consisting of peat moss and sand 1:1 (v/v) and successfully transferred to the greenhouse after 3 weeks.

Keywords: In vitro propagation; Micropropagation; Damask rose (*Rosa damascena* Mill.); Plant growth substances

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1. Introduction

Damask rose *(Rosa damascena* Mill.), an important species among the scented roses, yields a highly fragrant commerically 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.

Damask rose is commonly propagated by asexual methods (sucker, hardwood cutting, semi-hardwood cutting, budding and grafting) (Horn, 1992; Hudson et al., 2002). These methods are very slow and time-consuming and usually associated with various problems such as limitation of stock plant and prolonged production time (Skirvin et al., 1990).

Different aspects of tissue culture propagation systems for various rose species have been described (Alderson et al., 1988; Arnold et al., 1992; Bhat, 1992; Campos and Pais, 1990; Chu et al., 1993; Horn, 1992; Ishioka and Tanimoto, 1990; Khosh-Khui and Sink, 1982a, 1982b; Kumar et al., 2001; Rahman et al., 1992; Rout et al., 1999; Salehi and Khosh-Khui, 1996; Skirvin et al., 1990). However, no detailed report on Damask rose is available except a report by Kumar et al. (2001) who used thidiazuron. In this study, the complete stages of multiplication of Damask rose were studied through micropropagation.

2. Materials and methods

2.1. Plant material

Among different explants, single-node explants were selected and excised from shoots of a local cultivar of greenhouse-grown *R. damascena* Mill. The relative humidity in the greenhouse was $70 \pm 15\%$ and its temperature was $25 \pm 3\ ^{\circ}C$.

2.2. Culture establishment

Single-nodes, 1.5–2 cm long were prewashed in a commercial dish washer (Rika) solution (about 0.1%) for 15 min and then placed under running tap water until used. The explants were surface sterilised by immersion in 10% chlorox (5.25% NaOCl) for 15 min and then rinsed 3 times in autoclaved distilled water.

Explants, 6–10 mm long, were placed in culture vessels containing about 25 ml of MS (Murashige and Skoog, 1962) medium salts and vitamins plus 30 g/l sucrose and 8 g/l agar. After preliminary experiments, concentrations of plant growth regulators (PGRs) tested for shoot multiplication were initially 0–5 mg/l BA with 0–1 mg/l IBA, Kin 0–5 mg/l with IBA 0–1 mg/l and BA + Kin 0–3 mg/l with IBA 0–1 mg/l. Then, based on the above experiments, the combinations of 2.25, 2.50, 2.75, 3.00, 3.25, 3.50 or 3.75 mg/l BA with 0.0, 0.1 or 0.5 mg/l IBA were tested for shoot multiplication. Cultures were kept under a 16 h photoperiod of 1500 lux light intensity at $25 \pm 5\ ^{\circ}C$.

2.3. Explant orientation

Explants inserted in medium horizontally, vertically or obliquely, and their proliferation rate was evaluated.

2.4. Explant wounding

This treatment involved: removing a thin layer below the buds and making some horizontal and vertical cuts under the buds.

2.5. Subcultures

Subculturing was performed every 4 weeks using the MS medium with 2.5–3.00 BA and 0.1 IBA.

2.6. Rooting and acclimatisation

Media used for rooting (initiation and elongation) were 1/4, 1/3, 1/2 or full strength MS salts plus vitamins, 30 g/l sucrose and 8 g/l agar. Experiments were conducted on above media without any PGRs, with 0–5 mg/l IAA, IBA and NAA separately or in combination. In vitro derived shoots were cultured on MS medium after quick-dip in sterilised root aqueous solutions containing 0–2000 mg/l of IAA, IBA and NAA for 5 s. Other treatments such as using 0–2 mg/l ABA with 0–5 mg/l IAA, IBA and NAA or application of 20, 30 and 40 g/l sucrose and 4, 6 and 8 g/l agar or using liquid medium were evaluated. The other treatment was using 0–30 mg/l 2,4-D for 2 weeks and then transferring the explants to MS medium without any PGRs.

After 2 weeks, rooted plantlets were transferred to a pasteurized soil mixture consisting of peat moss and sand 1:1 (v/v) and acclimatised plants were transferred to the greenhouse after 3 weeks First the plantlets were covered tightly in greenhouse trays and later the covers were gradually removed.

2.7. Statistical analysis

The proliferation rate of shoots was recorded after 4 weeks. All experiments were conducted as a completely randomized design with 8 replications and repeated three times. Data were statistically analysed and the means were compared using Duncan's new multiple range test (DNMRT).

3. Results

3.1. Culture establishment

Various concentrations of BA and IBA showed significant differences (Table 1). Combinations of 2.5–3 mg/l BA with 0.1 mg/l IBA were the most suitable PGR treatment.

Table 1
Comparison of shoot number per single-node explant of *R. damascena* on MS medium supplemented with various cocentrations of BA and IBA (mg/l)

IBA (mg/l)	BA (mg/l)							
	2.25	2.50	2.75	3.00	3.25	3.50	3.75	Mean
0	1.375 b	2.125 a,b	2.000 a	2.250 b	1.375 b	1.875 a,b	1.875 a,b	1.839 B
0.1	2.625 b	3.875 a	3.750 a	4.000 a	2.250 b	2.250 b	2.000 b	2.964 A
0.5	2.500 a,b	2.875 a	2.375 a,b	2.125 b	1.875 b,c	1.875 b,c	1.375 c	2.143 B
Mean	2.167 B	2.958 A	2.708 A	2.791 A	1.833 B	2.000 B	1.750 B	

In each row followed by the same small (capital in each row and column for means) letters are not significantly different at $P \leq 0.05$ by DNMRT.

Table 2
Comparison of shoot length (mm/shoot) of proliferated explants on MS media supplemented with various concentrations of BA and IBA (mg/l)

IBA (mg/l)	BA (mg/l)			
	2.50	2.75	3.00	Mean
0	15.760 b	16.000 b	16.125 b	15.961 B
0.1	28.250 a	25.625 a	25.000 a	26.291 A
Mean	22.005 A	20.813 A	20.563 A	

In each column followed by the same small (capital in each row and column for means) letters are not significantly different at $P \leq 0.05$ by DNMRT.

Explants cultured on BA alone proliferated but shoots did not elongate (Table 2). Addition of IBA to the medium resulted in shoot elongation.

3.1.1. Explant orientation

Inserting the explants horizontally, vertically or obliquely had no significant effect on proliferation rate.

Table 3
Comparison of the number of proliferated shoots at different multiplication stages in MS medium supplemented with 2.5–3 mg/l BA and 0.1 mg/l IBA

Multiplication stages	No. of shoots at different multiplication stages in MS medium supplemented with 2.5–3 mg/l BA and 0.1 mg/l IBA			Mean
	2.20	2.75	3.00	
Establishment	3.875 b	3.750 b	4.000 b	3.875 B
First subculture	7.375 a	7.375 a	7.625 a	7.458 A
Second subculture	3.250 b	3.250 b	3.125 b,c	3.208 B,C
Third subculture	2.275 b	2.500 b,c	2.625 b,c	2.625 C,D
Fourth subculture	1.625 b	1.750 c	1.500 c	1.625 D
Mean	3.775 A	3.725 A	3.775 A	

In each column followed by the same small (capital in each row and column for means) letters are not significantly different at $P \leq 0.05$ by DNMRT.

3.2. Explant wounding

Wounding the explants did not significantly affect the proliferation rate.

3.3. Subcultures

Established shoots were four times subcultured to fresh MS medium containing 2.5–3.00 BA and 0.1 IBA.

Among the multiplication stages, the first subculture resulted in the highest proliferation rate and the differences between second to forth subcultures were negligible (Table 3 and Fig. 1).

3.4. Rooting and acclimatisation

Application of different media (MS, 1/2 MS, 1/3 MS and 1/4 MS) with different concentrations of auxins did not produce satisfactory results. Similarly, explants failed to

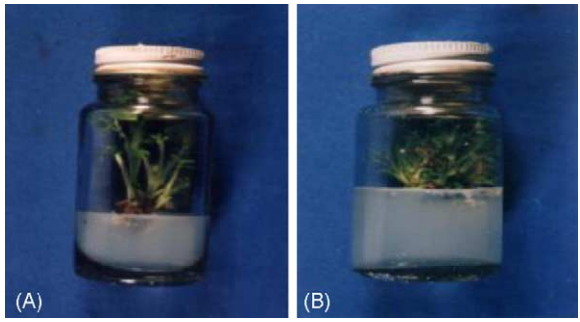

Fig. 1. Comparison of the number of proliferated shoots at establishment (A) and first subculture (B).

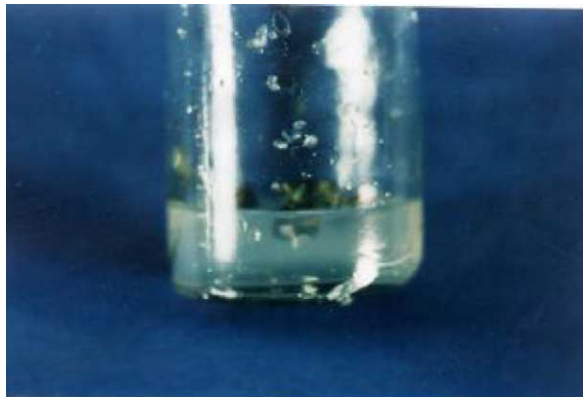

Fig. 2. In vitro root initiation of *R. damascena* was obtained by using MS medium supplemented with 2.5 mg/l 2,4-D and then transferring explants to MS medium without any PGRs.

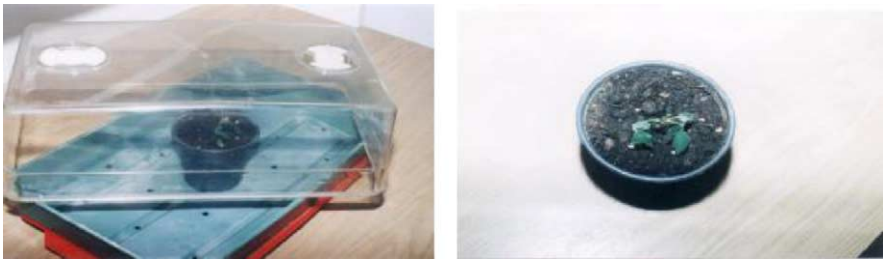

Fig. 3. Plantlets successfully acclimatised to ex vivo conditions and after 3 weeks transferred to the greenhouse.

produce roots in quick-dip method using sterilised 0–2000 mg/l auxin solutions. Other treatments such as using various concentrations of ABA with various concentrations of IAA, IBA and NAA also applying different concentrations of sucrose and agar did not result in rooting. At last, among the treatments the successful treatment for rooting of shoots was using 2.5 mg/l 2,4-D for 2 weeks in MS medium and then transferring the explants to MS medium without any PGRs (Fig. 2).

Plantlets were acclimatised using a soil mixture consisting of peat moss and sand 1:1 (v/v) and successfully transferred to the greenhouse after 3 weeks (Fig. 3).

4. Discussion

Based on the results obtained in this study, BA at concentrations of 2.5–3 mg/l in combination with a low rate of IBA was the most suitable treatment for in vitro multiplication of Damask rose. This is in accord with the results obtained by other investigators for other rose species (Campos and Pais, 1990; Khosh-Khui and Sink, 1982a; Kumar et al., 2001; Mederos and Rodriguez Enriquez, 1987; Salehi and Khosh-Khui, 1996; Skirvin et al., 1990).

In this study, explants tolerated a higher concentration of PGRs without showing any abnormality in morphology and also without any increase in proliferation rate. Although some proliferation was observed on media containing BA alone, a low level of auxin was also necessary for optimum proliferation. In apple, a related woody species, only BA was required for initial growth and development of shoot tip explants, which very frequently produced proliferated cultures (Lane, 1978). Shoot orientation did not influence shoot multiplication rate as was shown by Lane (1979) for ‘Bartlett’ pear.

According to some reports, wounding caused root formation in some species (Edwin and Paul, 1984) but in *R. damascena* rooting ability was not affected by this treatment.

In the present study, the general reduction in proliferation rate after first subculture might be due to an altered endogenous cytokinin level in plant tissue after continous subcultures (Mederos and Rodriguez Enriquez, 1987; Salehi and Khosh-Khui, 1996). In contrast to this result, Campos and Pais (1990) reported the highest shoot proliferation of a dwarf rose ‘Rosamini’ at third subculture and Chu et al. (1993) observed the same proliferation rate in all four subcultures of *R. chinensis* Jaccq. ‘Minma’. Genotypical differences may be responsible for this contradiction.

According to our results, application of MS medium supplemented with 2.5 mg/l 2,4-D for 2 weeks followed by transferring the explants to MS medium free from any PGRs was successful for root formation in Damask rose. 2,4-D is one of the important phenolic compounds with auxinic effect, at low concentrations and acts as a rooting cofactor and prevents breaking of endogenous auxin by oxidase enzyme results in rooting (Hudson et al., 2002). Effective use of 2,4-D has been also reported for in vitro rooting of other plants (Edwin and Paul, 1984).

When shoots were kept in the rooting medium for more than 2 weeks, root-tips became brown in color and plantlets died after a few days. The problem was solved by transferring the plantlets from rooting medium to root elongation medium (without any PGRs). It may be concluded that auxins are necessary just for root initiation, but not for subsequent root development of Damask rose.

It was observed that gradual acclimatisation was essential for this species of roses. After 3 weeks, the plants were sufficiently conditional, so were ready to be transferred to the greenhouse.

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