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In Vitro Flowering from Cultured Nodal Explants of Rose (*Rosa hybrida* L.)

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Abstract

Roses are one of the world's most important ornamentals for a long time and are most often used for ornamental, medicinal and aromatic purposes. The study reports in vitro multiple shoot formation and flower induction of *Rosa hybrida* L. cv. 'Red Masterpiece' with maximum number of 5 shoots per explant on MS medium supplemented with 3 mg/l BA and 1 mg/l kinetin, followed by flower induction on MS medium containing 2 mg/l BA for 9 weeks. The shoots readily rooted on ¼MS medium devoid of growth regulators. Shoots cultured under various photoperiods did not flower. Rooted plantlets were hardened and established in pots with 100% survival.

Keywords: in vitro flowering, micropropagation, nodal culture, *Rosa hybrida*, subculture time

Introduction

Roses are one of the world's most important ornamentals for a long time. They belong to the Rosaceae and are grown world wide as cut flowers, potted plants and home gardens. Rose is an important perennial flower shrub or vine of the genus *Rosa,* within the family Rosaceae that contains over 100 species and comes in a variety of colors.

The switch from vegetative stage to reproductive stage of growth is one of the most critical events in the life of a plant. An *in vitro* flowering system is considered to be a convenient tool to study specific aspects of flowering and whole mechanisms of the reproductive process such as floral initiation, floral organ development and floral senescence (Goh, 1992). Several successful attempts to induce *in vitro* flowering of roses have been reported (Vu *et al*., 2006; Wang *et al*., 2002). The application of cytokinins, photoperiod and subculture time to promote *in vitro* flowering is well documented in many plant species including roses (Vu *et al*., 2006; Wang *et al*., 2002).

The study aimed to develop a protocol for in vitro flowering of *Rosa hybrida* L. cv. 'Red Masterpiece' with aim to provide convenience to study specific aspects of flowering and mechanism of reproduction of this important rose cultivar.

Materials and methods

Plant materials

The Rosa hybrida L. cv. 'Red Masterpiece' was used in the experiment. Nodal explants containing lateral buds of actively field-grown 'Red Masterpiece' roses were cut into 3 cm length segments and washed in running water to remove dirt. These segments were surface disinfested using 70% ethanol for 15 sec and then immersed in 20% (v/v) of commercial laundry bleach CloroxTM solution (5.25% NaOCl) containing 2 drops of Tween-20 emulsifier to aid wetting for 20 min. Thereafter, the sterilized explants were rinsed 2-3 times with sterile distilled water. The explants were trimmed to 1 cm long pieces before transfer to the culture medium.

Medium preparation and culture conditions

MS (Murashige and Skoog, 1962) salts supplemented with 3% sucrose and 0.82 %agar (Mermaid™) was used to prepare the culture medium. The pH of all media was adjusted to 5.8 with 1 N NaOH or 1 N HCl before autoclaving at 1.05 kg/cm^2, 121°C for 20 min. Cultures were maintained at 25±1°C air temperatures in a culture room with a 16-h light photoperiod unless otherwise stated. Each treatment contained 20 capped glass jars with 20 ml of regeneration medium and each jar (115 ml capacity) contained 1 cm long one explant with a culture cycle of 3 weeks. Thereafter, the regenerating explants were subcultured to fresh medium or developing green and normal adventitious shoots were rooted on growth regulators free ¼MS medium. After development of adequate length of shoots and roots, the plantlets were transferred to 330 ml screw-topped jars containing sterile vermiculite for 2 weeks for hardening off.

Influence of BA and kinetin on in vitro flowering

To test the effect of BA (N6-benzyladenine) and kinetin on in vitro flowering, the nodal explants were aseptically cultured on MS medium containing 0, 1, 2, or 3 mg/l BA or kinetin singly or in combination.

Influence of photoperiod on in vitro flowering

Three photoperiods (light/dark cycle) i.e. 12/12, 16/8 and 8/16 were used to monitor *in vitro* flowering under an illumination of 20 µmol $m^{-2}s^{-1}$ photosynthetic photon flux density provided by cool-white fluorescent light.

Influence of subculture time

Explants were subcultured to fresh MS medium supplemented with 2 mg/l BA every 3 weeks resulted in 3, 6, 9, 12, and 15 weeks-interval.

Statistical analysis

All experiments were conducted on three different days. Analysis of variance was done using standard statistical techniques and the difference between means was compared using Tukey's test at $p \leq 0.05$.

Results and discussion

Multiple shoots were recorded on all explants after 3 weeks of culture, on MS medium containing various concentrations of BA and kinetin. Variable number of shoots was recorded on all culture media. Maximum number of 5.3±0.7 shoots per explant ($p \leq 0.05$) was recorded on MS medium containing 3mg/l BA in combination with 1 mg/l kinetin (Tab. 1). This suggests that in this cultivar required cytokinins to proliferate high number of shoots per explant without intervening callus stage. Previous research shows on other rose species regarding development of multiple shoot on regeneration media containing BA and IBA (Khosh-Khui and Jabbarzadeh, 2007; Kumar *et al.*, 2001) or BA and NAA (Drefahl *et al.*, 2007; Vu *et al.*, 2006; Wang *et al.*, 2002) with intervening callus phase. Therefore MS medium containing 3 mg/l BA and 1 mg/l kinetin was considered as optimal for shoot proliferation. These in vitro regenerated shoots were further multiplied on this medium by successive subculture, after every three weeks.

In vitro flowering was observed on MS medium containing 2 mg/l BA after 9 weeks of culture (Tab. 1). The flowers had normal petals and sepals at opening (Fig. 1). Initially the flower was pale red in color becoming red in the later stages of growth. BA is widely used for *in vitro* flowering in many roses (Dobres *et al*., 1998, Wang *et al*., 2002, Vu *et al*., 2006) and a number of other plant species (Lin *et al*., 2004, Taylor *et al*., 2005). The application of cytokinins induces molecular changes associated with the floral transition (Bernier *et al*., 2002).

Effect of photoperiod on individual shoots was recorded after 4 weeks of culture. Length of photoperiod had no significant effect on shoot multiplication and the photoperiod did not influence *in vitro* flowering (Tab. 2). The age of mother plants influenced flower induction (Demeulemeester and DeProft, 1999) therefore, it is possible that the explants used in this study remained at the vegetative phase. Tab. 3 shows the effect of subculture time on flower induction *in vitro*. After the 3-week-intervals for three consecutive subcultures, the percentage of in vitro flowering was 50%. The flower buds were small; however, the flowers were normal. It seems that a period of nine weeks in culture was appropriate for flowering in the present study. Wang *et al*. (2002) stated that there has been no report on the effect of subculture time on *in vitro* flowering and the subculture time could substantially affect *in vitro* flowering.

To establish complete rose plants, regenerated shoots were excised and transferred to ¼MS medium without growth regulators. Roots that developed on this medium were thick, long and fibrous. Two weeks of rooting was ad-

Tab. 1 Effect of different combinations of BA and kinetin on multiple shoot formation and *in vitro* flowering of *Rosa hybrida* L. cv. 'Red Masterpiece'

MS medium			
BA (mg/l)	Kinetin (mg/l)	Number of shoots per explant (Mean ± SE)	Explants producing flower (%)
0	0	1.0 ± 1.1^{d}	0
1	0	1.9 ± 0.2^{bcd}	0
2	0	2.8 ± 0.3^{bcd}	5
3	0	2.5 ± 0.3^{bcd}	0
0	1	3.0 ± 0.3^{bc}	0
0	2	2.4 ± 0.3^{bcd}	0
0	3	1.4 ± 0.2^{cd}	0
1	1	1.4 ± 0.1^{cd}	0
1	2	2.1 ± 0.2^{bcd}	0
1	3	1.0 ± 0.0^{d}	0
2	1	4.7 ± 0.5^{a}	0
2	2	3.7 ± 0.3^{ab}	0
2	3	4.8 ± 0.7^{a}	0
3	1	5.3 ± 0.7^{a}	0
3	2	2.6 ± 0.3^{bcd}	0
3	3	2.0 ± 0.3^{bcd}	0

The different letters within column show significant difference analyzed by Tukey's test at $p \leq 0.05$.

Fig. 1. In vitro flowering of the *Rosa hybrida* L. cv. 'Red Masterpiece' shoots cultured on MS medium supplemented with 2mg/l BA for 9 weeks.

equate before transplanting to polystyrene pots containing soil mixture (1 sand: 1 manure: 1 decayed leaves). *In vitro*-derived plants did not display any phenotypic variation during subsequent vegetative development.

Conclusions

Tab. 2 Effect of different photoperiod on multiple shoot formation of *Rosa hybrida* L. cv.'Red Masterpiece'

Photoperiod (light/dark)	Number of shoots per explant (Mean ± SE)
12/12	2.6 ± 0.4
16/8	2.8 ± 0.4
8/16	2.0 ± 0.2

Tab. 3 Effect of subculture time on *in vitro* flowering in *Rosa hybrida* L.
cv. 'Red Masterpiece'

Subculture period (weeks)	Explants flower *in vitro* (%)
3	0
6	0
9	50
12	0
15	0

A micropropagation system for *Rosa hybrida* cv. 'Red Masterpiece' has been worked out utilizing nodal explants. BA and subculture time have a promoting effect on *in vitro* flower initiation. Although 50% of flowering was observed, more reliable culture regimes for *in vitro* flower need to be elucidated.

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