Optimizing *in vitro* propagation of *Rosa persica*

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Abstract

Rosa persica with a distinctive red eye is a good candidate for addition to the breeding pool of roses. In order to optimize micropropagation of *R. persica*, three culture media (MS, QL and VS) were compared. The maximum growth rate was observed in the VS medium. Addition of FeEDTA (Fe: 65.4 mg/l), calcium gluconate (Cg: 2.7 g/l) and Gamborg vitamins (B5) instead of VS vitamins, alone or in combinationwith VS medium were compared. The maximum growth rate was obtained in the medium containing VS + Cg + B5 (VSCgB5). Various concentrations of BAP (0, 2, 4 and 8 μM) in combination with 3 μM GA3 showed that maximum growth rate was attained in the medium containing 8 μM of BAP. To reduce vitrification of *Rosa persica*, effects of the type of gelling agent (8 g/l agar, 4 g/l phytagel and 2 g/l agar + 3 g/l phytagel) and sucrose concentrations (30, 45 and 60 g/l) were investigated. The results showed that agar could completely eliminate vitrification and was the superior gelling agent for *R. persica*, whereas different sucrose concentrations did not have a significant effect on vitrification. At rooting stage, half-strength VS mineral salts and vitamins containing IBA (0, 0.25, 0.5, and 1 μM) and NAA (0 and 0.5 μM) were investigated. The results showed that there was not a significant difference between different rooting media in percentage of rooting or the number of roots per plantlet.

Keywords: micropropagation, *Rosa persica*, rooting, vitrification.

Introduction

Rosa persica Michx. ex Juss, syn. *Hulthemia persica* (Michx.), is a dwarf suckering and procumbent shrub of up to 50 cm in height which produces solitary flowers with yellow petals of up to 15 mm long with a red base, purple anthers and rounded, bristly, blackish hips (Fig 1). It is native to the steppes and desert regions of Iran, Afghanistan, central Asia, and north to western Siberia. *R. persica* is common in Iran, often growing as a weed and being used for fuel after harvest (http://www.rogerroses.com). Rose breeders have spent much time on hybridizing *R. persica* with the view that the distinctive red eye would be an important addition to the breeding pool of roses. For instance, the 'Hardy rose' ('*R. x hardyi*') was obtained from a cross between *R. clinophylla* (Sect. *Bracteatae*) and *R. persica* by J.A. Hardy about 1830 (Joyaux, 2001).

The conventional propagating methods are very slow, time consuming, and tiring. Tissue culture on the other hand is becoming increasingly popular as an alternative means of plant vegetative propagation (Khosravi *et al.*, 2007). The most commonly used method of micropropagation of roses is based on shoot multiplication, which is the *in vitro* equivalent of *in vivo* propagation from cuttings (Roberts and Schum, 2003).

Fig. 1: *Rosa persica* produces solitary flowers with yellow petals and a red base.

Preservation of valuable germplasm is undoubtedly another important issue facing plant breeders, as the genetic relatedness of the current crop varieties is becoming higher than ever. This obviously requires preservation of plant genetic resources for their potential use in the future. Plant tissue culture methods are being extensively used in this area (Kazan, 2002). In general, growth and development of plantlets *in vitro* are faster and more uniform, plantlets *in vitro* have less physiological and morphological disorders, biological contamination *in vitro* is less, plantlets have a higher percentage of survival during acclimatization *ex vitro*. Hence, production costs could be reduced and plant quality could be improved significantly with photoautotrophic micropropagation (Kozai *et al.*, 2005).

Different aspects of tissue culture propagation system for various roses have been described by many authors (Pati *et al*., 2006; Kumar *et al*., 2001; Rosu *et al*., 2000; Rout *et al*., 1999 and Aslam *et al*., 2004). However, no detailed report on *in vitro* propagation of *Rosa persica* is available. In the present investigation, at proliferating stage, three culture media (MS, QL and VS) and addition of FeEDTA (Fe: 65.4 mg/l), calcium gluconate (Cg: 2.7 g/l) and Gamborg vitamins (B5) were investigated. To optimize growth rate, different concentration of BAP were compared and to reduce vitrification, interactive effect of two gelling agents and different sucrose concentrations were compared. Finally at rooting stage, half-strength VS mineral salts and vitamins containing IBA (0, 0.25, 0.5, and 1 μM) and NAA (0 and 0.5 μM) were compared.

Materials and methods

Plant material and general considerations

Plants of *R. persica* were supplied by the Rose Germplasm Collection maintained at the Agricultural Biotechnology Research Institute of Iran (ABRII). Nodal segments (1-1.5 cm) were washed thoroughly with running tap water for half an hour and surface sterilized for 30 seconds in 70% ethanol, followed by a 15 min soak in 2.5% sodium hypochlorite solution with a few drops of Tween-20, and rinsed three times with sterile distilled water.

All media were prepared according to their general procedures, 30 g/l sucrose and 8 g/l agar (unless otherwise stated) was added to them. The pH of all media was adjusted to 5.8 before adding the gelling agent. Media were autoclaved for 15 min at 121 ºC and 1.2 kPa pressure. Cultures were placed under high pressure metal halide lamps on a 16/8 hour light/dark cycle in a culture room maintained at 21 ± 1 ºC.

In vitro preservation and shoot proliferation

Three basal media *i.e.* Murashige and Skoog (MS) (1962), Quoirin and Lepovier (QL) (1977) and van der Salm (VS) (1994) were compared to find the best medium for micropropagation. Addition of FeEDTA

(Fe: 65.4 mg/l), calcium gluconate (Cg: 2.7 g/l) and Gamborg vitamins (B5) instead of VS vitamins, alone or in combination with VS medium were investigated. To reduce vitrification, interactive effect of gelling agents (8 g/l plant agar, 4 g/l phytagel, and 2 g/l plant agar + 3 g/l phytagel) and sucrose concentrations (30, 45 and 60 g/l) were tested. Each treatment involved 5 repeats with 4 explants (20 explants). Number of axillary shoots, number of new leaves produced and height of shoots (cm) were recorded after 21 days for three consecutive subcultures and the weekly average was calculated

Shoot proliferation media contained full strength VS salts and vitamins with various concentrations of BAP (0, 2, 4 and 8 μM) in combination with 3 μM GA_3. In each treatment 5 repeats with 3 explants (15 explants) were used in total. Then umber of axillary shoots, number of new leaves produced, ratio of green leaves to total number of leaves and height of shoots (cm) were recorded after 21 days for three consecutive subcultures and the weekly average was calculated.

Root formation

Shoots were cultured on shoot elongation medium (VS mineral salts and vitamins without hormones) for 21 days prior to rooting treatments. For rooting, half-strength concentration of VS mineral salts and vitamins containing IBA (3-Indolebutyric acid) (0, 0.25, 0.5, and 1 μM) and NAA (0 and 0.5 μM) were tested in liquid media. For liquid medium, Sorbarods (Cellulose support plugs; Sorbarod, Ilacon, UK) were used. Each treatment involved 5 repeats with 3 explants (15 explants) in total. After 21 days, number of roots and their lengths were recorded.

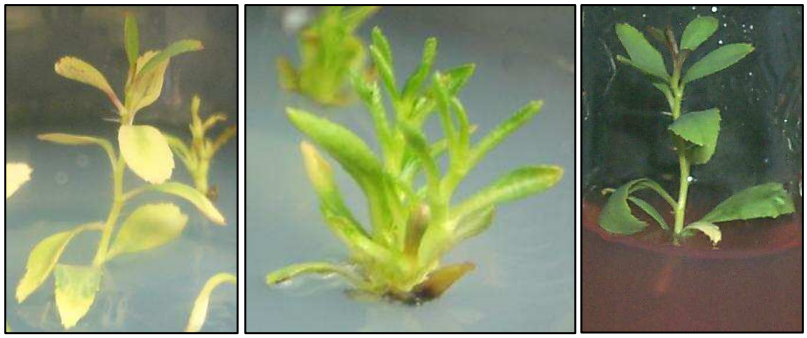
Experimental design and statistical analysis

Experiments were analyzed in a factorial based, completely randomized design and completely randomized design with unequal numbers of repetitions. Analysis of variance was performed and comparisons of means were conducted by Duncan's Multiple Range Test. All analyses were performed using SAS V8 GLM (SAS, 1999) and MSTAT-C statistical software (MSTAT-C, 1989). Differences were regarded as significant at $P \leq 0.05$.

Results

Shoot proliferation of *R. persica*

Comparing MS, QL and VS media indicated that using VS medium resulted in healthier and better growth (Fig. 2). Maximum increase in the number of axillary shoots (1.08), the number of new leaves (5.52) and the height of the shoots (0.43) were also observed in VS medium (Table 1). The addition of calcium gluconate (Cg) and Gamborg vitamins (B5) to VS medium (VSCgB5 medium) resulted in a maximum average number of axillary shoots (1.24), maximum number new leaves per explant (7.08) and maximum increase in height (0.74 cm) in average growth cycle (Table 1). Vitrification was reduced to 0% in the medium containing plant agar, while 55% and 95% of vitrification were observed in the media containing plant agar + phytagel and phytagel, alone, respectively. When plant agar was used as the gelling agent 0% vitrification was observed at all sucrose concentrations, however, when phytagel was used, 95%, 70% and 40% vitrification was observed in the media containing 30, 45 and 60 g/l sucrose, respectively (Table 2). A significant difference was observed between the different levels of BAP in the number of axillary shoots (Table 3). These results showed the highest number of axillary shoots (4.067), the highest ratio of green leaves to the total number of leaves (0.875), and the maximum height (0.413 cm) in medium containing 2 μM BAP.



(A) **(B)** **(C)**

Fig. 2: *In vitro* micropropagation of *R. persica* in **A:** QL medium; **B:** MS medium and; **C:** VS medium.

Table 1: Comparing the effect of different media on growth parameters. Means in each column with different letters show significant differences according to Duncan's multiple range test ($P \leq 0.05$).

media	plant height	number of	axillary shoot
MS	0.30^{bc}	3.24^{b}	1.08^{abc}
QL	0.24^{c}	0.57^{c}	0.28^{d}
VS	0.43^{bc}	5.52^{ab}	1.08^{abc}
VSCg	0.55^{ab}	4.72^{ab}	0.88^{bc}
VSFe	0.44^{bc}	5.76^{ab}	1.56^{a}
VSB5	0.70^{a}	5.48^{ab}	0.52^{dc}
VSCgB5	0.74^{a}	7.08^{a}	1.24^{ab}
VSFeB5	0.31^{bc}	3.76^{b}	1.20^{ab}

Table 2. Comparing the effect of different gelling agents and sugar concentrations on vitrification. Means in each column with different letters show significant differences according to Duncan's multiple range test ($P \leq 0.05$).

gelling agent	sugar (g/l)	vitrification
agar	30	0^{a}
agar	45	0^{a}
agar	60	0^{a}
agar + phytagel	30	55^{cd}
agar + phytagel	45	25^{ab}
agar + phytagel	60	30^{bc}
phytagel	30	95^{e}
phytagel	45	70^{d}

phytagel	60	40[bc]

Although the highest (12.867) and the lowest (4.267) number of new leaves produced were achieved in the media containing 8 and 0 µM BAP, respectively, but there was not a significant difference between other levels of BAP such as 0, 2 and 4 µM.

Table 3. Analysis of variance for the effect of different levels of BAP on the number of new leaves, the ratio of green leaves to total leaves, height, and axillary shoots.

Source	df	Mean square number of new leaves produced	Mean square ratio of green leaves to total leaves	Mean square height	Mean square number of axillary shoots
BAP	3	225.44**	0.049[ns]	0.06[ns]	40.68**
Q	1	0.96[ns]	0.02[ns]	0.02[ns]	0.55**
Experimental Error	16	15.50	0.04	0.07	0.59
Personal Error	40	6.35	0.02	0.02	0.48

** significant at 1% level, * significant at 5% level, [ns] not significant.

Root formation of *R. persica*

Table 4 illustrates the analysis of variance for the average percentage of rooting, the number of roots, and root length. The results indicated that the highest percentage of rooting (33.33%), highest number of roots (2), and maximum root length (7 mm) were obtained in the medium containing 1 µM + 0.25 µM IBA + 0 µM NAA, 1 µM IBA + 0.5 µM NAA and 0 µM IBA + 0.5 µM NAA, respectively. However, there were not significant differences between media containing auxins with basal medium. Plantlets rooted easily in the hormone-free medium (Fig. 3).

Table 4. Analysis of variance for effect of IBA and NAA in **A:** the average percentage of rooting, **B:** the average number of roots, and root length. [ns] not significant.

A

source	df	Mean square percentage of rooting
IBA	3	175.93[ns]
Q	1	92.75[ns]
NAA	1	1360.95[ns]
IBA×NAA	3	768.52[ns]
Error	32	416.65

B

rce	df	Mean square number of roots	Mean square root length
Treatment	7	0.47[ns]	4.9[ns]
Q	1	0.22[ns]	11.57[ns]
Error	11	0.75	8.09

Fig. 3. *R. persica* cultured in medium without BAP.

Discussion

Van der Salm *et al.* (1994) showed that the growth of the rose rootstock cultivar 'Moneyway' was faster on MS than on QL or Woody Plant medium. They also showed that the replacement of FeNaEDTA by FeEDDHA in QL and MS media resulted in the development of green shoots for more than three months in the *in vitro* propagation of the rose rootstock 'Moneyway'. The present investigation showed that shoots growing in MS and QL media, despite good growth, developed chlorosis in newly formed leaves whereas, replacement of FeNaEDTA by FeEDDHA in MS. *i.e*. VS medium, resulted in better growth.

Addition of Cg and B5 to VS medium (VSCgB5 medium) resulted in a better growth measured by the number of axillary shoots, new leaves per explant and increase in height. Some roses require more calcium ions than is provided in the basal medium and calcium deficiency results in shoot-tip necrosis and hyperhydration of stem and leaf tissues. These effects can be controlled by the addition of calcium chloride but, as this salt can have toxic side-effects, the use of calcium gluconate is preferred. According to Roberts and Schum (2003) in those roses that express calcium deficiency, the inclusion of Cg (6 µM) in the multiplication medium, significantly increased shoot multiplication rates. They also suggested that calcium gluconate has no negative effects on roses that do not suffer from calcium deficiency, so its routine inclusion in the multiplication medium is recommended. Inclusion of B5 vitamins from Gamborg medium has been defined for the growth of cell suspensions of soybean root cells. In this medium thiamine, which is known to be an essential vitamin for cell growth, is increased to 10 mg/l.

Although the medium containing BAP is essential for bud break and shoot multiplication of *R. persica*, but the results showed that application of BAP in combination with GA_3 periodically caused to preserve this species for a long time. Roberts and Schum (2003) indicated that for introduction into culture and multiplication, BAP at a concentration of 2 µM is adequate for most species and cultivars of roses. Vijaya *et al.* (1991) also reported that BAP was the most effective growth regulator in stimulating shoot proliferation. Kim *et al.* (2003) obtained the best shoot proliferation in the presence of 2 mg/l of BAP and 0.01 mg/l of NAA in full-strength MS salts. They also showed that *in vitro* shoot proliferation and multiplication are largely based on media formulations containing cytokinins as a major plant growth regulators, whereas, in some cases, low concentrations of auxins and gibberellic acid 3 (GA_3) were also used.

Vitrification is one of the main problems of long term *in vitro* propagation of *R. persica* which results in abnormal growth with very few tall stems and curled succulent, translucent and brittle leaves.

Podwyszynska and Olszewski (1995) compared gelling agents on modified MS medium, and showed that higher levels of calcium and magnesium in Bacto agar medium improved the quality of shoots of the rose 'White Gem', while medium containing phytagel increased the multiplication rate. Kumar *et al.*, (2003) compared the effects of agar and phytagel on micropropagation of *R. damascena* and observed enhanced growth and better shoot proliferation in cultures grown on medium containing phytagel. However, rooting was much more pronounced, with a greater number of roots per rooted shoots when micro-shoots were grown on medium gelled with agar compared to phytagel. Agar is a natural product derived from seaweed and gives a semi-opaque medium, whereas phytagel is a product of bacterial fermentation and gives a much clearer medium. phytagel has a higher matric potential than agar (Roberts and Schum, 2003). In some plant species over a long period of time, cultured tissues become hyperhydrated and shoot growth is distorted. In the present investigation vitrification was reduced to 0% in the medium containing plant agar. Thus, VS medium containing calcium gluconate (Cg: 2.7 g/l), Gamborg vitamins (B5) and agar as the gelling agent is recommended for *in vitro* micropropagation of *R. persica.*

Our investigation showed that the highest percentage of rooting, root length and number of roots were observed in half-strength medium. This is in accordance with Faisal and Al-Amin (2000), who reported that using 0.2 mg/l of IBA and 0.2 mg/l of IAA with half strength MS were the best combination for root formation in *Chrysanthemum morifolium.*

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References

Aslam K. M., Khurram Z., and Iftikhar A. (2004). Effect of various hormones and different rootstocks on rose propagation. *Pakistan Journal of Biological Sciences*, 7: 1643-1646.

Faisal S. M., and Al-Amin M. (2000). Rapid multiplication of two *Chrysanthemum* cultivars through *in vitro* shoot tip culture. *Plant Tissue Culture*, 10: 131-136.

Joyaux F. (2001). La rose, une passion française. Bruxelles: Editions Complexe.

Kazan, K. (2002). Book Review. In Plant Biotechnology I, Tissue Culture and Its Applications (Babaoglu M., Gurel, E. Ozcan S.). *Turkish Journal of Botany*, 26: 51-53.

Khosravi P., Kermani J. M., Nematzadeh G. A., and Bihamta, M. R. (2007). A protocol for mass production of *Rosa hybrida* cv. Iceberg through *in vitro* propagation. *Iranian Journal of Biotechnology*, 5: 100-104.

Kim C. K., Oh J. Y. Jee S. O., and Chung J. D. (2003). *In vitro* micropropagation of *Rosa hybrida* L. *Plant Biotechnology*, 5: 115-119.

Kozai T., Xiao Y., Nguyen Q. T., Afreen F. and Zobayed S. M. A. (2005). Photoautotrophic (sugar- free medium) micropropagation system for large-scale commercialization. *Propagation of Ornamental Plants*, 5: 23-34.

Kumar A., Plani L. M. S., and Nandi S. K. (2003). The effect of light source and gelling agent on micropropagation of *Rosa damascena* Mill. and *Phynchostylis retusa* (L.) Bl. *Journal of Horticultural Science and Biotechnology*, 78: 786-792.

Kumar A., Sood A., Plani U. T., Gupa A. K., and Plani L. M. S. (2001). Micropropagation of *Rosa damascena* Mill. using thidiazuron. *Journal of Horticultural Science and Biotechnology*, 76: 30-34.

MSTAT-C (1989). MSTAT-C, a microcomputer program for the design, management, and analysis of agronomic

research experiments. Michigan State Univ., East Lansing, MI.

Murashige T., and Skoog F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant*, 15: 473-497.

Pati P. K., Rath S. P., Sharma M., Sood A., and Ahuja P. S. (2006). *In vitro* propagation of rose: a review. *Biotechnology Advances*, 24: 94-114.

Podwyszynska M., and Olszewski T. (1995). Influence of gelling agent on shoot multiplication and the uptake of macroelements by *in vitro* culture of rose, *Cordyline* and *Homalomena*. *Scientia Horticulturae*, 616: 231-235.

Quoirin M., and Lepoivre Ph. (1977). Etude de milieux adaptés aux cultures *in vitro* de Prunus. *Acta. Horticulturae*, 78: 437-442.

Roberts A. V., and Schum A. (2003). Cell, tissue and organ culture. In encyclopedia of rose science (Roberts A. V., Debener T., and Gudin, S.). Elsevier Academic Press, 1: 60-61.

Rosu A., Mitica O., and Ariton S. M. (2000). Biotechnological methods for the enhancement of the propagation rate in roses. *Biotechnology and Biotechnological Equipment*, 14: 32-36.

Rout G. R., Samantaray S., Mottley J., Das P. (1999). Biotechnology of the rose: a review of recent progress. *Scientia Horticulturae*, 81: 201-228.

SAS Inc (1999). The SAS system for Windows, version 8.0. SAS Inst., Inc., Cary, N.C.

van der Salm T. P. M., van der Toorn C. J. G., and Hanisch ten Cate C. H. (1994). Importannce of the iron chelate formula for micropropagation of *Rosa hybrida* L. 'Moneyway'. *Plant Cell Tissue and Organ Culture*, 37: 73-77.

Vijaya N., Satyanarayana G., Prakash J., and Pierik R. L. M. (1991). Effect of culture media and growth regulators on *in vitro* propagation of rose. *Current Plant Science and Biotechnology in Agriculture*, 12: 209-214.