ELSEVIER

Scientia Horticulturae 92 (2002) 69–74

SCIENTIA HORTICULTURÆ

www.elsevier.com/locate/scihorti

Short communication

An improved system for the in vitro propagation of rose cultivars

B.P. Carelli, S. Echeverrigaray*

Institute of Biotechnology, University of Caxias do Sul, R. Francisco Getúlio Vargas 1130, Caxias do Sul 95001-970, Rio Grande do Sul, Brazil

Accepted 11 April 2001

Abstract

The effects of growth regulators, carbon sources and salt media were studied on in vitro shoot proliferation from lateral bud explants obtained from 2- to 5-year-old plants of *Rosa hybrida* cv. Baronesse. The final medium adopted included the salt formulation of Quoirin and Lepoivre [Acta Hortic. 78 (1977) 437], 30 g l^{-1} of sucrose, benzyladenine (3.0 mg l^{-1}) and naphthaleneacetic acid (0.5 mg l^{-1}). Under these conditions, a multiplication rate of 30.3 plantlets per explant was obtained after 180 days. This system was also effective for the multiplication of six out of the other nine cultivars evaluated with a multiplication rate of 1.85–2.88 plantlets per explant after 60 days in culture. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: *Rosa hybrida*; Micropropagation; Growth factors; Multiplication rate

1. Introduction

The types of *Rosa hybrida* L. used in floriculture are the so-called hybrid tea, floribunda, and hybrid polyantha roses, which are mostly tetraploid. Commercial roses are usually propagated by grafting of hybrid scion cultivars onto the rootstock species, although they can also be propagated by seed, cuttings, and budding.

Since the reports of Skirvin and Chu (1979) and Hasegawa (1979) on the micropropagation of *Rosa hybrida* L. by proliferation of axillary buds, several studies concerning different aspects of commercial rose multiplication have been published (Davies, 1980; Bressan et al., 1982; Curir et al., 1986; Valles and

* Corresponding author. Tel./fax: +55-54-218-2149.
E-mail address: selaguna@ucs.tche.br (S. Echeverrigaray).

0304-4238/02/$ – see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0304-4238(01)00280-1

Boxus, 1987; Horn et al., 1988; Horn, 1992). However, there is only limited commercial use of the technique because of contradictory results and the low multiplication rate achieved with the most important rose cultivars. Several results concerning the effect of different factors on the micropropagation of the rose cultivar 'Baronesse', and the response of nine other cultivars on the selected medium are presented here.

2. Material and methods

Single undeveloped axillary buds were excised from the middle part of vegetative shoots of plants of cv. Baronesse, as well as other cultivars (Table 3), that were 2–5-years-old. The explants were excised at the beginning of spring, and were surface sterilized by a treatment with 70% ethanol for 20 s, then incubated in a 5% solution of sodium hypochloride for 20 min, followed by three rinses in sterile distilled water. Axillary buds were transferred to test tubes (2.5 cm × 15 cm tubes, with plastic closures) containing 5 ml of culture medium. After establishment (30 days), the plantlets were transferred to 120 ml glass flasks (5 plantlets per flask) containing 30 ml of culture medium.

The effects of cytokinins (benzyladenine (BA), kinetin (KIN) and 2-isopenteny-ladenine (2-iP)) alone or combined with auxins (naphthaleneacetic acid (NAA), indoleacetic acid (IAA) and indolebutyric acid (IBA)) were evaluated on basal Murashige and Skoog (1962) medium. The effects of polyvinylpyrrolidone (PVP, 0.5 and 10 mg l^{-1}) were also examined. Five carbon sources (sucrose, glucose, mannitol, sorbitol and galactose), as well as three different basic components, MS (Murashige and Skoog, 1962), B5 (Gamborg et al., 1968) and QL (Quoirin and Lepoivre, 1977), all supplemented with MS vitamins, were tested. The pH was adjusted to 5.6 with 1 M KOH before agar was added.

The cultures were maintained at 25 ± 2°C with 16 h photoperiod at a photosynthetic photon flux density of 10–20 μmol $m^{-2} s^{-1}$ (Phillips "cool-white" fluorescent tubes). Rooting was obtained on MS medium supplemented with 0.1 mg l^{-1} NAA and 2.5 g l^{-1} activated charcoal. Subculturing was carried out at 30-day intervals. All treatments were performed on three replications of 10 explants in experiments employing a completely randomized design. All the experiments were conducted twice.

3. Results and discussion

3.1. The effect of growth regulators

BA promotes a higher number of shoots per explant compared with KIN, and 2-iP gave intermediary results (Table 1). However, the shoots developed in the

Table 1
The effect of different cytokinins (3.0 mg l^{-1}) on the in vitro establishment of rose cv. Baronesse. The experiments were carried out on MS basal medium, and the explants were recultured after 30 days[a]

Cytokinin	No. of shoots per explant	Shoot length (cm)
KIN	1.73 ± 0.18 B	0.74 ± 0.063 AB
2-iP	2.17 ± 0.23 AB	0.91 ± 0.065 A
BA	2.57 ± 0.20 A	0.57 ± 0.059 B

[a] Values with different letters are significantly different ($p \leq 0.05$) with Tukey's test.

presence of 2-iP or KIN did not survive upon transferring. In the absence of cytokinins (data not shown), all the shoots died within 2 weeks. The presence of BA significantly reduced shoot elongation compared with 2-iP. The results are in accordance with those obtained with other rose cultivars by Skirvin and Chu (1979), Bressan et al. (1982), Short and Roberts (1991), and Horn (1992).

The number of shoots increased with increasing concentration of BA in the media, but the elongation decreases (Table 2). With 3.0 mg l^{-1} BA, we obtained a high number of shoots per explant and a non-significant reduction of plant height. In 5.0 mg l^{-1} of BA, hyperhydricity was observed.

The association of 1.0 mg l^{-1} BA and 0.5 mg l^{-1} NAA positively affected the multiplication of the Baronesse cultivar compared with 1.0 mg l^{-1} BA, leading to an increase in the number of developing shoots (Table 2). However, this association did not influence the elongation of the plantlets. At high concentrations of BA, the presence of NAA reduced the incidence of hyperhydricity maintaining a high number of developing shoots.

Based on these results, the combination of 3.0 mg l^{-1} of BA and 0.5 mg l^{-1} of NAA was adopted in the following experiments.

Table 2
Axillary budding of rose cv. Baronesse on MS medium supplemented with various concentrations of BA and NAA (60 days of cultivation)[a]

Concentration of BA and NAA (mg l^{-1})	No. of shoots per explant	Shoot length (cm)
0.5 BA	1.00 ± 0.00 C	1.54 ± 0.18 A
1.0 BA	1.12 ± 0.12 BC	1.31 ± 0.18 AB
3.0 BA	2.50 ± 0.19 A	1.27 ± 0.15 AB
5.0 BA	2.25 ± 0.45 AB	0.70 ± 0.10 B
0.5 BA + 0.5 NAA	2.10 ± 0.31 ABC	1.81 ± 0.19 A
1.0 BA + 0.5 NAA	2.50 ± 0.34 A	1.18 ± 0.23 AB
3.0 BA + 0.5 NAA	2.89 ± 0.26 A	1.24 ± 0.07 AB
5.0 BA + 0.5 NAA	2.67 ± 0.23 A	1.26 ± 0.11 AB

[a] Values with different letters are significantly different ($p \leq 0.05$) with Tukey's test.

3.2. The effect of carbon source and salt mixture

Five carbon sources were evaluated at a concentration of 20 g l^{-1} (glucose, mannitol, sorbitol, galactose and sucrose). Shoots grown on glucose and sucrose were taller, and exhibited a larger number of new shoots (data not shown). Based on these preliminary results, different concentrations of glucose and sucrose were tested. The results showed that the growth and the multiplication of rose cv. Baronesse were not highly influenced by the kind (sucrose or glucose) and the concentration (10, 30 and 60 mg l^{-1}) of sugar. However, based on the low frequency of hyperhydricity, 30 g l^{-1} sucrose was chosen.

A low percent survival (35–45%) of the explants during the first 30 days after inoculation was observed. To prevent this problem, several salt formulations (MS, QL and B5), and the addition of PVP (0.5 and 10 mg l^{-1}), were assayed. PVP was not effective but the QL medium drastically reduced explant oxidation giving a percent survival of 77.5% compared to only 45 and 25% on MS and B5 media, respectively.

In comparison with MS and B5 media, the ammonium ion concentration in the QL medium is strongly reduced, calcium is increased, and chlorine ions are almost eliminated (George, 1992). These differences may enable hyperhydricity problems to be avoided (Valles and Boxus, 1987), they may also be responsible

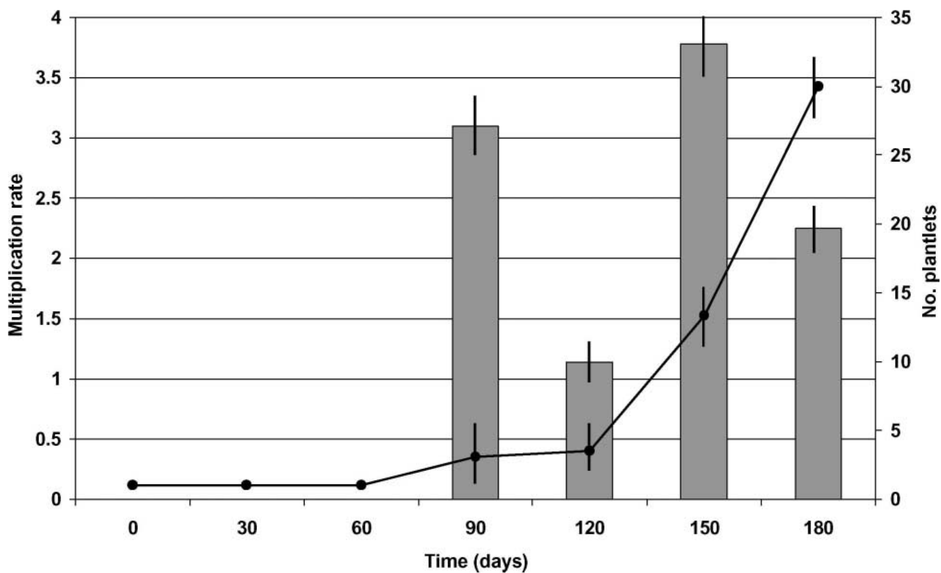

Fig. 1. Multiplication rate (bars) and number of plantlets per initial explant (line) obtained during the micropropagation of Baronesse on QL medium supplemented with 3.0 mg l^{-1} of BA and 0.5 mg l^{-1} of NAA. Data correspond to the mean and standard deviation value obtained with the 30 initial explants.

for the low oxidation of the explants during the first 30 days in culture, and the survival of the shoots after their replication.

Based on the above observations, the in vitro multiplication of 'Baronesse' was conducted during 180 days with subcultures at a 30-day interval on QL medium with 30 g l^{-1} sucrose supplemented with 3.0 mg l^{-1} of BA and 0.5 mg l^{-1} of NAA (Fig. 1). During the first 60 days in culture, the explants and their developing shoots were just transferred to new media, and the time was spent waiting for the adaptation to the in vitro condition and development of the new shoots. After 90 days, the plantlets with shoots longer than 0.8 mm were excised and transferred separately. At the end of 180 days, we obtained a multiplication rate of 30.3 plantlets from each initial explant. This multiplication rate is comparable to that obtained by Horn (1992), i.e. 9.7 plantlets per explant in 140 days.

All the plantlets obtained after 180 days of culture rooted on MS medium supplemented with 0.1 mg l^{-1} of NAA, and activated charcoal (2.5 g l^{-1}), and 92% were established in outdoor conditions. This result shows that this multiplication system can be used for the commercial propagation of the Baronesse cultivar.

3.3. The response of different cultivars on the selected medium

Nine cultivars, other than Baronesse, were assayed on QL medium supplemented with 3.0 mg l^{-1} of BA and 0.5 mg l^{-1} of NAA. The results, presented in Table 3, indicate that this medium can be used with success for the multiplication of 70% of the cultivars evaluated, with an average multiplication rate of 2.2 shoots per explant after 60 days. The influence of the genotype on the in vitro behavior of roses has been observed by Khosh-Khui and Sink (1982),

Table 3
Micropropagation of different rose cultivars on QL medium supplemented with 3.0 mg l^{-1} of BA and 0.5 mg l^{-1} NAA (60 days of cultivation)[a]

Cultivars	No. of shoots per explant	Shoot length (cm)
Baronesse	2.88 ± 0.16 A	1.49 ± 0.092 A
Rafaela	2.22 ± 0.173 AB	1.47 ± 0.067 A
Confet	2.16 ± 0.401 AB	1.18 ± 0.091 ABC
Roseana	2.14 ± 0.404 AB	0.96 ± 0.087 BC
Dukat	2.11 ± 0.26 AB	0.78 ± 0.076 C
Scala	2.04 ± 0.18 AB	1.32 ± 0.047 AB
Supless	1.85 ± 0.260 AB	1.36 ± 0.099 AB
Oceânica	1.50 ± 0.17 B	1.01 ± 0.057 BC
Juvena	1.23 ± 0.17 B	1.00 ± 0.058 BC
Vegas	1.22 ± 0.15 B	0.81 ± 0.054 C

[a] Values with different letters are significantly different ($p \leq 0.05$) with Tukey's test.

Horn et al. (1988), Short and Roberts (1991), Horn (1992), and Arnold et al. (1995). Horn (1992) observed a clear effect of genotypes when comparing the micropropagation of nine rose cultivars on a defined medium, in which just 33.3% of cultivars showed satisfactory results. The present results show that the system optimized for the propagation of 'Baronesse' can be used for multiplication of several other cultivars.

Acknowledgements

This research was supported by a grant from FAPERGS, and CAPES, Brazil. The authors wish to express their gratitude to Mr. Shigeu (Floricultura Feliz, RS) for supplying shoot samples of the rose cultivars.

References

Arnold, N.P., Binns, M.R., Cloutier, D.C., Barthakur, N.N., Pellerin, R., 1995. Auxins, salt concentrations, and their interactions during in vitro rooting of winter-hardy and hybrid tea roses. HortSci. 30, 1436–1440.

Bressan, P.H., Kim, Y.J., Hyndman, S.E., Hasegawa, P.M., Bressan, R.A., 1982. Factors affecting in vitro propagation of rose. Am. J. Hortic. Sci. 107, 979–990.

Curir, P., Damiano, C., Cosmo, T., 1986. In vitro propagation of some rose cultivars. Acta Hortic. 189, 221–224.

Davies, D.R., 1980. Rapid propagation of roses in vitro. Sci. Hortic. 13, 385–389.

Gamborg, O.L., Miller, R.A., Ojima, H., 1968. Nutrient requirements of suspension cultures of soybean root cell. Exp. Cell Res. 50, 151–158.

George, E.F., 1992. Plant Propagation by Tissue Culture. Part 1. The Technology. Exegetics, Edington, UK.

Hasegawa, P.M., 1979. In vitro propagation of rose. HortSci. 14, 610–612.

Horn, W.A.H., 1992. Micropropagation of rose (*Rosa* L.). In: Bajaj, Y.P.S. (Ed.), Biotechnology in Agriculture and Forestry, Vol. 20. Springer, Berlin, pp. 320–342.

Horn, W.A.H., Schlegel, G., Lerstuhl, K.J., 1988. Micropropagation of roses (*Rosa hybrida*). Acta Hortic. 226, 623–627.

Khosh-Khui, M., Sink, K.C., 1982. Micropropagation of new and old world Rosa species. Am. J. Hortic. Sci. 57, 315–319.

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco cultures. Physiol. Plant. 15, 473–497.

Quoirin, M., Lepoivre, P., 1977. Étude des milieux adaptés aux cultures in vitro de *Prunus*. Acta Hortic. 78, 437–442.

Short, K.C., Roberts, A.V., 1991. *Rosa* spp. (roses): in vitro culture, micropropagation, and the production of secondary products. In: Bajaj, Y.P.S. (Ed.), Biotechnology in Agriculture and Forestry, Vol. 15. Springer, Berlin, pp. 376–397.

Skirvin, R.M., Chu, M.C., 1979. In vitro propagation of 'Forever Yours' rose. HortSci. 14, 608–610.

Valles, M., Boxus, P., 1987. Micropropagation of several *Rosa hybrida* L. cultivars. Acta Hortic. 212, 611–617.