POLISH SOCIETY FOR HORTICULTURAL SCIENCE

Folia Hort. 23/2 (2011): 145-149

DOI: 10.2478/v10245-011-0022-5

FOLIA HORTICULTURAE

Published by the Polish Society
for Horticultural Science since 1989

The effect of BA and GA_3 on the shoot multiplication of *in vitro* cultures of Polish wild roses

Bożena Pawłowska

Department of Ornamental Plants
University of Agriculture in Krakow
29 Listopada 54, 31-425 Kraków, Poland
e-mail: ropawlow@cyf-kr.edu.pl

ABSTRACT

The experiment was conducted using five species of roses naturally occurring in Poland: *Rosa agrestis* (fieldbriar rose), *R. canina* (dog rose), *R. dumalis* (glaucous dog rose), *R. rubiginosa* (sweetbriar rose), and *R. tomentosa* (whitewooly rose), from the *in vitro* collection of the Department of Ornamental Plants of the University of Agriculture in Kraków. We examined the effect of cytokinin BA (1-10 μM) added to an MS medium (Murashige and Skoog 1962) on auxiliary shoot multiplication. The second group of test media contained BA (1-5 μM) and gibberellin GA_3 (0.3-1.5 μM). The cultures were maintained at a phytotron temperature of 23/25°C (night/day), 80% relative humidity, with a 16-hour photoperiod and PPFD of 30 μmol m^{-2} s^{-1}, and cultured in five-week cycles. The highest multiplication rate was obtained for *R. canina* and *R. rubiginosa* (4.1 shoots per one explant) and *R. dumalis* (2.9 shoots per one explant), when shoots were multiplied on an MS medium supplemented with 1 μM BA and 1.5 μM GA_3. Multiplication was the weakest in *Rosa tomentosa* independent of the medium used.

Key words: Polish wild roses, auxiliary shoots, multiplication

INTRODUCTION

In vitro cultures are one of the key tools in plant biotechnology. They are used for large-scale multiplication of plant clones and contribute to biodiversity conservation. The *in vitro* technique significantly influences the popularity and cultivation of roses, and is used for the propagation of new culture lines, for the production of material for rootstock for grafting on mass-scale and for the quick multiplication and production of disease-free plants (Pati et al. 2006, Rout et al. 2006, Canli and Kazaz 2009). Studies of *in vitro* rose cultures conducted previously indicate that genotype is a decisive factor for micropropagation. The high heterozygosity and polyploidy of roses hinders and limits their propagation and necessitates the study of each genotype separately. Roses are propagated most often by auxiliary bud induction, which guarantees the highest genetic stability of the obtained plant material, whereas only a few studies have reported successful somatic embryogenesis (Roy et al. 2004, Bhardwaj et al. 2006, Hameed et al. 2006, Oo et al. 2008, Nak-Udom et al. 2009, Murali and Sindhu 2011).

The aim of the present research was to determine the effect of cytokinin BA and gibberellin GA_3 added to an MS medium on the axillary bud multiplication of *in vitro* cultures of five rose species that are naturally occurring in Poland: *Rosa agrestis*, *R. canina*, *R. dumalis, R. rubiginosa* and *R. tomentosa*.

MATERIAL AND METHODS

The experiments were conducted on *Rosa agrestis* (fieldbriar rose), *R. canina* (dog rose), *R. dumalis* (glaucous dog rose), *R. rubiginosa* (sweetbriar rose), and *R. tomentosa* (whitewooly rose) shoots, which were procured from the *in vitro* collection of the

Department of Ornamental Plants (Pawłowska et al. 2009). The study aimed to investigate the effect of media with basic composition according to Murashige and Skoog (1962), containing 30 g dm^3 sucrose and 7 g dm^3 agar Difco Bacto, pH 5.7. The first group of test media was supplemented with BA at concentrations of 1, 2.5, 5 or 10 µM. The second group contained cytokinin BA and gibberellin GA_3 at 5 µM BA + 0.3 µM GA_3, 1 µM BA + 1.5 µM GA_3 or 1 µM BA + 1 µM GA_3 (Tab. 1). Rose shoots with three to four leaves were placed on media, five shoots per 250 ml Erlenmeyer flask. The plants were cultivated for five weeks in a phytotron under a 16-hour photoperiod, at a temperature of 23/25°C (night/day) and 80% relative humidity. When a growth cycle was over, the shoot multiplication rate was calculated (number of shoots per regenerating explant), and shoot height and number of leaves per shoot were recorded.

All experiments were performed in five replicates, 25 explants in each. Data significance was evaluated using a combined two-factor analysis for independent data. The level of significance was set to $\alpha = 0.05$. Data were analysed by the Duncan test.

RESULTS AND DISCUSSION

Polish species of roses originating from natural habitats can be used in ornamental horticulture for their decorative values. They are also an important raw material for the pharmaceutical and cosmetic industries and a source of food and shelter for animals. *In vitro* cultures of roses are used for the rapid multiplication of superior cultivars and wild roses, the production of disease-free plants and in order to expedite breeding programs. Wild rose species also play a role in rose breeding, which is based on a very narrow genetic base. Currently, only eight species have made a significant contribution to the gene pool of modern roses (De Vries and Dubois 1996, Engelmann and Engels 2002, Bhoomsiri and Masomboon 2003, Khosh-Khui and Teixeira da Silva 2006, Kornova and Michailova 2008, Pawłowska et al. 2009).

The experiments on micropropagation of five rose species, *Rosa agrestis*, *R. canina*, *R. dumalis, R. rubiginosa* and *R. tomentosa,* on test media showed the formation of new shoots with a rosette growth. The multiplication rate ranged from 1 to 4.1 shoots per one regenerating micro-explant, depending on rose species and medium tested (Figs 1 and 2). Newly formed plants had 5-6 leaves on average, while their length was 7.5-10 mm; shoots cultivated on the medium supplemented with 1 µM BA had the greatest height (Tab. 1). Barna and Wakhlu (1995) used media with BA or thidiazuron (1-10 µM) for the multiplication of *Rosa hybrida* and obtained plantlets that were 5-11 mm high.

The experiment evaluating the effect of cytokinine BA (1-10 µM) present in an MS medium (Murashige and Skoog 1962) on the multiplication of axillary shoots showed that the highest shoot multiplication rate (2.5 shoots per one regenerating explant) was obtained in *Rosa canina* when BA concentration in the medium was 1 µM (Fig. 1). The highest BA concentration in the medium inhibited shoot multiplication, and the shoot multiplication rate at the 10 µM BA concentration was 1.6. A similar relationship was observed in *Rosa rubiginosa* (2.1 at 1 µM BA and 1.6 at 10 µM BA). Murali and Sindhu (2011) obtained the best results with *Rosa bourboniana* multiplication when BA concentration in the medium was 0.5 µM. In my experiment, we did not observe differences in the multiplication rate of the remaining species, *Rosa agrestis, R. dumalis, R. tomentosa,* when the BA concentration in the medium varied in the range of 1-10 µM. Pawłowska et al. (2009) obtained similar results – namely, the abovementioned rose species showed poor multiplication (1-1.3) on QL media supplemented with BA (5-10 µM), NAA (0.03-0.2 µM) and GA_3 (0.3-0.5 µM). BA is the most often

Table 1. The effect of the medium on the regeneration of wild roses independent of the species

MS medium composition	Multiplication rate	Mean shoot length (mm)	Mean number of leaves
1 µM BA	1.9 c*	10.0 d	6.1 e
2.5 µM BA	1.7 bc	9.1 c	5.9 de
5 µM BA	1.8 c	8.1 ab	5.6 bc
10 µM BA	1.4 a	8.2 ab	6.1 e
5 µM BA + 0.3 µM GA_3	1.7 bc	8.5 bc	5.4 b
1 µM BA + 1.5 µM GA_3	2.9 d	8.9 bc	4.9 a
1 µM BA + 1 µM GA_3	1.5 ab	7.5 a	5.7 cd

*Means with the same letter do not differ significantly at $\alpha = 0.05$

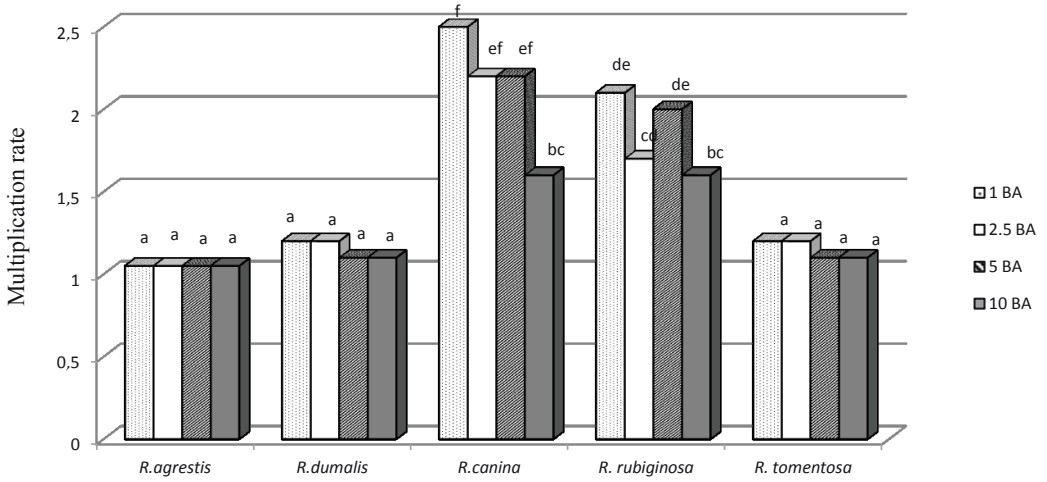

Figure 1. The effect of BA (μM) in the medium on shoot multiplication in a five-week cycle in five species of roses naturally occurring in Poland

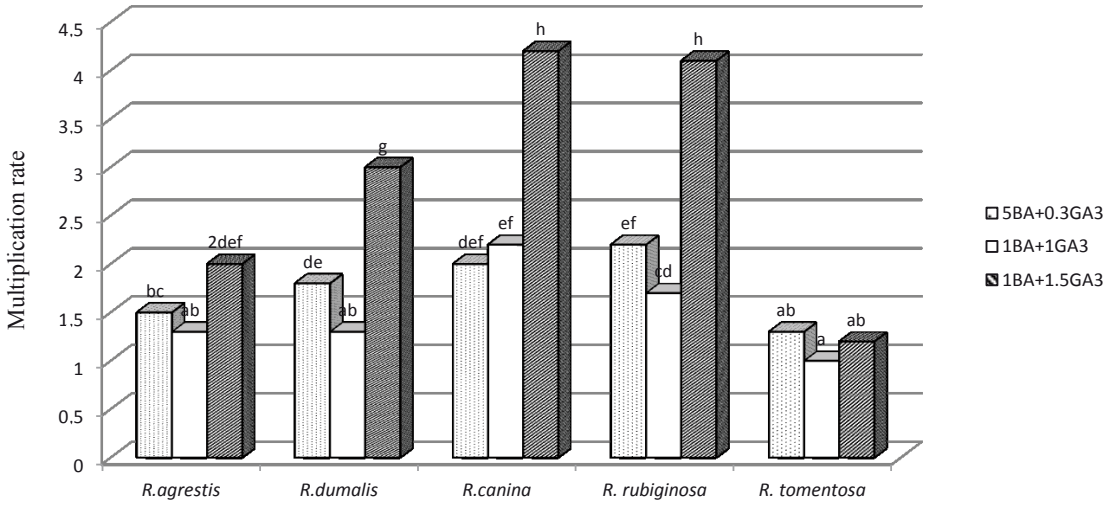

Figure 2. The effect of BA and GA_3 (μM) in the medium on shoot multiplication in a five-week cycle in five species of roses naturally occurring in Poland

used cytokinin in rose propagation (Khosh-Khui and Teixeira da Silva 2006). Barna and Wakhlu (1995) investigated the BA effect on *Rosa hybryda* multiplication and obtained the best results with a 10 μM BA concentration in the medium. Pati et al. (2001) optimized the BA concentration at 5 μM for shoot proliferation in *R. damascena* and *R. bourboniana*. *In vitro* shoot multiplication of roses is largely based on media containing cytokinins as a major PGR, whereas, in some cases, low concentrations of auxins and GA_3 were also used (Pati et al. 2006). In the present experiments, three media containing BA and GA_3 in different proportions were tested: 5 μM BA + 0.3 μM GA_3, 1 μM BA + 1.5 μM GA_3 or 1 μM BA + 1 μM GA_3. The highest shoot multiplication rate (over four) was observed when the medium contained 1 μM BA and 1.5 μM GA_3 (Fig. 2). *Rosa dumalis* and *R. agrestis* shoots multiplied the best on the previously mentioned medium (with a multiplication rate of two to three), although the multiplication level was lower than in *R. canina* and *R. rubiginosa.* Halmagi and Pinker (2006) multiplied roses from the 'Kardinal', 'Fairy' and 'Maidy' cultivars for cryopreservation of apical buds on an identical medium. The weakest multiplication was observed for *R. tomentosa* independently of the medium used (Figs 1 and 2). In some plants, GA_3 alone can induce adventitious shoot formation or can be a replacement for auxin in the induction of shoot formation (George et al. 2008).

The present study revealed that the use of a combination of BA and GA_3 in a multiplication medium can significantly increase the shoot

propagation rate of some wild roses and makes the micropropagation of this plant more efficient.

CONCLUSIONS

1. The best results concerning shoot multiplication in roses naturally occurring in Poland were obtained using an MS medium supplemented with cytokinin BA and gibberellin GA_3 as compared with media containing only BA.
2. The greatest shoot multiplication rate (over 4 shoots per one explant in a five-week cycle) was observed for *Rosa canina* and *R. rubiginosa* on a medium containing 1 μM BA + 1.5 μM GA_3. *R. agrestis* and *R. dumalis* multiplication was also the best on this medium, though the multiplication level was lower (1.9-2.9).
3. *Rosa tomentosa* multiplication was the weakest independent of the medium used and this species requires a separate study.

REFERENCES

Barna K.S., Wakhlu A.K., 1995. Effects of thidiazuron on micropropagation of rose. In Vitro Cell. Dev. Biol. 31: 44-46.

Bhardwaj R., Singh S.K., Pal S., Kumar S., 2006. An improved protocol for micro-propagation of miniature rose (*Rosa chinensis* Jacq. var. minima) cultivars. J. Ornam. Hort. 9(4): 238-242.

Bhoomsiri Ch., Masomboon N., 2003. Multiple Shoot Induction and Plant Regeneration of *Rosa damascena* Mill. Silpakorn University International Journal 3(12): 230-237.

Canli F.A., Kazaz S., 2009. Biotechnology of roses: Progress and future prospects: 167-183.

De Vries D.P., Dubois L.A.M., 1996. Rose Breeding: past, present, prospect. Acta Hort. 424: 241-248.

Engelmann F., Engels J.M.M., 2002. Managing plant genetic diversity. Engels J.M.M., Ramanatha Rao V., Brown A.H.D., Jackson M.T. (eds), Wallingford and Rome, CAB International and IPGRI: 89-104.

George E.F., Hall M.A., De Klerk G.J., 2008. Plant propagation by tissue culture. Vol. 1. The background. Springer.

Halmagyi A., Pinker I., 2006. Plant regeneration from *Rosa* shoot tips cryopreserved by a combined droplet verification method. Plant Cell Tiss. Org. Cult. 84: 145-153.

Hameed N., Shabbir A., Ali A., Bajwa R., 2006. *In vitro* micropropagation of disease free rose (*Rosa indica* L.). Mycopath 4(2): 35-38.

Khosh-Khui M., Teixeira da Silva J.A., 2006. In vitro culture of the Rosa species. Floriculture, Ornamental and Plant Biotechnology Vol. II. Global Science Books, UK.

Kornova K., Michailova J., 2008. Optimizing the rooting process in propagation of Kazanlak oil-bearing rose (*Rosa damascena* Mill.) *in vitro*. Propag. Ornam. Plants 8(4): 224-229.

Murali R., Sindhu K., 2011. In vitro multiplication of rose (*Rosa bourboniana*). Int. J. Curr. Res. 3(4): 100-103.

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

Nak-Udom N., Kanchanapoom K., Kanchanapoom K., 2009. Micropropagation from cultured nodal explants of rose (*Rosa hybrida* L. cv. 'Perfume Delight'). Songklanakarin Journal of Science and Technology 31(6): 583-586.

Oo K.T., Khai A.A., Lwin K.M., 2008. Micropropagation of rose (*hybrid Rosa* spp.) by in vitro culture technigue. GMSARN International Conference on Sustainable Development: Issues and Prospects for the GMS: 12-14.

Pawłowska B., Bach A., Popek R., 2009. Wykorzystanie kultur *in vitro* rodzimych gatunków róż do ochrony zasobów genowych. Zesz. Probl. Post. Nauk Roln. 534: 199-206.

Pati P.K., Sharma M., Ahuja P.S., 2001. Micropropagation, protoplast culture and its implications in the improvement of scented rose. Acta Hort. 547: 147-148.

Pati P.K., Rath S.P., Sharma M., Sood A., Ahuja P.S., 2006. In vitro propagation of rose - a review. Biotechnol. Adv. 24: 94-114.

Rout G.R., Mohapatra A., Mohan Jain S., 2006. Tissue culture of ornamental pot plant: A critical review on present scenario and future prospects. Biotechnol. Adv. 24: 531-560.

Roy P.K., Mamun A.N.K., Golam A., 2004. In vitro plantlets regeneration of rose. Plant Tissue Cult. 14(2): 149-154.

WPŁYW BA I GA_3 NA NAMNAŻANIE PĘDÓW RÓŻ DZIKOROSNĄCYCH W KULTURACH *IN VITRO*

Streszczenie: Badaniami objęto pięć gatunków róż rosnących dziko w Polsce: *Rosa agrestis* (róża polna), *R. canina* (róża dzika), *R. dumalis* (róża sina), *R. rubiginosa* (róża rdzawa), *R. tomentosa* (róża kutnerowata), znajdujących się w kolekcji *in vitro* Katedry Roślin Ozdobnych Uniwersytetu Rolniczego w Krakowie. Badano wpływ cytokininy BA (1-10 μM) w pożywce MS (Murashige i Skroog 1962) na namnażanie pędów bocznych. Druga grupa testowanych pożywek zawierała BA (1-5 μM) oraz giberelinę GA_3 (0,3-1,5 μM). Kultury prowadzono w fitotronie, w temperaturze 23/25°C (noc/dzień), wilgotności względnej 80%, przy

16-godzinnym dniu i natężeniu PPFD 30 µmol m^{-2} s^{-1} i przenoszono na świeże pożywki co 5 tygodni.

Najwyższy współczynnik namnażania uzyskano dla róży dzikiej (*R. canina*) i rdzawej (*R. rubiginosa*) (4,1 pędów na jeden eksplantat) oraz sinej (2,9 sztuk), gdy pędy były namnażane na pożywce MS zawierającej 1 µM BA i 1,5 µM GA_3. Najsłabiej, bez względu na użytą pożywkę, namnażała się róża kutnerowata (*Rosa tomentosa*).

Received May 26, 2011; accepted December 12, 2011