POLISH SOCIETY FOR HORTICULTURAL SCIENCE

Folia Hort. 30(2), 2018, 259-267

DOI: 10.2478/fhort-2018-0022

FOLIA HORTICULTURAE

Published by the Polish Society for Horticultural Science since 1989

ORIGINAL ARTICLE | Open access | www.foliahort.ogr.ur.krakow.pl

In vitro propagation of *Rosa* 'Konstancin' (*R. rugosa* × *R. beggeriana*), a plant with high nutritional and pro-health value

Agnieszka Wojtania, Bożena Matysiak*

Department of Applied Biology
Research Institute of Horticulture
Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland

ABSTRACT

The aim of the study was to develop an efficient micropropagation system for *Rosa* 'Konstancin', an interspecific hybrid between *R. rugosa* and *R. beggeriana*, whose fruits have high pro-health value. Shoot cultures were initiated from shoot buds collected in May and August from 15-year-old field-grown *Rosa* 'Konstancin' shrubs. The effect and interaction of different concentrations of phytohormones, sucrose and iron sources on *in vitro* initiation, multiplication and rooting of shoots were studied.

The time of collecting explants from donor plants significantly affected the initiation of shoot culture of *Rosa* 'Konstancin'. Considerably higher frequency of bud break (100%) was obtained in explants isolated in August as compared to those collected at the end of May (30%). All buds developed into single shoots after 2-4 weeks of growing on the basal Murashige and Skoog medium containing 2.2 µM BAP, 0.3 µM GA_3 and 88 mM of sucrose. The highest multiplication rate (4.8 shoots/explant) in a 5-week period was obtained on MS medium containing 50% of nitrogen salts, 3.1 µM BAP, 0.9 µM GA_3 and 58 mM sucrose. High rooting frequency (100%) and quality of rooted plantlets was obtained on a medium containing 0.5 µM IBA, 138 µM Fe-EDDHA and 88 mM sucrose. Fe-EDDHA had a beneficial effect on the growth and photosynthetic activity of *Rosa* 'Konstancin' plantlets, which were successfully acclimatized *ex vitro*, with a more than 90% survival rate.

Keywords: fruit rose, Fe-EDDHA, micropropagation, photosynthetic activity, sucrose

INTRODUCTION

The genus *Rosa* L. (Rosaceae) contains approximately 150 species, from which 40 wild species are present in Europe (MacPhail and Kevan, 2009). Roses are some of the most important commercial crops in ornamental horticulture. They are highly popular as ornamental garden plants, cut flowers and potted plants. Roses have also been cultivated since antiquity as medicinal plants. The rosehips contain large amounts of pharmacologically active compounds such as organic acids, flavonoids, carotenoids and tannins (Adamczak et al., 2012). Although the high value of rosehips has been recognized throughout the centuries, it is only recently that the wild roses are being domesticated and cultivated for their fruits, and to develop agronomic practices. This is due to an improved understanding of the important role of dietary fruit in enhancing human health and reducing the risk of diseases (Uggla, 2004; Fofana et al., 2013). One of the problematic issues for the

*Corresponding author.
e-mail: agnieszka.wojtania@inhort.pl (A. Wojtania).

sciendo

establishment of commercial rosehip plantations is plant material availability for large acreages.

Rosa 'Konstancin' is an interspecific hybrid between *R. rugosa* and *R. beggeriana*, introduced by J. Milewski in Poland (Milewski, 1974). It has been demonstrated that the fruits of this hybrid contain relatively high levels of ascorbic acid (3000 -3500 mg 100 g^{-1} fresh mass) and carotenoids. The hips of *Rosa* 'Konstancin' also have ornamental value (Fig. 1). Despite all these features, *Rosa* 'Konstancin' plants are not cultivated on a large scale in Poland. A few plants can be found on private

Figure 1. The hips of *Rosa* 'Konstancin'

plantations and in the germplasm collection at the Research Institute of Horticulture in Skierniewice. A low rooting ability of stem cuttings, especially of those collected from mature donor plants, is a major limiting factor in the conventional propagation of *Rosa* 'Konstancin' and its implementation into horticultural production (personal communication by B. Matysiak).

In vitro propagation of rose has played a very important role in the rapid multiplication of species and cultivars with desirable traits and in the production of healthy and disease-free plants (Previati et al., 2008). Development of *in vitro* propagation methods combined with cryopreservation is very useful in gene banks for rose germplasm protection (Pawłowska and Szewczyk-Taranek, 2014, 2015; Kwaśniewska et al., 2017). So far, there has been no information on the micropropagation of *Rosa* 'Konstancin' (*R. rugosa* × *R. beggeriana*). *In vitro* propagation methods have been demonstrated for other fruit-bearing rose genotypes, including *R. pomifera* 'Karpatia' (Sedlak and Paprstein, 2014; Kwaśniewska et al., 2017), *R. canina* (Kucharska et al., 2006; Pawłowska, 2011; Pahnekolayi et al., 2014; Ambros et al., 2016), *R. beggeriana* (Pahnekolayi et al., 2014), *R. multiflora* (Kucharska et al., 2006) and *R. rugosa* (Xing et al., 2010).

Various factors have been found to influence the morphogenesis of rose *in vitro*, including salt concentration of the medium, type and combination of plant growth regulators, carbon source and gelling agent (Bressan et al., 1982; Rout et al., 1999; Pati et al., 2006). A number of papers have reported that the decisive factor for effective micropropagation of rose is the genotype. The high heterozygosity and polyploidy of roses hinder and limit their propagation and necessitate studies on each genotype (Kucharska et al., 2006; Pawłowska, 2011; Kwaśniewska and Pawłowska, 2017).

The aim of the study was to develop an efficient micropropagation system for *Rosa* 'Konstancin' using axillary buds of mature 15-year-old plants. The effects and interaction of different concentrations of phytohormones, sucrose and iron sources on *in vitro* initiation, multiplication and rooting of shoots, as well as the photosynthetic performance of plants, assessed by the chlorophyll fluorescence method, were studied. Physiological parameters at subcellular levels, such as chlorophyll fluorescence, are widely proposed as a useful indicator of plant quality of *in vitro* developed plants (Gago et al., 2014).

MATERIAL AND METHODS

For culture initiation, actively growing shoots (15-20 cm long) of *Rosa* 'Konstancin' were collected in May and August from 15-year-old field-grown shrubs in the germplasm collection at the Research Institute of Horticulture in Skierniewice. After removing all expanded leaves, the shoots were cut into 1.5-2 cm long segments. The shoot explants (shoot tips and internodes with axillary buds) were first washed in running tap water and then in a 3% Chloramine T solution (with a few drops of detergent), rinsed with sterile distilled water, soaked in 0.1% $HgCl_2$ for 5 min. and then rinsed once with sterile water. These surface-sterilized shoot segments were trimmed at the edges to remove the exposed brown dead tissues, and then the explants were placed on the shoot induction medium.

The Murashige and Skoog (1962) medium (MS) with the nitrogen salts reduced to half strength were used as the basal medium. All culture media included 100 mg L^{-1} *myo*-inositol, vitamins – nicotinic acid, pyridoxine and thiamine (1.0 mg L^{-1} each), 2 mg L^{-1} glycine, and 10 mg L^{-1} ascorbic acid. The pH of the medium was adjusted to 5.6 before adding the gelling agents, a mixture of 3 g L^{-1} agar (Biocorp, Poland) and 1.2 g L^{-1} gelrite (Duchefa, Netherlands). Our previous study had shown that

the mixture of gelling agents had a better influence on the growth of *Rosa* 'Konstancin' shoots than agar or gelrite used singly (data not shown). The shoots were subcultured on a fresh medium every 5 weeks. All cultures were kept at 23 ± 2ºC under a 16/8 h day/night photoperiod provided by cool-white fluorescent lamps at 40 µmol m^{-2} s^{-1} (Philips TLD 36W/95).

For shoot initiation, the explants were placed in 50 ml Erlenmeyer flasks (one shoot per flask) on MS medium supplemented with 2.2 µM benzylaminopurine (BAP), 0.3 µM gibberellic acid (GA_3) and 88 mM sucrose. After 5 weeks of culture, the survival rate and the number of developed shoots were determined. The experiment was conducted twice. After 3 subcultures (each lasting 5 weeks), the single shoots were subjected to multiplication experiments.

To obtain effective shoot multiplication, the single shoots were cultured on the basal medium containing different concentrations of BAP (2.2 or 3.1 µM) alone or combined with GA_3 (0.3, 0.9 or 1.4 µM) (Experiment 1). In Experiment 2, the shoots were cultured on media containing 3.1 µM BAP and 0.9 µM GA_3. The effects of iron sources – ethylenediaminetetraacetic acid (Fe-EDTA) or ethylenediamine di-2-hydroxy-phenylacetic acid (Fe-EDDHA), both at a concentration of 138 µM, and of sucrose concentrations of 58, 73 and 88 mM were examined. After 5 weeks of subculture, the number and length of shoots as well as the number of leaves were determined.

For rooting, shoots longer than 2 cm were cultured on the basal MS medium containing different concentrations of sucrose (88 or 117 mM), without or supplemented with 0.5 µM indole-3-butyric acid (IBA). The effect of iron chelate (Fe-EDTA or Fe-EDDHA, both at a concentration of 138 µM) on the rooting ability and plantlet quality was also examined. After 4 weeks, the number of rooted shoots, the number of roots per explant, and the photosynthetic performance of plantlets, assessed by the chlorophyll fluorescence method, were determined. The rooted plantlets were transplanted into Paper-Pots (Ceres, Poland) soaked in water and placed in trays under a clear transparent plastic cover (100% air humidity). The plantlets were kept in a growth chamber (maintained at 50 µmol m^{-2} s^{-1} and 25 ± 2ºC). The plants were gradually acclimatized by opening the cover over 30 days.

Shoot multiplication and rooting were performed in 330 ml glass jars. Eight different media were tested in Experiment 1, six media in Experiment 2, and 8 media in the rooting experiment. All treatments contained 5 replications, with 6 explants each. The experiments were carried out twice. The final data were the means of two experiments. The results of the above experiments were elaborated statistically by analysis of variance, and the significance of the differences between means was evaluated by Duncan's test at $p = 0.05$.

Chlorophyll fluorescence of plantlets was determined using a Photosynthesis Yield Analyzer (MINI-PAM, Walz, Germany) equipped with a leaf clip holder. Chlorophyll fluorescence was measured on the *in vitro* developed leaves of 30 plantlets for each sucrose level and iron source treatment. Prior to measurements, the glass culture vessels were kept in darkness for 30 minutes to provide an equilibrium state for the photosynthetic electron transport, and then the maximal photochemical quantum yield of PS II in dark-adapted plantlets (Fv/Fm) was measured. The measurements were made in a darkened room to minimize ambient light. The other four parameters including Yield (effective photochemical quantum yield of PS II), qP (coefficient of photochemical fluorescence quenching), qN and NPQ (coefficient of non-photochemical fluorescence quenching) were measured with the plants exposed to actinic light according to the procedure by Murchie and Lawson (2013). Data were analyzed with two-way ANOVA and Tukey's tests were performed to evaluate the significance of differences at $p = 0.05$.

RESULTS AND DISCUSSION

Shoot initiation

Roses are propagated *in vitro* by the activation of axillary buds, which guarantees high stability of the obtained plant material (Bressan et al., 1982). On the basal MS medium supplemented with 2.2 µM BAP and 0.3 µM GA_3, shoot tips and axillary buds of *Rosa* 'Konstancin' developed into single shoots in 2-4 weeks (Fig. 2A). The efficiency of this process depended on the time of collecting explants from donor plants. A considerably higher frequency of bud break (100% of uncontaminated buds) was obtained in explants isolated in August as compared to those collected at the end of May (30%) (Tab. 1). Similar results had been obtained by Allahverdi Mamaghani et al. (2010) in 3 cultivars of *R. damascena*. In contrast, the buds of *Rosa canina* showed higher activity when they were taken from donor plants in September-October (50%) than in April-May (14.6%) and in July-August (0%)

Figure 2. The different stages of growth and development of *Rosa* 'Konstancin' plantlets. A. Axillary buds after 2 weeks of culturing on basal MS medium containing BAP and GA_3 (5.0 mm size bar). B. Multiplied shoots after a 4-week subculture period on basal MS medium supplemented with BAP, GA_3, 75 mM sucrose and Fe-EDTA (on the left) and Fe-EDDHA (on the right) (13.0 mm size bar). C. *In vitro* rooting of rose 'Konstancin' on basal MS medium supplemented with Fe-EDDHA, 88 mM sucrose and IBA (on the left) or without auxin (on the right) (10.0 mm bar). D. The plants of rose after 4-weeks of growing in the high humidity condition (15.0 mm bar)

Table 1. Effect of the time of collecting explants on the initiation of shoot cultures

Explant collection time	Total number of explants	Contaminated explants (%)	Uncontaminated explants that did not develop shoots (%)	Uncontaminated explants that developed shoots (%)
May	50	28	42	30
August	50	8.3	0	91.7

(Ambros et al., 2016). The authors explained that it may have been due to the different levels of phenolic content in the explants. It has been reported that bud break in rose depends on many other factors, including the size of explants, position of the bud on the stem, composition of basal salts, plant growth regulators and genotype (Rout et al., 1999). Murashige and Skoog's (1962) medium has been found to be the most effective in shoot induction and multiplication in different rose species and cultivars (Xing et al., 2010; Sedlak and Paprstein, 2014; Kwaśniewska and Pawłowska, 2017). The important factor stimulating the activity of rose buds was BAP added singly (Pahnekolayi et al., 2014; Sedlak and Paprstein, 2014; Kwaśniewska and Pawłowska, 2017), together with an auxin (Ambros et al., 2016; Senapati and Rout, 2008), GA_3 (Shirdel et al., 2012; Pawłowska and Szewczyk-Taranek, 2014), or NAA and GA_3 (Xing et al., 2010).

The single shoots of *Rosa* 'Konstancin' obtained on the medium supplemented with BAP and GA_3 started to form new shoots after being transferred to a fresh medium.

Effect of growth regulators, iron sources and sucrose levels on shoot multiplication

It has been reported that among the cytokinins tested, BAP has been found to be more effective than isopentenyladenine (2iP), kinetin and thidiazuron (TDZ) for axillary multiplication in different *Rosa* genotypes (Carelli and Echeverrigaray, 2002; Sedlak and Paprstein, 2014). The optimal BAP concentration ranged from 2.2 to 22.2 µM depending on the rose species and cultivar (Kucharska et al., 2006; Senapati and Rout, 2008; Xing et al., 2010). However, varied multiplication response of rose genotypes to BAP has also been reported. Kucharska et al. (2006) showed that *R. indica* 'Major' on a medium containing

6.66 µM BAP produced 6.0 shoots per explant, while *R. multiflora* only 3.4 shoots per explant. In *R. canina*, Ambros et al. (2016) obtained the highest multiplication rate (7.5 shoots/explant) using 4.44 µM BAP. Pahnekolayi et al. (2014), however, reported the best multiplication (4.2 shoots/explant) on a medium containing 8.88 µM BAP. Pawłowska (2011) did not observe significant differences in the multiplication rate of the remaining species, *R. agrestis*, *R. dumalis*, *R. tomentosa*, when the BAP concentration varied in the range of 1-10 µM.

The results of our first experiment showed that increasing BAP concentration from 2.2 to 3.1 µM had no effect on the multiplication rate of *Rosa* 'Konstancin' (Fig. 3A). The use of BAP at the concentration of 4.4 µM not only did not enhance the multiplication rate of shoots but also resulted in the hyperhydricity of the shoots (data not shown). As shown in Figure 3A, the combination of GA_3 (0.3-1.4 µM) with BAP had no significant effect on axillary shoot formation of *Rosa* 'Konstancin', but it stimulated leaf formation and growth of shoots (Fig. 3B, 3C). The presence of 0.3 µM GA_3 together with BAP was found by Valles and Boxus (1987) to prevent apical necrosis in rose cv. White Dream. In the available literature there are some reports showing that the addition of GA_3 to a BAP-containing medium significantly enhanced (Xing et al., 2010; Pawłowska, 2011) or inhibited (Pahnekolayi et al., 2014) the multiplication of rose shoots. This probably depended on the genotype, other culture components and the physiological status of the shoots.

The results of the next experiment showed that the shoot formation in *Rosa* 'Konstancin' significantly depended on the sucrose level and iron source in the medium (Fig. 4A). The highest multiplication rate (4.8 shoots/explant) was obtained on the basal MS medium supplemented with growth regulators (3.1 µM BAP + 0.9 µM GA_3) and 58 mM sucrose (Fig. 4A). In the presence of Fe-EDTA, increasing sucrose concentration from 58 to 88 mM resulted in a reduction of the multiplication rate by 46% (Fig. 4A). The sucrose-induced inhibition of shoot formation had been previously observed in other woody plant species, such as *Camellia japonica* (Wojtania et al., 2011), *Syringa vulgaris* (Gabryszewska, 2011), *Magnolia* × *soulangiana* (Wojtania et al., 2015) and *Magnolia* × 'Spectrum' (Wojtania et al., 2016). It is suggested that a high sucrose level induces bud dormancy (Chao et al. 2006). Langford and Wainwright (1987) demonstrated that decreasing sucrose concentration in the medium from 117 to 29 mM increased the photosynthetic ability of rose cv. Iceberg and Peace. As shown in Figure 4, the morphogenic response of *Rosa* 'Konstancin' to different sucrose levels differed depending on the iron source in the medium. Iron is

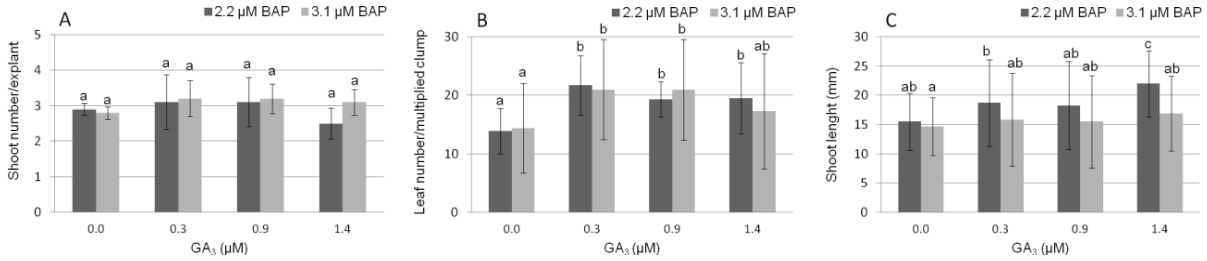

Figure 3. Effect of different BAP and GA_3 concentrations on shoot formation (A), leaf number (B) and shoot length (C) after a 5-week subculture period. Means of each parameter marked with the same letter do not differ significantly ($p = 0.05$) according to Duncan's test

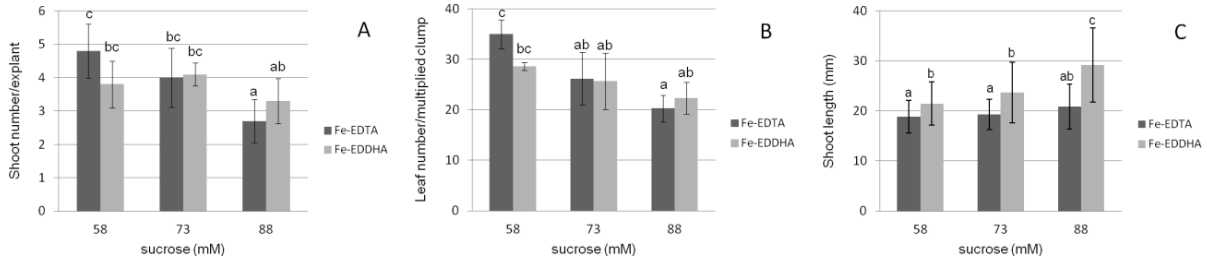

Figure 4. Effect of sucrose levels and iron sources (EDTA or EDDHA), added to basal MS medium containing 3.1 µM BAP and 0.9 µM GA_3, on shoot formation (A), leaf number (B) and shoot length (C) after a 5-week subculture period. Means of each parameter marked with the same letter do not differ significantly ($p = 0.05$) according to Duncan's test

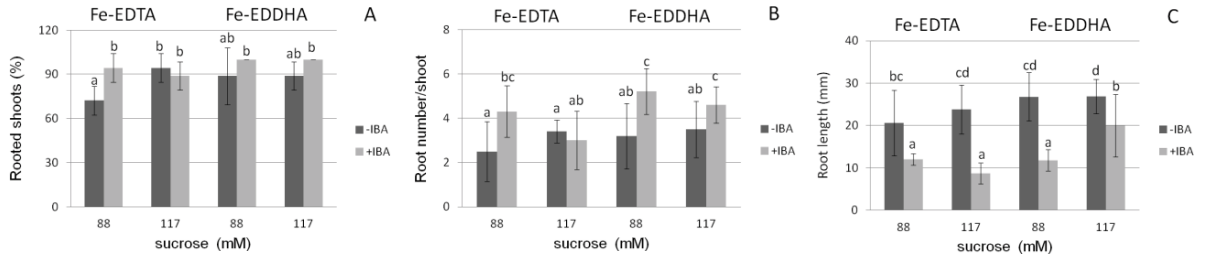

Figure 5. Effect of sucrose levels and iron sources (EDTA or EDDHA), added to basal MS without auxin or supplemented with 0.5 µM IBA, on rooting frequency (A), root number (B) and root length (C) after a 4-week subculture period. Means of each parameter marked with the same letter do not differ significantly ($p = 0.05$) according to Duncan's test

involved in fundamental physiological processes as an enzyme cofactor in photosynthesis, respiration, nitrogen fixation, synthesis of DNA, lipids and growth regulators (Dalton et al., 1983). Fe-EDTA has been the more widely used chelator *in vitro* in many plant species, including rose. However, Fe-EDTA photooxidizes at pH of 5.7 and quickly forms insoluble ferric oxide, which is unavailable to plant tissue (Dalton et al., 1983). In a comparative study of different iron chelates, Fe-EDDHA retained more chelated Fe in solution, iron was available for longer, and plants required less energy to utilize it effectively for growth as compared to Fe-EDTA (Alvarez-Fernandez et al., 1997; Molassiotis et al., 2003). The replacement of Fe-EDTA with Fe-EDDHA had positive effects on *in vitro* shoot formation of *Carica papaya* (Castillo et al., 1997) and *Rubus* spp. (Zawadzka and Orlikowska, 2006). As in *Rosa hybrida* 'Moneyway' (Van der Salm et al., 1994) and hybrid hazelnut 'Geneva' (Garrison et al., 2013), our study showed that Fe-EDDHA form was superior to Fe-EDTA in producing longer shoots (Fig. 2B, 4C), which were easier to subculture and better for the rooting process. As shown in Figure 4C, Fe-EDDHA stimulated the growth of rose shoots in a manner dependent on sucrose concentration.

Rooting and acclimatization

Rooting of microcuttings is a critical step in micropropagation. It has been shown that root formation in different rose genotypes is enhanced by lowering the amount of macronutrients to half strength, optimizing auxin concentration, using a combination of auxins, increasing magnesium level by 50%, and application of activated charcoal, phroglucinol or cold treatment (Ma et al., 1996; Podwyszyńska, 1996; Podwyszyńska and Goszczyńska, 1998; Xing et al., 2010; Kwaśniewska et al., 2017). Podwyszyńska (2003) underlined the importance of the physiological status and quality of microcuttings, especially in recalcitrant rose cultivars. It is known that rose genotypes differ in their rooting capacity. A lower rooting frequency was recorded in old world species (*Rosa canina*, *Rosa damascena*) when compared with *Rosa hybrida* (Ginova et al., 2012). In some cases, conflicting data were presented. Sedlak and Paprstein (2014) achieved the highest rooting frequency (40%) in *Rosa pomifera* 'Karpatia' on a 50% MS medium supplemented with 5.4 µM NAA. Kwaśniewska et al. (2017) reported 97% rooting frequency of rose 'Karpatia' shoots on a 50% MS medium without any growth regulators.

For the rooting of *Rosa* 'Konstancin' shoots, we studied the effect of IBA (0 and 0.5 µM), iron sources (Fe-EDTA and Fe-EDDHA) and sucrose levels (88 and 117 mM). As shown in Fig. 5A, *Rosa* 'Konstancin' is a plant that is easy to root *in vitro.* It was found to have a high rooting frequency (90%) on a medium without the auxin (Fig. 2C, 5A). The addition of IBA at a low level had no significant effect on rooting frequency, but significantly increased root number and reduced root length (Fig. 5A, 5B, 5C). The highest rooting frequency (100%) and the greatest root formation (5.4 roots/shoot) occurred on the IBA-medium with Fe-EDDHA and 88 mM sucrose. In the presence of IBA, the higher level of sucrose (117 mM) lowered the number of roots and induced leaf senescence. The presence of Fe-EDDHA in the rooting medium had a beneficial effect on the growth and photosynthetic activity of *Rosa* 'Konstancin' plantlets.

The maximal photochemical activity Fv/Fm of *in vitro* cultures on the basal MS medium containing 88 mM sucrose and Fe-EDTA was below optimal, as indicated by the value of 0.73 (Tab. 2). For unstressed leaves, the value of Fv/Fm is highly consistent, with values of ~0.83, and correlates with the maximum quantum yield of photosynthesis (Björkman and Demmig, 1987; Maxwell and Johnson, 2000). Lowering of Fv/Fm indicates the existence of some type of stress that results in inactivation damage of PS II (photoinhibition) or the induction of sustained

Table 2. Chlorophyll fluorescence parameters in *in vitro* cultivated *Rosa* 'Konstancin'

Fe source	Sucrose level (mM)	Fv/Fm	Yield	qP	qN	NPQ
EDTA	88	0.730 a	0.049 a	0.076 a	0.321 a	0.339 a
EDTA	117	0.726 a	0.059 a	0.094 ab	0.337 a	0.367 a
EDDHA	88	0.761 b	0.076 b	0.114 bc	0.337 a	0.377 a
EDDHA	117	0.755 b	0.081 b	0.127 c	0.379 a	0.441 a

Different letters indicate significant differences among the treatments according to Tukey's test ($p = 0.05$)

quenching (Murchie and Lawson, 2013). A low photosynthetic photon flux, the presence of sugars in the medium and large diurnal fluctuations in CO_2 in *in vitro* cultures result in poor development of the photosynthetic apparatus of micropropagated plants, including roses (Capellades et al., 1991; Kozai et al., 1992; Genoud-Gourichon et al., 1996; Pospíšilová et al., 2007). For *Rosa* plantlets grown *in vitro* on the basal MS medium, the maximal quantum yield of photosynthesis (Fv/Fm) under a low irradiance level during the rooting phase was 0.8 (Sallanon et al., 1998) and 0.64-0.66 (Genoud et al., 1999).

In our study, the physiological activity of *Rosa* 'Konstancin' plantlets was improved with the supplementation with iron in the form of Fe-EDDHA rather than Fe-EDTA (Tab. 2). This might be attributed to a greater amount of iron used in the photosynthetic electron transport chain, and also to a greater enrichment in iron of the chloroplasts when Fe-EDDTA was used as iron source. The maximal photochemical activity (Fv/Fm) for plants on the medium supplemented with Fe-EDDHA was significantly higher (0.76) than in the case of supplementation with Fe-EDTA (0.73). An increase in Fv/Fm was found in both Fe-EDDHA treatments, i.e. with 88 and 117 mM sucrose levels, mainly in consequence of significant increases in the values of Fm. Yield (Y), i.e. effective photochemical activity, was 45% higher for plants growing on a medium containing Fe-EDDHA than Fe-EDTA. Also the quenching analysis showed that the supplementation with Fe-EDDHA was more favourable for converting light energy into chemical form, represented by the coefficient qP. Non-photochemical fluorescence quenching was not dependent on the Fe chelate. Sucrose is a source of carbon for plants in sterile, heterotrophic conditions inside vessels and supports the maintenance of osmotic potential and conservation of water in cells. There is some evidence that the level of sucrose in the culture medium, combined with other *in vitro* factors, can alleviate or intensify the potential photoinhibition of plantlets (Capellades et al., 1991; Serret et al., 2001; Genoud-Gourichon et al., 1996; Gago et al., 2014; Matysiak and Gabryszewska, 2016). Our results have shown that increasing the level of sucrose from 88 to 117 mM did not affect the potential efficiency of the photosynthetic apparatus of *in vitro* developed *Rosa* 'Konstancin'.

The rooted plantlets were transplanted into Paper-pots (Ceres, Poland) and kept in a growth chamber at 25 ± 2ºC, in plastic boxes with transparent covers that were subsequently lifted to reduce humidity. After 4-5 weeks of acclimatization in the growth chamber, the plantlets were successfully established under greenhouse conditions. The survival rate was at least 90%. The regenerated plantlets did not show any morphological abnormalities during an observation period of 5 months, and 100% of the plants survived after overwintering in a cold greenhouse.

CONCLUSIONS

1. Our study yielded a practical protocol for mass propagation of *Rosa* 'Konstancin' *in vitro*. It enhances the availability of plant material for the establishment of commercial rosehip plantations.
2. The results presented here indicate an important role of sucrose in the regulation of shoot formation of *Rosa* 'Konstancin' *in vitro.*
3. It was found that the morphogenic response to sucrose levels differed depending on the iron source in the medium.
4. Replacement of Fe-EDTA with Fe-EDDHA increased the length of shoots and the photosynthetic ability of rose plantlets.
5. Some modification of the culture media for *Rosa* 'Konstancin' may be useful for other rose species and cultivars.

FUNDING

This research was supported by Poland's Ministry of Science and Higher Education as part of the statutory activities of the Department of Applied

Biology, Research Institute of Horticulture in Skierniewice, Poland.

AUTHOR CONTRIBUTIONS

A.W. – concept of the study and final approval of the version to be published; A.W., B.M. – analysis and interpretation of data for publishing, photographs, writing of the manuscript.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

REFERENCES

Adamczak A., Buchwald W., Zieliński J., Mielcarek S., 2012. Flavonoid and organic acid content in rose hips (*Rosa* L., sect. *Caninae* DC. EM. Christ.). Acta Biol. Cracov. Bot. 54, 105-112.

Alvarez-Fernandez A., Garate A., Lucena J.J., 1997. Interaction of iron chelates with several soil materials and with a soil standard. J. Plant Nutr. 20, 559-572.

Ambros E.V., Vasilyeva O.YR., Novikova T., 2016. Effects of *in vitro* propagation on ontogeny of *Rosa canina* L. micropropagated plants as a promising rootstock for ornamental roses. Plant Cell Biotechnol. Mol. Biol. 17, 72-78.

Björkman O., Demmig B., 1987. Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. Planta 170, 489-504.

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

Capellades M., Lemeur R., Debergh P., 1991. Effect of sucrose on starch accumulation and rate of photosynthesis in *Rosa* cultured *in vitro*. Plant Cell Tiss. Organ Cult. 25, 21-26.

Carelli B.P., Echeverrigaray S., 2002. An improved system for the *in vitro* propagation of rose cultivars. Sci. Hortic. 92, 69-74.

Castillo B., Smith M.A.L., Madhavi D.L., Yadava U.L., 1997. Interaction of irradiance level and iron chelate source during shoot tip culture of *Carica papaya* L. HortScience 32, 1120-1123.

Chao W.S., Serpe M.D., Anderson J.V., Gesch R.W., Horvath D.P., 2006. Sugars, hormones, and environment affect the dormancy status in underground buds of leafy spurge (*Euphorbia esula*). Weed Sci. 54, 59-68.

Dalton C.C., Iqbal K., Turner D.A., 1983. Iron phosphate precipitation in Murashige and Skoog media. Physiol. Plant. 57, 472-476.

Fofana B., Ghose K., Chapman B., Sanderson K., 2013. Genetic, agronomy, and metabolomics of Prince Edwards Island wild rose collection and promise for cultivar development. In: Using Old Solutions to New Problems. Natural Drug Discovery in the 21st Century. M. Kulka (Ed.), IntechOpen Limited, London, UK, http://dx.doi.org/10.5772/54688.

Gago J., Martinez-Nunez L., Landin M., Flexas J., Gallego P.P., 2014. Modeling the effects of light and sucrose on *in vitro* propagated plants: a multiscale system analysis using artificial intelligence technology. PLoS ONE 9, e85989.

Gabryszewska E., 2011. Effect of various levels of sucrose, nitrogen salts and temperature on the growth and development of *Syringa vulgaris* L. shoots *in vitro*. J. Fruit Ornam. Plant Res. 19, 133-148.

Garrison W., Dale A., Saxena P.K., 2013. Improved shoot multiplication and development in hybrid hazelnut nodal cultures by ethylenediamine di-2-hydroxy-phenylacetic acid (Fe-EDDHA). Can. J. Plant Sci. 93, 511-521.

Genoud C., Coudret A., Amalric C., Sallanon H., 1999. Effect of micropropagation conditions of rose shootlets on chlorophyll fluorescence. Photosynthetica 36, 243-251.

Genoud-Gourichon C., Sallanon H., Coudret A., 1996. Effects of sucrose, agar, irradiance and CO_2 concentration during the rooting phase on the acclimation of *Rosa hybrida* plantlets to *ex vitro* conditions. Photosynthetica 32, 263-270.

Ginova A., Tsvetkov I., Kondakova V., 2012. *Rosa damascena* Mill. – an overview for evaluation of propagation methods. Bulg. J. Agric. Sci. 18, 545-556.

Kozai T, Fujiwara K, Hayashi M, Aitken-Christie J., 1992. The *in vitro* environment and its control in micropropagation. In: Transplant Production Systems. K. Kurata and T. Kozai (Eds), Springer, Dordrecht, Netherlands, 247-282.

Kucharska D., Gruchała A., Orlikowska T., 2006. *In vitro* propagation of four rose rootstocks. Propag. Ornam. Plants 6, 44-50.

Kwaśniewska E., Pawłowska B., 2017. Efficient *in vitro* propagation of historical roses for biodiversity conservation. Propag. Ornam. Plants 17, 3-11.

Kwaśniewska E., Dziedzic E., Pawłowska B., 2017. Integration of cryopreservation and tissue culture for germplasm conservation and propagation of *Rosa pomifera* 'Karpatia'. Not. Bot. Horti. Agrobot. Cluj Napoca 45, 208-214.

Langford P.J., Wainwright H., 1987. Effects of sucrose concentration on the photosynthetic ability of rose shoots *in vitro*. Ann. Bot. 60, 633-640.

Ma Y., Byrne D.H., Chen J., 1996. Propagation of rose species *in vitro*. In Vitro Cell. Dev. Biol.-Plant 32, 103-108.

MacPhail V.J, Kevan P.G., 2009. Review of breeding systems of wild roses (*Rosa spp*.). Floricult. Ornam. Biotechnol. 3, 1-13.

Mamaghani A.B., Ghorbani M., Assareh M.H., Ghamari Zare A., 2010. *In vitro* propagation of three damask roses accessions. Iran. J. Plant Physiol. 1, 85-94.

Matysiak N., Gabryszewska E., 2016. The effect of *in vitro* culture conditions on the pattern of maximum photochemical efficiency of photosystem II during acclimatisation of *Helleborus niger* plantlets to *ex vitro* conditions. Plant Cell Tiss. Organ Cult. 125, 585-593.

Maxwell K., Johnson G. N., 2000. Chlorophyll fluorescence – a practical guide. J. Exp. Bot. 51, 659-668.

Milewski J.C., 1974. Hybrydyzacja róż owocodajnych w celu uzyskania wysokiej zawartości witaminy C w owocniach. Prace Instytutu Badawczego Leśnictwa, 474, 127-172.

Molassiotis A.N., Dimassi K., Therios I., Diamantidis G., 2003. Fe-EDDHA promotes rooting of rootstock GF-677 (*Prunus amygdalus* × *P. persica*) explants *in vitro*. Biol. Plant. 47, 141-144.

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

Murchie E.H., Lawson T., 2013. Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. J. Exp. Bot. 64, 3983-3998.

Pahnekolayi M.D., Tehranifar A., Samiei L., Shoor M., 2014. Micropropagation of *Rosa canina* through axillary shoot proliferation. J. Ornam. Plants 1, 45-51.

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.

Pawłowska B., 2011. The effect of BAP and GA_3 on the shoot multiplication of *in vitro* cultures of Polish wild roses. Folia Hort. 23(2), 145-149.

Pawłowska B., Szewczyk-Taranek B., 2014. Droplet vitrification cryopreservation of *Rosa canina* and *Rosa rubiginosa* using shoot tips from *in situ* plants. Sci. Hortic. 168, 151-156.

Pawłowska B., Szewczyk-Taranek B., 2015. Efficient cryopreservation by droplet vitrification of pentaploid roses and the phenotype of regenerated plants. Acta Soc. Bot. Pol. 84, 439-442.

Pospíšilová J., Synková H., Haisel D., Semorád Š., 2007. Acclimation of plantlets to *ex vitro* conditions: effects of air humidity, irradiance, CO_2 concentration and abscisic acid (a review). Acta Hortic. 748, 29-38.

Previati A., Benelli C., Da Re F., Ozudogru A., Lambardi M., 2008. Micropropagation and *in vitro* conservation of virus-free rose germplasm. Propag. Ornam. Plants 8, 93-98.

Podwyszyńska M., 1996. Practical method for overcoming shoot senescence and difficulties with rooting of rose shoots propagated *in vitro*. Acta Hortic. 424, 363-365.

Podwyszyńska M., Goszczyńska D., 1998. Effect of inhibitors of ethylene biosynthesis and action, as well as calcium and magnesium on rose shoot rooting, shoot-tip necrosis and leaf senescence *in vitro*. Acta Physiol. Plant. 20, 91-98.

Podwyszyńska M., 2003. Rooting of micropropagated shoots. In: Encyclopedia of Rose Science. A.V. Roberts, T. Debener, and S. Gudin (Eds), Academic Press, Vol. 1, 66-76.

Rout G.R., Samantaray S., Mottley J., Das P., 1999. Biotechnology of rose: a review of recent progress. Sci. Hortic. 81, 201-228.

Sallanon H., Berger M., Genoud C., Coudret A., 1998. Water stress and photoinhibition in acclimatization of *Rosa hybrida* plantlets. In Vitro Cell. Dev. Biol. Plant 34, 169-172.

Sedlak J., Paprstein F., 2014. Micropropagation of *Rosa pomifera*. Acta Hortic. 1048, 215-220.

Senapati S.K., Rout G.R., 2008. Study of culture conditions for improved micropropagation of hybrid rose. Hort. Sci. (Prague) 35, 27-35.

Serret M.D, Trillas M.I., Araus J.L., 2001. The effect of *in vitro* culture conditions on the pattern of photoinhibition during acclimation of gardenia plantlets to *ex vitro* conditions. Photosynthetica 39, 67-73.

Shirdel M., Motallebi-Azar A., Mahna N., 2012. *In vitro* micropropagation of dog rose (*Rosa canina* L.). Acta Hortic. 937, 911-913.

Uggla M., 2004. Domestication of wild roses for fruit production. Doctoral thesis. ISSN 1401-6249, ISBN 91-576-6751-9.

Valles M., Boxus P.H., 1987. Regeneration from *Rosa* callus. Acta Hortic. 212, 691-696.

Van der Salm T.P.M., Van der Toorn C.J.G., Hänisch ten Cate C.H., Dubois L.A.M., De Vries D.P., Dons H.J.M., 1994. Importance of the iron chelate formula for micropropagation of *Rosa hybrida* L. 'Moneyway'. Plant Cell Tiss. Organ Cult. 37, 73-77.

Wojtania A., Gabryszewska E., Podwyszyńska M., 2011. The effect of growth regulators and sucrose concentration on *in vitro* propagation of *Camellia japonica* L. Propag. Ornam. Plants 11, 177-183.

Wojtania A., Skrzypek E., Gabryszewska E., 2015. Effect of cytokinin, sucrose and nitrogen salts concentrations on the growth and development and phenolics content in *Magnolia* × *soulangiana* 'Coates' shoots *in vitro*. Acta Sci. Pol. Hortorum Cultus 14, 51-62.

Wojtania A., Skrzypek E., Gabryszewska E., 2016. Morphological and biochemical responses to gibberellic acid in *Magnolia* × 'Spectrum' *in vitro*. Acta Biol. Cracov. Bot. 58, 103-111.

Zawadzka M., Orlikowska T., 2006. The influence of FeEDDHA in red raspberry cultures during shoot multiplication and adventitious regeneration from leaf explants. Plant Cell Tiss. Organ Cult. 85, 145-149.

Xing W., Bao M., Qin H., Ning G., 2010. Micropropagation of *Rosa rugosa* through axillary shoot proliferation. Acta Biol. Cracov. Bot. 52, 69-75.

Received August 31, 2017; accepted February 22, 2018