https://doi.org/10.17221/239/2017-HORTSCI

Cryopreservation of Caninae rose shoot tips guarantees biochemical stability and pollination potential monitored in four-year-old regenerants

Bożena Pawłowska[1], Jarmila Neugebauerová[2], Monika Bieniasz[3], Bożena Szewczyk-Taranek[1], Ivo Ondrasek[4]*

[1]*Department of Ornamental Plants, University of Agriculture in Krakow, Kraków, Poland*
[2]*Department of Vegetable Growing and Floriculture, Mendel University in Brno, Lednice, Czech Republic*
[3]*Department of Pomology and Apiculture, University of Agriculture in Krakow, Kraków, Poland*
[4]*Department of Fruit Growing, Mendel University in Brno, Lednice, Czech Republic*

**Corresponding author: ropawlow@cyf-kr.edu.pl*

Citation: Pawłowska B., Neugebauerová J., Bieniasz M., Szewczyk-Taranek B., Ondrášek I. (2019): Cryopreservation of Caninae rose shoot tips guarantees biochemical stability and pollination potential monitored in four-year-old regenerants. Hort. Sci. (Prague), 46: 90–97.

Abstract: Stability of *Rosa agrestis*, *R. canina*, *R. dumalis* and *R. rubiginosa* regenerants after shoot tip cryopreservation was monitored with the use of biochemical markers and pollen quality assessment in matured plants cultivated in the field. The results of rosa pollen and hip evaluation revealed no significant differences between post-cryopreserved regenerants and control plants; however, variations in the assessed markers were noted between *Rosa* species. Analyses of hips confirmed high levels of vitamin C in these plants; the highest was observed in *R. dumalis* (750–870 mg/100 g fresh weight). High total antioxidant activity (TAC) was as follows: DPPH – 50–250 mM TE/100 dry weight, FRAP – 80–180 mM TE/100 g dry weight. The present study showed a high content of flavonoids (TFC) and phenols (TPC). Post-cryopreserved plants retained pollen specific for Caninae: numerous anthers, abundant pollen, poor pollen germination and viability and typical pollen morphology. This study demonstrates new tools for evaluating the fidelity of post-cryopreserved regenerants.

Keywords: hypanthia; pollen; TAC; TFC; TPC; vitamin C

The genus *Rosa* contains approximately 100 species that are widely distributed in Europe, Asia, the Middle East, and North America. Roses from the section Caninae are one of the most important among wild grown species in Europe. Rosehips (hypantia) have traditionally been used for food and medicinal purposes in all Carpathians and Balkan countries, consumed fresh or as variously processed products (Quave, Pieroni 2015; Stanila et al. 2015). Recently, fruits of wild roses have been the focus of several studies due to their human nutraceutical properties and beneficial health effects, resulting from the abundance of fruit extracted compounds, such as polyphenols (flavonoids, anthocyanins), carotenoids, vitamin C, vitamin E and their immense antioxidative potential. The clinical efficacy of rose hip properties has been utilized in many pharmaceuticals and cosmetics, having a great impact on novel European foods, diet supplements and drug trade (Roman et al. 2013; Nybom, Werlemark 2017).

There is a large genetic biodiversity among *Rosa* species from Caninae, which consists mainly of pentaploids (characterized by specific meiosis) with uneven chromosomal contribution from both parents. Those species are self-fertile, sometimes apomictic, they have reduced pollen viability and germination (Nybom et al. 2005). Pollen quality is usually very low in the section Caninae, as the percentage of empty pollen grains varies from 30

https://doi.org/10.17221/239/2017-HORTSCI

to 70%. Pollen grain image analysis with the use of SEM methods is very well documented and provides useful diagnostic criteria for taxonomy and sectional level of wild roses (Ercisli 2007a; Wrońska-Pilarek 2011; Fatemi et al. 2012).

The intervention of biotechnological tools will be beneficial for the conservation of roses, as the natural source of important health-promoting substances. For long-term preservation, cryogenic storage is the only method available. Maintaining genetic stability of plants regenerated after cryopreservation is important to ensure sustainable use of conserved resources in the future. Biochemical markers are one of the scientifically validated tools applied to prove the fidelity and integrity of regenerants after cryopreservation. Usually, secondary metabolites are of crucial importance for medicinal plants. In post-cryopreserved regenerants the concentrations of proline, carotenoids and hypericin were determined in *Hypericum perforatum* (Skyba et al. 2010), and the accumulation of malondialdehyde (MDA), free radicals (ROS), phenols and flavonoids in *Hypericum rumeriacum* (Yordanova et al. 2011), while 35 different biochemical components in *Chrysanthemum morifolium* (Bi et al. 2016) and bacoside A content in *Bacopa moninieri* (Sharma et al. 2017). It is also important to evaluate field performance and plant functions, but up to now, studies on these topics are rather limited. There are works on papaya clones (Kaity et al. 2009, 2013) and *Argyranthemum* in the reproductive growth phase (Zhang et al. 2015). Those observations did not prove any adverse effects on morphology and plant biochemical activity in regenerants.

We have previously reported a successful and efficient droplet vitrification cryopreservation of *Rosa agrestis*, *R. canina*, *R. dumalis* and *R. rubiginosa*, and assessed the morphological markers of regenerants *in vitro*. After shoot tip cryopreservation, 95–100% of axillary shoots were propagated, rooted and successfully acclimatised. Phenotype biometric examination during the *in vitro* growth stage did not reveal any morphological abnormalities compared with non-cryopreserved plants (Pawłowska, Szewczyk-Taranek 2015). The observations described here are the last step of integrated study scheme of complete cryopreservation procedure, describing the quality of matured, four-year-old roses after shoot tips cryopreservation in field conditions. The present study was, therefore, conducted to assess the stability and confirm "true to type" of regenerants from cryopreserved shoot tips with the use of biochemical markers and pollen quality characteristics. In order to implement the complete *Rosa* cryopreservation procedure to gene banks, long-term monitoring of regenerants has to be accomplished.

MATERIAL AND METHODS

Plant material. Pollen and hypanthia from field cultivation of rose regenerants after shoot tip cryopreservation were analyzed. The study covered four species of wild roses from the section Caninae: fieldbriar rose *Rosa agrestis* Savi, dog rose *R. canina* L., glaucous dog rose *R. dumalis* Bechst. and sweetbriar rose *R. rubiginosa* L. Shoots regenerated from *in vitro* culture without cryopreservation were designated as the controls. Shoot tips of roses that survived droplet vitrification cryopreservation were subsequently subject to *in vitro* propagation, rooting and acclimation (Pawłowska, Szewczyk-Taranek 2015). Microplants were potted in pots (∅ 10.5 cm) filled with TS2 substrate (Klasmann-Deilmann) at pH 6.0, mixed with sand in a 3 : 1 ratio, and cultivated in the greenhouse for 12 months (April 2012 to April 2013), 80 plants of each combination. Plants were overwintered in a non-heated polyethylene greenhouse. In the springtime, roses were transplanted to the field (Fig. 1a) at the Experimental Station of the Department of Pomology and Apiculture in Garlica Murowana (GPS 50.140, 19.930). Twelve plants were randomly selected out of the greenhouse plot from each combination (Fig. 1b). Rose bushes did not bloom in the first year of cultivation (Fig. 1c); in the second year, there were few flowers observed. Roses (Fig. 1d) reached full flowering and fruiting potential in the third year of field cultivation, and this material was collected for analysis (Fig. 1e, f).

Standard agronomic practices were followed for raising the crop: spring and summer weeding and chemical protection for mildew (0.05% Domark 100EC in May, 0.15% Tiofan 500SC in June). The experiment concerning the field performance was set up based on a completely random design.

Pollen characterization. Ten flowers (stage of bursting bud) were collected from each plant on a rainless day, at the end of May, and the number of anthers per flower was counted. The anthers were cut off from the filament and air dried for 12 h in

https://doi.org/10.17221/239/2017-HORTSCI

Fig. 1. Roses regenerants after shoot tips cryopreservation: (a) container cultivation in greenhouse; (b) establishment of openfield trial; (c) 1st year of field cultivation; (d) 3rd year of cultivation, plant material for assessment regenerants fidelity; *Rosa dumalis* samples collected for experiments: (e) flowers; and (f) hips.

open Eppendorf at 23°C. The numbers of pollen grains per anther were determined using a hemocytometer in six replicates (Bieniasz et al. 2017). The pollen viability was assessed according to the Alexander (1969) method. Pollen germination capacity was determined *in vitro* on 0.6% agar medium and 15% sucrose. Fresh pollen was dusted directly on the medium in sterile Petri dishes and incubated at 24°C for 24 h to stimulate germination. The germination criterion was a well-formed pollen tube, which was three times longer than the pollen diameter. All observations were made in 5 replicates, each 200 grains and the experiment was repeated twice. Observations were made using electronic imaging (LM microscope Carl Zeiss Image M2 AXIO, Axio Vision 2.0).

For scanning electron microscopy (SEM), the sampled air dry pollen grains were mounted on stubs, 12.5 mm in diameter, and then sputtered with approximately 25 nm of chrome. Specimens were examined and photographed with a PhenomWorld PRO scanning electron microscope.

Rose hip phytochemical composition. Fruits (100 per combination) were collected according to the uniformity of shape and colour. Achenes were discarded, and fruit flesh was used for vitamin C analysis, while the remaining were stored in polyethene bag at –20°C for 30 days until analyses (total antioxidant capacity – TAC, total flavonoids content – TFC and total content of phenols – TPC).

The concentration of ascorbic acid was determined by high-performance liquid chromatography (HPLC), according to Arya et al. (2000). Cleaned fresh hips of rose species were homogenized. For analyses, 5 g of the mixture was homogenized in a blender with 20 ml of 0.1M oxalic acid. The homogenate was filtered and topped up with oxalic acid to a volume of 100 ml. Then, 20 ml of mixture solution was centrifuged at 3,500 rpm for 10 minutes. The supernatant was filtered through a microfilter (PVDF 0.45 micrometre) and used for the measurements. The analyses were performed by RP-HPLC (ECOM, 1998) at 254 nm using a UV-VIS detector. The amount of ascorbic acid is expressed as mg/100 g fresh weight (FW).

Extracts for TAC, TFC and TPZ determination were prepared according to Shan et al. (2005). Frozen and homogenized plant material (1 g) was ex-

https://doi.org/10.17221/239/2017-HORTSCI

tracted using 75% methanol. After 24 h of extraction at room temperature, samples were filtered through filter paper (2R80; 80 ± 4 g/m^2) and stored in a refrigerator until further analysis. The total antioxidant capacity (TAC) was determined by DPPH and FRAP methods.

DPPH radical scavenging assay. The assay is based on the relative antioxidant activity to quench the radical DPPH$^+$ cation (2,2-diphenyl-picrylhydrazyl). The purple-coloured radical changes into yellow reduced DPPH after reaction with radical scavenger. DPPH solution (100 µM/l) was prepared in methanol. Two hundred ml of the extracts was added to 3.8 ml of DPPH solution. After 30 minutes of incubation at room temperature, the absorbance was measured at 515 nm. The standard curve was prepared using different Trolox concentrations. The results were expressed as mM Trolox equivalent per 100 g of dry weight (DW) (mM TE/100 g DW) (Zloch et al. 2004).

Ferric reducing antioxidant power (*FRAP*). The FRAP method is based on the reduction of the ferric complex to its ferrous coloured form in the presence of antioxidants. A mixture of acetate buffer (pH 3.6), TPTZ and ferric chloride (10 : 1 : 1) was made to prepare the FRAP reagent. Fifty µl of Trolox was added to 4 ml of the reagent. The reaction was monitored after 10 min at the absorption maximum of 593 nm. Trolox solution was used to make calibration curves. The results were expressed as mM Trolox equivalent per 100 g of DW (mM TE/100 g DW) (Zloch et al. 2004).

The content of total phenols (TPC) in each methanol extracts was determined according to the Folin-Ciocalteu procedure (Lachman et al. 2006). All extracts were obtained from 1 g of plant material. The diluted methanol extract (0.5 ml) was mixed with 9 ml of distilled water and 1 ml of Folin-Ciocalteu reagent and allowed to stand at room temperature for 10 min. Sodium bicarbonate solution (10 ml, 7%) was added to the mixture and incubated at room temperature for 90 min, and absorbance was measured at 765 nm using a spectrophotometer (JENWAY 6100). The total phenolic content was expressed as mg Gallic Acid/100 g dry weight (mg GAE/100 g DW).

Determination of total flavonoids (TFC). The aluminium chloride spectrophotometric method was used to determine the total content of flavonoids (Zloch et al. 2004). The diluted methanol extract (0.5 ml) was mixed with 1.5 ml of distilled water and 0.2 ml $NaNO_2$ (5%) and kept at room temperature for 5 minutes. Aluminium chloride solution (0.2 ml, 10%) was added to the mixture, shaken vigorously and incubated for 5 min. Finally, sodium hydroxide solvent (1.5 ml, 1M) and 1 ml of distilled water were added to the mixture, shaken and incubated for 15 minutes. The absorbance was measured at 510 nm, using a spectrophotometer (JENWAY 6100). The total flavonoid content was expressed as mg catechin/100 g dry weight (mg CE/100 g DW).

Statistical data analysis. The experiments were set as a completely randomized design with three replicates. Data were subjected to the analysis of variance (ANOVA) and means were separated by Duncan multiple range test at the significance level of $P \leq 0.05$.

RESULTS AND DISCUSSION

Cryopreservation is the main technique used to conserve plant natural resources that assumes that tissues stored in liquid nitrogen retain their properties (Ružić et al. 2014; Kulus, Mikuła 2016). Much attention is devoted to cryopreservation techniques, but the issue of genetic stability of biological material is not always explained by scientists. The reliability of cryopreservation protocol is essential for its practical application and includes the last step of monitoring true to type regenerants using genotypic and phenotypic markers, monitoring of biochemical status and assessment of generative reproduction functions (Harding 2004; Kaity et al. 2009; Martin et al. 2015). Evaluation of these features has become an essential part of developing new plant cryopreservation protocols. The priorities of our scientific cryopreservation program of biodiversity protection of *Rosa* natural resources in Central Europe assume the following steps: (1) successful and efficient cryopreservation protocol; (2) monitoring the fidelity of plants regenerated after shoot tip cryopreservation (Pawłowska, Szewczyk-Taranek 2014, 2015); (3) continuation of previous studies is now the first report on the stability of matured *Rosa* regenerants with the use of biochemical markers and pollen quality assessment.

To investigate the pollination potential we estimated pollen quantity and viability as well as pollen germination capacity. The examined species of roses differed statistically in terms of pollen characteristics (anther per flower, pollen per anther,

https://doi.org/10.17221/239/2017-HORTSCI

Table 1. Pollination potential of rose regenerants from cryopreserved shoot tips

Genotype	Treatment	Anthers per flower	Pollen per anther	Pollen viability (%)	Pollen germination (%)
R. agrestis	control	89.8 ± 7.4^{c*}	2,075 ± 217^{bc}	64.6 ± 3.2^{b}	63.0 ± 4.0^{c}
	cryo	86.8 ± 14.5^{bc}	2,160 ± 496.45^{bc}	69.7 ± 6.8^{bc}	68.0 ± 2.0^{c}
R. canina	control	94.5 ± 3.4^{c}	2,550 ± 182.03^{c}	54.6 ± 6.6^{a}	36.0 ± 3.0^{a}
	cryo	76.5 ± 9.5^{ab}	1,742 ± 635.31^{ab}	52.8 ± 5.7^{a}	39.0 ± 5.0^{ab}
R. dumalis	control	73.3 ± 3.2^{a}	2,245 ± 778^{bc}	75.9 ± 3.9^{c}	35.0 ± 4.0^{a}
	cryo	70.5 ± 3.3^{a}	1,750 ± 331^{ab}	74.3 ± 4.9^{c}	36.0 ± 4.0^{a}
R. rubiginosa	control	93.3 ± 8.8^{c}	1,845 ± 458$^{a–c}$	71.9 ± 2.5^{bc}	47.3 ± 6.0^{b}
	cryo	95.5 ± 8.4^{c}	1,140 ± 269^{a}	75.2 ± 1.1^{c}	42.7 ± 2.1^{ab}
Significance of factors and their interaction estimated by ANOVA					
Genotype		***	**	***	***
Treatment		ns	***	ns	ns
Genotype × treatment		ns	ns	ns	ns

*means ± standard deviations within a column followed by the same letter are not significantly different according Duncan's multiple range test at α = 0.05; #significant effect: **at $P < 0.05$; ***at $P < 0.01$; ns – not significant

and pollen viability and germination). There were no differences between postcryopreserved regenerants and control, except for *Rosa canina*, where postcryopreserved regenerants had fewer anthers per flowers and less pollen per anther (Table 1). Similarly, the quality of pollen did not depend on the origin of the plant, but on the genotype. The smallest pollen viability was noted in *R. canina* and its pollen also germinated poorly. The pollen of *R. agrestis* showed the best germination (Table 1, Fig. 2a, b). The plants regenerated after cryopreservation retained typical features for roses from the section Caninae, i.e., poor germination and pollen viability and characteristic pollen grain structure. It has been reported that the male meiosis in Caninae roses is often unsuccessful and that pollen quality in this section is very poor, and a great number of malformed, collapsed pollen grains, usually without cytoplasm have been reported (Ercli 2007a). The results of the study of Wrońska-Pilarek and Tomlik-Wyremblewska (2010) confirmed that pollen viability had different values ranging from 29.43% in *R. agrestis* to 54.56% in *R. rubiginosa*. According to those authors, *R. dumalis* and *R. rubiginosa* seemed to have the most viable pollen. After testing the germination of rose pollen grains, they found that *R. agrestis* pollen demonstrated the best germination (in 10.26%), while *R. canina* exhibited the lowest germination percentage of 2.65% (Wrońska-Pilarek, Tomlik-Wyremblewska 2010), which was confirmed in our study. SEM observations revealed large and fully developed pollen grains (mean 40 μm), but also significantly smaller (2–3 times shorter) and not completely developed pollen. The basic shape of pollen grains is subprolate and trizonocolporate aperture consisting of 3 ectocolpi and 3 endospores located in the middle part of ectocolpi (Fig. 2c–e). Striate sculpting is the main ornamentation of pollen grains, similar to other Rosaceae, which is consistent with Wrońska-Pilarek (2011) and Fatemi et al. (2012) observations.

Analyses of pro-health properties of rose hypanthia (vitamine C, TAC DPPH, TAC FRAP, flavonoids and phenols) did not show differences between post-cryopreserved regenerants and controls. At the same time, we found differences between the tested genotypes. However, it was observed that *R. dumalis* and *R. rubiginosa* regenerants after cryopreservation had less vitamin C, as compared to control (Table 2). The highest content of vitamin C was observed in *R. dumalis* hypanthia, statistically fewer in *R. agrestis*. *R. canina*, and *R. rubiginosa* hypanthia contained less vitamin C (by 40–60%) than *R. dumalis* hypanthia. The analysis of vitamin C content has confirmed that hypanthia are rich in this compound, but slight differences in the contents may depend on exposure to light and crop conditions. In the available literature, there are many reports on high vitamin C content, e.g., a small fruit group, rose deep-coloured hips, are an excellent source of vitamin C. According to observations, the concentration of vitamin C in rose hips from Caninae varies from 400–900 mg/100 g FW

https://doi.org/10.17221/239/2017-HORTSCI

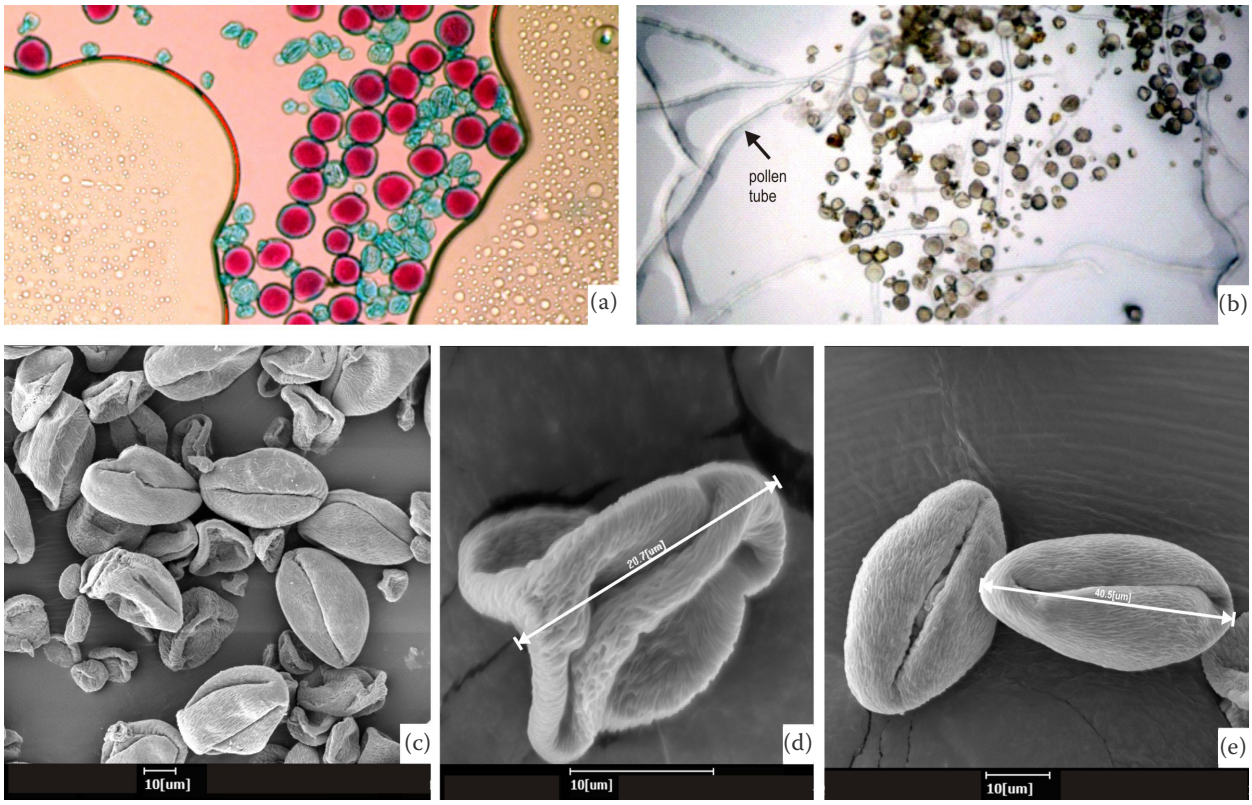

Fig. 2. *Rosa dumalis* pollen quality observations: (a) viability, fuchsia colour viable; (b) germination; SEM micrographs: (c) pollens sample; (d) smaller, not completely developed pollen grain; (e) large and fully developed pollen grain, in equatorial view

and depends on the genotype and cultivation (Ercisli 2007b; Alp et al. 2016). These values are similar to the values obtained in our experiment.

The high antioxidant activity of rose hips is another important trait of interest. Measurements of antioxidant properties showed the difference re-

Table 2. Bioactive content and antioxidant activity of rose hips of rose regenerants from cryopreserved shoot tips

Genotype	Treatment	Vitamin C	TAC DPPH	TAC FRAP	TFC	TPC
		mg/100 g FW	mM TE/100 g DW	mM TE/100 g DW	mg CE/100 g DW	mg GAE/100 g DW
R. agrestis	control	553.9^{c*}	50.0^{a}	102.5^{ab}	13.2^{a}	52.9^{a}
	cryo	589.2^{c}	71.6^{a}	79.7^{a}	11.4^{a}	44.7^{a}
R. canina	control	254.0^{a}	175.8^{b}	182.8^{c}	38.1^{bc}	77.9^{b}
	cryo	308.5^{ab}	158.8^{b}	171.2^{c}	42.6^{c}	82.6^{b}
R. dumalis	control	869.5^{e}	181.3^{b}	165.6^{c}	39.7^{bc}	101.9^{c}
	cryo	761.7^{d}	255.4^{c}	168.9^{c}	38.9^{bc}	108.1^{c}
R. rubiginosa	control	382.7^{b}	153.0^{b}	117.2^{b}	32.7^{b}	75.0^{b}
	cryo	268.2^{a}	165.9^{b}	127.2^{b}	41.5^{c}	81.3^{b}
Significance of factors and their interaction estimated by ANOVA						
Genotype		*** $^{\#}$	***	***	***	***
Treatment		ns	ns	ns	ns	ns
Genotype × treatment		**	**	ns	ns	ns

*means ± standard deviations within a column followed by the same letter are not significantly different according Duncan's multiple range test at α = 0.05.;$^{\#}$significant effect: **at $P < 0.05$; ***at $P < 0.01$; ns – not significant; TAC DPPH – total antioxidant activity; TFC – total flavonoids content; TPC – total content of phenols; TE – Trolox equivalent; CE – catechin/100 g DW; GAE – Gallic acid equivalent

https://doi.org/10.17221/239/2017-HORTSCI

sulting from the origin of regenerants only in two cases. Namely post-cryo regenerants of *R. dumalis* had a 30% higher DPPH, and the level of flavonoids in *R. rubiginosa* was also about 30% higher in post-cryo regenerants. The highest value of DPPH and FRAP was recorded for *R. dumalis* and *R. canina* hypanthia, *R. agrestis* showed the lowest antioxidant properties, which also had the lowest level of DPPH and FRAP. Total phenol content was the highest in *R. dumalis*, regardless of the origin of regenerants; generally, this species was characterized by the best parameters of vitamin C content and antioxidant properties. The high level of bioactive content in *R. dumalis* was emphasized in the literature (Alp et al. 2016). Rose hips are known to have the highest antioxidant activity, which plays an important role in maintaining fruit quality (Chrubasik et al. 2008). Our findings were consistent with the results obtained by other authors, as all our samples exhibited strong antioxidant activity (Alp et al. 2016; Taneva et al. 2016). Physiological functions of Rosaceae fruits may be partly attributed to their high phenolic content. *R. canina* was shown to contain phenolics at the level of 96 mg GAE/g DW (Ercisli 2007b), which is in accordance with our results. Total phenolic contents of rose species in our experiment were found to range from 50 mg GAE/100 g DW (*R. agrestis*) to 105 mg GAE/100 g FW (*R. dumalis*) (Table 2).

The research results published so far in the literature have proven that the regenerants obtained after cryopreservation repeat the characteristics of maternal plants. Information on field performance of plants after cryopreservation is crucial for conservation in gene banks of true to type plants, tested for genetic fidelity. Our observations proved that cryopreservation did not alter the biochemical quality of hips and pollen properties of roses regenerants. This work has contributed to the possibility of a wider and safer application of cryopreservation methods to protect the biodiversity of wild European roses, plants with potential pharmaceutical and medicinal use.

References

Alexander M.P. (1969): Differential staining of aborted and nonaborted pollen. Journal of Stain Technology, 44: 117–122.

Alp S., Ercisli S., Jurikova T., Cakir O., Gozlekci S. (2016): Bioactive content of rose hips of different wildly grown *Rosa dumalis* genotypes. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 44: 472–476.

Arya S.P., Mahajan M., Jain P. (2000): Non-spectrophotometric methods for the determination of vitamin C. Analytica Chimica Acta, 417: 1–14.

Bi W.L., Pan C., Liu J., Wang Q.C. (2016): Greenhouse performance, genetic stability and biochemical compounds in *Chrysanthemum morifolium* 'Hangju' plants regenerated form cryopreserved shoot tips. Acta Physiologia Plantarum, 38: 38–268.

Bieniasz M., Necas T., Dziedzic E., Ondrasek I., Pawłowska B. (2017): Evaluation of pollen quality and self-fertility in selected cultivars of Asian and European pears. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 45: 375–382.

Chrubasik C., Roufogalis B. D., Müller-Ladner U., Chrubasik S. (2008): A systematic review on the *Rosa canina* effect and efficacy profiles. Phytotherapy Research, 22: 725–733.

Ercisli S. (2007a): Determination of pollen viability and *in vitro* pollen germination of *Rosa dumalis* and *Rosa villosa*. Bangladesh Journal of Botany, 36: 185–187.

Ercisli S. (2007b): Chemical composition of fruits in some rose (*Rosa* spp.) species. Food Chemistry, 104: 1379–1384.

Fatemi N., Attar F., Assareh M.H., Hamzehee B. (2012): Pollen morphology of the genus *Rosa* L. (Rosaceae) in Iran. Iran Journal of Botany, 18: 284–293.

Harding K. (2004): Genetic integrity of cryopreserved plant cells: a review. CryoLetters, 25: 3–22.

Kaity A., Ashmore S.E., Drew R.A. (2009): Field performance evaluation and genetic integrity assessment of cryopreserved papaya clones. Plant Cell Report, 28: 1421–1430.

Kaity A., Drew R.A., Ashmore S.E. (2013): Genetic and epigenetic integrity assessment of acclimatised papaya plants regenerated directly from shoot-tips following short- and long-term cryopreservation. Plant Cell Tissue and Organ Culture, 112: 75–86.

Kulus D., Mikuła A. (2016): Cryopreservation in securing the stability and physiological activity of plant cells. Acta Scientiarum Polonorum Biotechnologia, 15: 27–38 (in Polish).

Lachman J., Hamouz K., Čepl J., Pivec V., Šulc M., Dvořák P. (2006): Vliv vybraných faktoru na obsah polyfenolu a antioxidační aktivitu hlíz brambor. Chemické listy, 100: 522–527.

Martin C., Kremer C., Gonzalez I., Gonzalez-Benito M.E. (2015): Influence of the cryopreservation technique, recovery medium and genotype on genetic stability of mint cryopreserved shoot tips. Plant Cell Tissue and Organ Culture, 122: 185–195.

Nybom H., Werlemark G., Esselink D.G., Vosman B. (2005): Sexual preferences linked to *Rose* taxonomy and cytology. Acta Horticulturae (ISHS), 690: 21–28.

Nybom H., Werlemark G. (2017): Realizing the potential of health-promoting rosehips form dogroses (*Rosa* sec. Caninae). Current Bioactive Compounds, 13: 3–17.

https://doi.org/10.17221/239/2017-HORTSCI

Pawłowska B., Szewczyk-Taranek B. (2014): Droplet vitrification cryopreservation of *Rosa canina* and *Rosa rubiginosa* using shoot tips from in situ plants. Scientia Horticulturae, 168: 151–156

Pawłowska B., Szewczyk-Taranek B. (2015): Efficient cryopreservation by droplet vitrification of pentaploid roses and the phenotype of regenerated plants. Acta Societatis Botanicorum Poloniae, 84: 439–442.

Quave C.L., Pieroni A. (2015): A reservoir of ethnobotanics knowledge informs resilient food security and health strategies in the Balkans. Nature Plants: 14021. doi: 10.1038/nplants.2014.21

Roman I., Stanila A., Stanila S. (2013): Bioactive compounds and antioxidant activity of *Rosa canina* L. biotypes from spontaneous flora of Transylvania. Chemistry Central Journal, 7: 73.

Ružić DJ., Vujović T., Cerović R. (2014): Cryopreservation of cherry rootstock Gisela 5 using vitrification procedure. Horticultural Science (Prague), 41: 55–63.

Shan B., Yizhong Z.C., Mei S., Corke H. (2005): Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. Journal of Agricultural and Food Chemistry, 53: 7749–7759.

Sharma N., Singh R., Pandey R., Kaushik N. (2017): Genetic and biochemical stability assessment of plants regenerated from cryopreserved shoot tips of commercially valuable medicinal herb *Bacopa monnieri* (L.) Wettst. *In Vitro* Cellular and Developmental Biology - Plant, 53: 346–351.

Skyba M., Urbanowa M., Kapchina-Toteva V., Kosuth J., Harding K., Hellerova E. (2010): Physiological, biochemical and molecular characteristics of cryopreservated *Hyperium perforatum* L. shoot tips. CryoLetters, 31: 249–260.

Stanila A., Diaconeasa Z., Roman I., Sima N., Maniutiu D., Roman A., Sima R. (2015): Extraction and characterization of phenolic compounds form rose hip (*Rosa canina* L.) using liquid chromatography coupled with electrospray ionization – mass spectrometry. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 43: 349–354.

Taneva I., Petkova N., Dimov I., Ivan I., Denev P. (2016): Chracterization of rose hips (*Rosa canina* L.) fruits extracts and evaluation of their i*n vitro* antioxidant activity. Journal of Pharmacognosy and Phytochemistry, 5: 35–38,

Wrońska-Pilarek D., Tomlik-Wyremblewska A. (2010): Pollen viability and in vitro germination of selected Central European species from genus *Rosa* analysed with different methods. Dendrobiology, 64: 43–53.

Wrońska-Pilarek D. (2011): Pollen morphology of Polish native species of the rosa genus (Rosaceae) and its relation to systematic. Acta Societatis Botanicorum Poloniae, 80: 221–232.

Yordanowa Z.P., Dimitrova M.A., Čellárová E., Kapchin-Toteva V.M. (2011): Physiological evaluation of *Hypericum rumeliacum* Boiss. Plants regenerated after cryopreservation. Cryopreservation Symposium, COST Action 871 "Cryopreservation of crop species in Europe". Final meeting. Angers, France, February 8–11: 56–59

Zloch Z., Čelakovský J., Aujezdská A. (2004): Stanovení obsahu polyfenolu a celkové antioxidační kapacity v potravinách rostlinného původu. Závěrečná zpráva o plnění projektu podpořeného finančně Nadačním fondem Institutu Danone, Plzeň.

Zhang Z., Skjeseth G., Elameen A., Haugslien S., Silvertsen A., Clarke J.L., Wang Q.-Ch., Blystad D.-R. (2015): Field performance evaluation and genetic integrity assessment in *Argyranthemum* 'Yellow Empire' plant srecovered from cryopreserved shoot tips. In Vitro Cellular and Developmental Biology-Plants, 51: 505–513.

Received for publication December 9, 2017
Accpted after corrections July 27, 2018