UDC 635.076:57.043 **https://doi.org/10.15407/biotech12.05.089**

MATHEMATICAL MODEL FOR DESCRIBING THE POST-CRYOPRESERVATION VIABILITY OF FRUIT AND BERRY CUTTINGS

L. V. Gorbunov[1]
I. V. Petrov[2]
O. V. Zviahintseva[1]

[1] National Technical University "Kharkiv Polytechnical Institute", Ukraine
[2] Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, Kharkiv, Ukraine

E-mail: embiotech@icloud.com

Received 26.04.2019
Revised 21.07.2019
Accepted 30.10.2019

A mathematical model that simplifies the determination of optimal parameters ensuring the maximum viability of frozen-thawed fruit and berry cuttings was developed. Values of the minimum amount of intracellular water η1min, which minimizes the plasmolysis probability, and η2min, which minimizes the probability of intracellular ice formation, were determined with due account for the bioobject heterogeneity.

Free water amounts Δη, forming ice crystals inside the cell during cryopreservation of different of fruit and berry varieties, were calculated. The optimal conditions for cutting dehydration (temperature Ti and incubation time t2, minimum amount of intracellular water ηmin) ensuring the maximum viability after drying and low-temperature adaptation to cryopreservation were selected. The individual features of the viability of frozen-thawed cuttings of different species were quantitatively reflected in the free water index Δη. The maximum viability of frozen-thawed birch and blackcurrant cuttings was achieved, when intracellular water was in the bound, vitrified state $\Delta\eta = 0$. The calculated $\Delta\eta > 0$ for cuttings of different varieties of apple- and pear-trees as well as of raspberry-bushes leads to a decrease in the viability, and it is impossible to obtain viable plum, apricot or grape specimens after low-temperature cryopreservation with no bound water ηc at all.

Key words: mathematical model, cryopreservation, fruit and berry cuttings, viability.

In the gardening practice, it is conventional to use vegetative propagation methods, since segregation of multiple traits in the offspring occurs upon propagation by seeds [1, 2]. Therefore, in order to preserve valuable economic and biological features, propagation by cuttings is common. Ex situ preservation, in banks of plant genetic resources, is the most reliable way of long-term storage of plant samples [1, 3–7]. Creation of cryobanks makes it possible to preserve the natural diversity of plants, where genetically modified organisms are actively used.

Cryopreservation of apical meristems of shoots is the best way to preserve their gene pool for vegetatively propagating plants [8–10]. This method requires specific conditions of in vitro culturing of meristem cells, that is, sterile conditions, nutrient media, controlled temperature, lighting and humidity [11]. Therefore, there are difficulties while creating such cryobanks. There are only reports on regeneration of plants of about 16 species from meristems that had been in liquid nitrogen [1, 10, 12–14].

Cryopreservation difficulties for plant cells are associated with their relatively large volumes. Ninety percent of this volume is occupied by the central vacuole containing free water, which is not bound in hydration shells of macromolecules, and can freeze to form ice crystals. These crystals damage biological membranes causing the death of plant cells and organs [15]. Free water must be removed by preliminary partial dehydration of cells and reduction in freezing rates [12, 15–17]. Removing an excessively large portion of free water results in the protoplast compression,

which leads to irreversible changes and plasmolysis.

To ensure the integrity of cooled and frozen-thawed cells, many researchers use drying of fruit and berry specimens from 50–56% to 20–30% [1, 4, 18, 19]. It was noted that different frozen-thawed varieties and species of crops had various viability ranging from 0 to 100% [1, 4, 8, 9, 18–22]. At the same time, there are no data on the effect of water content in fruit and berry cuttings of different species on the probability of their germination; hence, it is necessary to study the effects of different factors on dehydration of cells of miscellaneous varieties of fruit and berry crops. We believe that this is one of the reasons for low reproducibility of cryopreservation results on fruit and berry cuttings of different varieties.

Our objective was to develop a mathematical model for optimizing the cryopreservation parameters for different fruit and berry varieties.

Materials and Methods

Plant cuttings were cryopreserved in the Laboratory of Preservation of the NCPGRU of the Plant Production Institute named after V. Ya. Yuriev of NAAS (Kharkiv) [18, 23, 24]. Cuttings of the following species and varieties were taken as the study object: blackcurrant (*Ribes nigrum* L.) — Dachnitsa, Kytaivska, Sofiivska, Yuvilei, Raduzhna, Titiniia, Dar Pavlovoi, Katyusha, Nadiia, Sofiia, Alta, Shedevr, Darunok Mliieva, Lentyay, Uvertyura, Mif, Halynka, Lybid, Ben Tiran, Biriulevska, Nimfa, Yuvilaina Kopania, Krasa Lvova, Biloruska Solodka, Slavuta, Vira, Chorna Krupnoplidna, Ametyst, Ozherelye; redcurrant (*Ribes rabrum*) — Kytaivska, Joker, Sviatkova; gooseberry (*Ribes uva-crispa*) — Krasen, Malakhit, Kolobok; raspberry (*Rubus idaeus* L.) — Novost Kuzmina, Skromnitsa, Struyka; grape (*Vitis labrusca* L.) — Lydiya, Rkatseteli, Aligote, Kober 5BB, Traminer Rozovyy; cherry (*Prunus cerasus* L.) — Stepnaya, Amulet, Pamiat Artemenka, Optymistka, Nochka; duke (cherry-sweet cherry hybrid) — Alpha; sweet cherry (*Prunus avium*) — Chitinskaya Chornaya, Donchanka, Lehenda Mliieva, Dar Mliieva; plum (*Prunus domestica* L.), reine-claude group — Altana, Pamiat Materi, Oposhnyanka; Hungarian plum group — Voloshka, Oda; apricot (*Prunus armeniaca* L.) — Moldavskyi Olimpiiets, Krymskyi Medunets; apple (*Malus domestica* L.) — Belyy Naliv, Amulet; Edera; Teremok; Radost; Katya; pear (*Pyrus communis* L.), summer group — Velyka Litnia, Uliublena Klapa, autumn group — Horodyshchenska, Osinnia Vdala, winter group — Bere Kyivska, Zelena Mliiska. Birch cuttings (*Betula pubescens*) were as a control for the selected cryopreservation methods.

Cuttings were cut from one-year shoots and divided into 10 individual specimens, with a length of 5–12 cm and a diameter of 0.5–1.0 cm. Cuttings had 2–5 vegetative buds. Before the specimens were dried, their viability and initial water content were determined.

The effects of low-temperature sublimation of intracellular water and plasmolysis were evaluated from the cutting viability. The control viability of cuttings was determined after each stage of drying and cooling. For this purpose, cuttings were placed in an exsiccator with distilled water at 5 °C for 14 days for their hydration, and then cultured in vitro (in glasses with water at 20–25 °C). Swelling and development of buds indicated that the specimens under investigation were viable. The percentage of viability was estimated as the ratio of the number of cuttings with evolved buds in vitro to the total number of buds in a specimen.

The water content in native cuttings varied within 50–20% at — 2 ± 2 °C. The water content was determined by weighing samples and calculating by the following formulae:

$$\eta_i = \frac{m_0 - m_k}{m_0} \times 100\%, \quad (1)$$

$$\eta_s = \frac{m_s}{m_0} \times \eta_0 \times 100\%, \quad (1a),$$

where: η_i — water content of a sample (%): i = 0 native sample; i = 1 after drying; i = 2 after cryopreservation; i = 3 after rehydration; m_0 — initial weight of a native sample (g); m_k — final weight of a sample after dehydration until constant weight (g); η_s — water content in a sample at a drying stage (%); m_s — sample weight at a drying stage (g).

To preserve the residual moisture, cuttings were waxed or paraffinned at both ends prior to cooling. Prior to freezing, they were exposed at 4 °C for 10–15 days and at −5 °C for 2 weeks to several months.

Fruit and berry cuttings were cooled on a step-by-step basis at a rate of 0.01–0.1 °C/h to −5÷−30 °C with the increment of 5 °C and exposed at these temperatures for 1, 3 or 7 days, respectively, in 2-liter household thermoses placed in a refrigerator. Specimens

were cooled from −20 and −30 °C to −196 °C by direct immersion into liquid nitrogen at a rate of 600–800 °C/min. Specimens were stored in liquid nitrogen for a period from 1 day to 1 year. They were thawed at the rate of 70 °C/min by direct placing in a room at 20 °C [25].

The data were statistically processed on a computer using conventional formulae in the standard program "Stadia" to compose regression equations.

Results and Discussion

The main criterion of the viability of a frozen-thawed bio-object is its ability for further development. Therefore, an in vitro culture is the simplest and most reliable way to quantify the viability of plant cuttings. Determination of the cutting viability is maximally reliable with the minimal usage of a bioobject, if the polynomial regression dependence (Fig. 1) is applied to experimental data of cultured specimens in combination with the dependence derived from Verhulst's formula

$$V_{k1}(t_1) = \frac{V_0 K e^{\mu_1 t_1}}{K - V_0 + V_0 e^{\mu_1 t_1}}, \quad (2)$$

where: $V_k(t_1)$ — change in the viability of native cuttings related to the culturing time $V_k(t_1) = n/n_0$, a relative value expressed in relative units; n — the number of viable cuttings at a culturing stage $t_1 > 0$; n_0 — the total number of cuttings at the start of culturing $t_1 = 0$; V_0 — initial viability of cuttings at $t_1 = 0$, $V_0 < 1$; K — the maximum theoretical value of the cutting viability $K = 1$; μ_1 — specific rate of decrease in the cutting viability in culture reflecting individual characteristics of a bioobject's resistance and determined by simulation modeling (Fig. 1) or in the inverse coordinate system, day^{-1}; t_1 — culturing time, days.

Cuttings were dehydrated stepwise at sub-zero temperatures of −5÷−10 °C with concurrent empirical determination of the minimum moisture η_{min}, at which a high viability was observed according to the results of culturing. The cutting viability after removal of excess moisture was evaluated using Moser's formula

$$V_\eta(\eta) = \eta_0 + \frac{\eta^\alpha}{K_\eta + \eta^\alpha}, \quad (3)$$

where η — water content in a cutting, η_0 — the empirically obtained correction coefficient, α and K_η — Moser's constants that reflect individual features of the bioobject viability, when its water content changes (determined by simulation modeling from experimental values, Fig. 2).

The post-dehydration viability was evaluated by the results of culturing

$$V_\eta(t_1\eta_{min}) = V_k(t_{1max}) \times V_\eta(\eta_{min}), \quad (4)$$

where η_{min} — the minimum water content in cuttings, which ensures their maximum viability $V_\eta(\eta_{min})$ (Fig. 2).

To bind the residual free intracellular water, temperature adaptation of cuttings was carried out at T = −20÷−30 °C. The cutting viability in the temperature range close to the

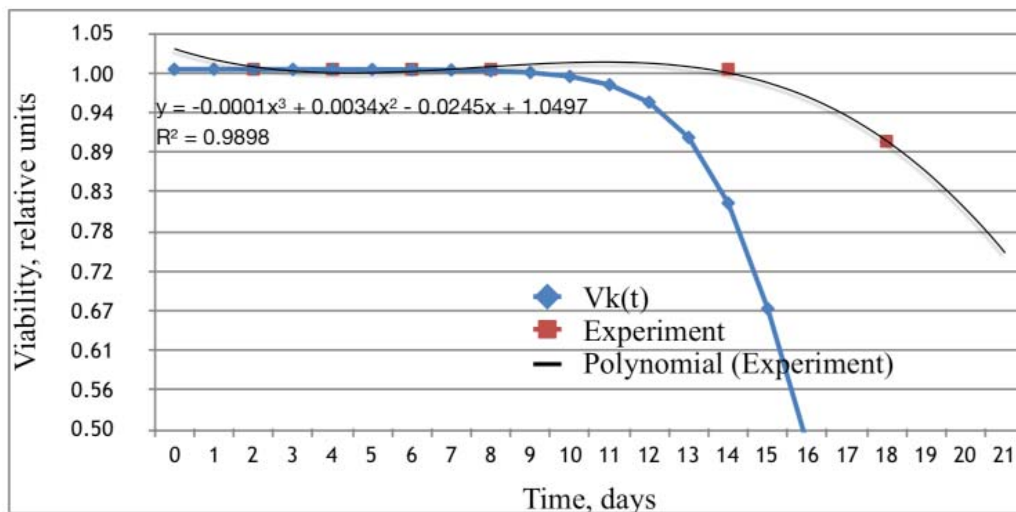

Fig. 1. **Changes in the viability of warty birch cuttings related to the *in vitro* culturing time**
Specific rate of decrease in the cutting viability — $\mu_1 = -0.78$; water content — $\eta = 38\%$

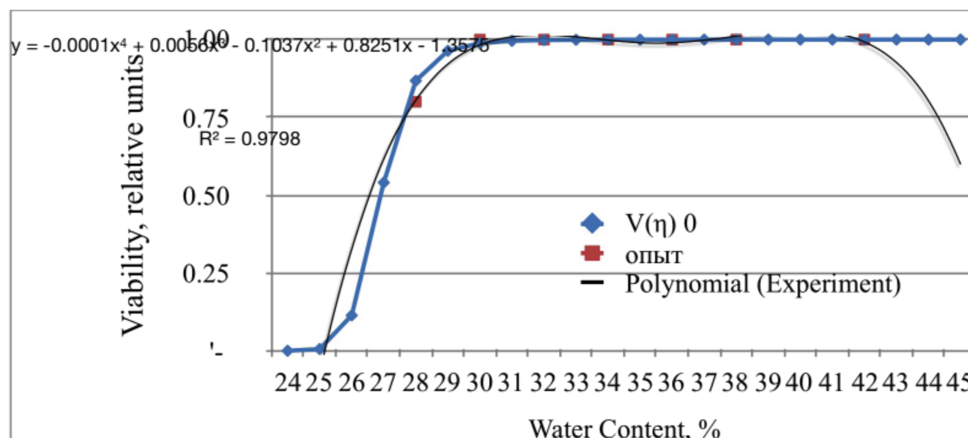

Fig. 2. **Changes in the viability of warty birch cuttings related to the *in vitro* culturing time after drying to a specified water content η_{min} = 30%**

Moser's constants K_s = 36770, α = 7.70, and the correction coefficients η_0 = 24

temperature of intracellular ice formation was determined experimentally and described by Verhulst's formula

$$V_T(t_2)=\frac{V_\eta(\eta_{\min})\times Ke^{\mu_2 t_2}}{K-V_\eta(\eta_{\min})+V_\eta(\eta_{\min})\times e^{\mu_2 t_2}}, \quad (5)$$

where: $V_T(t_2)$ — change in the cutting viability related to time at a specified temperature $V_T(t_2) = n/n_0$, a relative value expressed in relative units; n — the number of viable cuttings $t_2 > 0$; n_0 — the total number of cuttings in a sample $t_2 = 0$; V_η (η_{min}) — post-drying viability of cuttings at $t_2 = 0$, $K = 1$; μ_2 — specific rate of decrease in the cutting viability upon temperature adaptation reflecting individual characteristics of a bioobject's resistance and determined by simulation modeling (Fig. 3) or in the inverse coordinate system, day^{-1}; t_2 — exposure time, days.

The optimal exposure time t_2 at a temperature close to the intracellular crystallization point T^i and η_{2min} were determined experimentally from the obtained dependence ϕ (3) (Fig. 4).

The post-temperature adaptation viability were evaluated by the results of culturing

$$V_T(t_1,t_2,\eta_{2\min},T^i)=V_\eta(t_1,\eta_{2\min})\times V_T(t_2,T^i). \quad (6)$$

The viability of frozen-thawed cuttings V_d was estimated by the experimental results as the ratio of the number of viable cuttings n to the total number of cuttings in a sample no: $V_d = n/n_0$ (Fig. 5). This value depends on the initial viability $V_T(t_2,T^i)$ and the probability of intracellular crystallization P^i_k

$$V_d=V_T(t,T^i)\times(1-P_k^i). \quad (7)$$

The probability of intracellular crystallization can be calculated as follows:

$$\eta_c=\eta_{\min}\frac{V_d}{V_T}, \quad (8)$$

where η_c — the fraction of bound (vitrified) water, and $\Delta\eta = \eta_{min} - \eta_c$ — the fraction of free water forming crystals at a temperature below T^i.

The amount of bound water can be calculated as follows:

$$P_k^i=\frac{\eta_{\min}-\eta_c}{\eta_{\min}}. \quad (9)$$

The post-dehydration and post-temperature adaptation viability of frozen-thawed cuttings was estimated by the results of culturing

$$V_d(t_1,t_2,\eta_k,T,\eta_c)=V_T(t_1,t_2,\eta_k,T)\times V_d(\eta_c). \quad (10)$$

The results show that birch cuttings retain their initial viability after different drying regimes, step-wise cooling to −20÷−30 °C, provided they are cooled at the rate of 0.1 °C/h, long-term storage at — 196 °C and subsequent warming at the rate of 70 °C/min. At the same time, we found (Fig. 2) that the water content in specimens should not be below 32% upon their drying. Below this critical value, the viability of specimens decreases dramatically as a result of excessive dehydration of cells (plasmolysis effect). At −30÷−196 °C, this value can be reduced to 14% . The calculations show (Fig. 5) that all intracellular water is in a bound state. This ensures a high viability of frozen-thawed birch cuttings.

To determine the optimum parameters ensuring the maximum viability of frozen-thawed cuttings of different varieties of

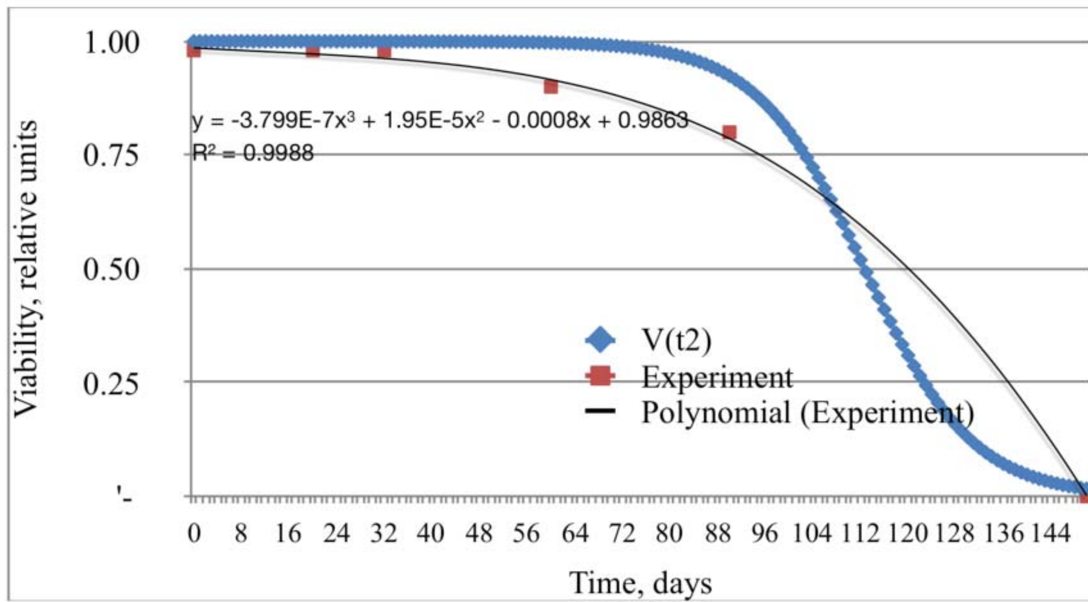

Fig. 3. **Changes in the viability of warty birch cuttings related to the exposure time at a temperature close to the temperature of intracellular crystal formation**
The initial viability of cuttings at a specified temperature — $V(t_2) = 0.99$;
specific rate of decrease in the cutting viability upon temperature adaptation $\mu_2 = -0.11$

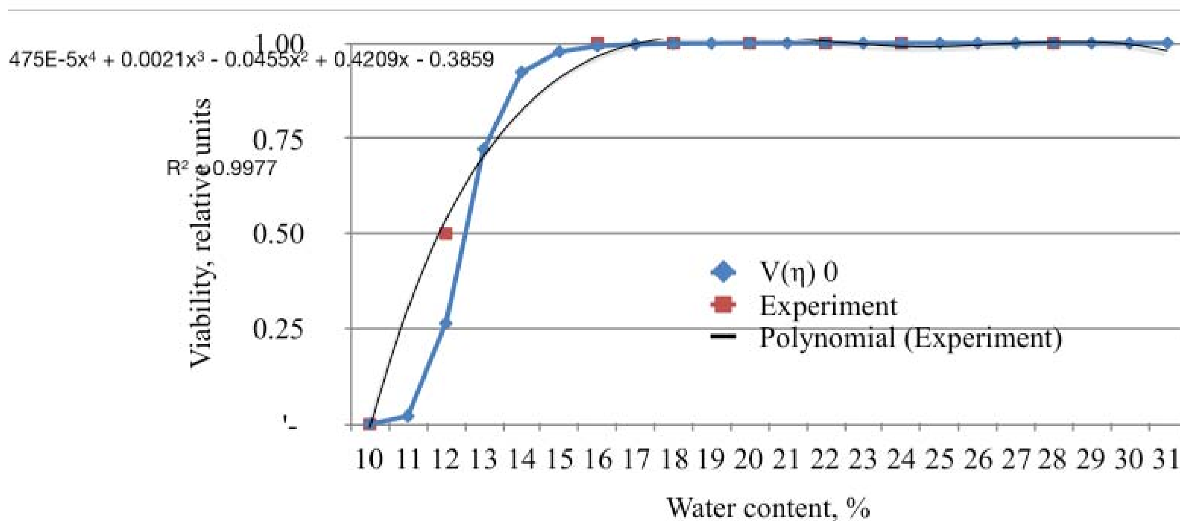

Fig. 4. **Changes in the viability of warty birch cuttings related to the in vitro culturing time after temperature adaptation to a specified water content η_{min} = 15%**
Exposure time at −20 °C t_2 = 30 days.
Moser's constants K_s = 36770, α = 7.70, and the correction coefficient η_0 = 10

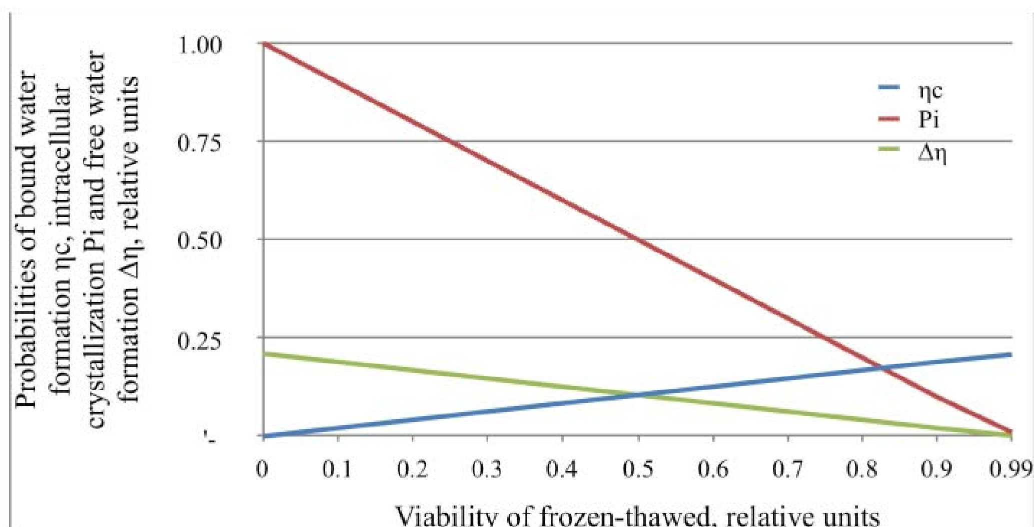

Fig. 5. **Dependence of the viability of frozen-thawed warty birch cuttings related to the *in vitro* culturing time after temperature adaptation to a specified water content η_{min} = 20%, t_2 = 30 days**

Table 1. **The viability *V* and water content η of cuttings of different berry varieties evaluated at the cryopreservation, stages**

Species	Variety	Cutting viability after cooling to					
		−10 °C		−30 °C		−196 °C	
		Experiment	η_{1min}, %	Experiment	η_{2min}, %	Experiment	Δη,%
Black-currant	Dachnitsa	98.0±2[a]	38	94.0 ±4[a]	30	92.0±5.8[a]	1
	Yuvilei	80.0±7.1[b]	37	62.0±8.6[b]	31	54.0±13.6 [b]	4
	Sofiivska	94.0±4	39	60.0±14.1[b]	30	6.0±2[c]	27
	Kytaivska	86.0±4	38	12.0±3.7[c]	32	0.0±0 [d]	32
Red-currant	Kytaivska	92±3.7[a]	40	84.0±6.8[a]	30	54.0±7.5[a]	11
	Joker	68.0±3.7 [b]	42	6.0±2.4[b]	33	4.0±2.4[b]	11
	Sviatkova	66.0±4.0	38	0.0±0[c]	30	0.0±0	-
Goose-berry	Krasen	84.0±8.1	35	70.0±7.1	25	30.0±7.1	14
	Malakhit	82.0±8.0[a]	36	68.0±7.3 [a]	26	48.0±15.9[a]	8
	Kolobok	40.0 ±3.2[b]	35	8.0±3.7 [b]	25	0.0±0[b]	25
Rasp-berry	Novost Kuzmina	96±2.4	37	82.0±8.0	21	70.0±7.1[a]	3
	Struyka	94±2.4[a]	37	58.0±12.8[a]	22	0.0±0[b]	22
	Skromnitsa	68.0±3.7[b]	37	4.0±2.4[b]	20	0.0±0	20
Grape	Lidiya	94.0±2.4	40	84.0±2.4[a]	28	0.0±0	28
	Rkatseteli	94.0±4.0	41	22.0±5.8[b]	27	0.0±0	27
	Aligote	92.0±3.7	40	20.0±7.1[b]	26	0.0±0	26
	Kober 5BB	90.0±3.2	42	18.0±3.7 [b]	27	0.0±0	27
	Traminer Rozovyy	86.0±4.2	40	0.0±0[c]	27	0.0±0	-

η_{1min} — the minimum amount of intracellular water minimizing the probability of plasmolysis and η_{2min} — the minimum amount of intracellular water minimizing the probability of intracellular ice formation, Δη — the amount of free intracellular water, %.

fruit and berry crops (Tables 1, 2 and 3), we carried out experiments in a similar fashion to those on birch cuttings. In the experiments, we investigated cuttings with the initial viability of ≥ 99%. To improve the result reproducibility, we used the proposed mathematical model ϕ (2–10) for evaluation of the viability at 3 stages of cryopreservation.

The technological parameters for each variety of blackcurrant, redcurrant, gooseberry, raspberry and grape were optimized via step-by-step cooling to −10 °C followed by temperature adaptation at −30 °C and freezing at −196 °C. The maximum viability was achieved by exposure at −5 and −10 °C for 14–60 days, stepwise cooling of specimens at a rate of 0.1–0.5 °C/h from −10 to −20 and −30 °C, 3–7-day exposure, direct immersion in liquid nitrogen, storage for 1 to 30 days, and warming at a rate of 70–100 °C/min. The exposure length at −5 and −10 °C was determined by monitoring the water content in specimens set in accordance with the maximum permissible value established for each plant variety.

Analysis of the viability of frozen-thawed cuttings of berry crops showed that within one species the average values for different varieties varied by up to 92% in blackcurrant, 54% in redcurrant,

Table 2. **The viability V and water content η of cuttings of different drupaceous varieties evaluated at the cryopreservation stages**

Species	Variety	Cutting viability after cooling to					
		−10 °C		−30 °C		−196 °C	
		$M \pm m$, %	η_{1min}, %	$M \pm m$, %	η_{2min}, %	$M \pm m$, %	$\Delta\eta$, %
Cherry	Stepnaya	96.0±2.4	34	78.0±8.6	33	64.0±12.9	6
	Amulet	94.0±4.5	35	90.0±8.6[a]	32	68.0±8.6	8
	Optymistka	92.0±3.7	34	62.0±10.2[b]	33	48.0±10.7	7
	Pamiat Artemenka	96.0±2.4	35	94.0±2.4[a]	34	34.0±2.4[a]	22
	Nochka	94.0±2.4	35	64.0±9.3[b]	35	8.0±3.7[b]	31
Sweet cherry	Alpha*	98.0±2.0	35	76.0±9.3	33	60.0±13.0	7
	Donchanka	90.0±9.7	35	72.0±10.8[a]	35	54.0±10.8[a]	9
	Lehenda Mliieva	94.0±2.4[a]	36	28.0±8.6[b]	31	0.0±0.0[b]	31
	Chitinskaya Chornaya	58.0±12.0[b]	37	12.0±3.7	30	0.0±0.0	30
Plum	Oposhnyanka	92.0±3.7	40	28.0±8.6	28	0.0±0.0	28
	Pamiat Materi	90.0±3.2	41	22.0±8.6	20	0.0±0.0	20
	Voloshka	86.0±4.0	40	12.0±3.7	19	0.0±0.0	19
	Oda	78.0±7.3	42	10.0±3.2	18	0.0±0.0	18
	Altana	80.0±7.1	40	8.0±3.7	18	0.0±0.0	18
Apricot	Krymskyi Medunets	54.0±10.8	35	12.0±3.7	21	0.0±0.0	21
	Moldavskyi Olimpiiets	40.0±7.1	40	8.0±3.7	28	0.0±0.0	28

Footnote, see Table 1.

Table 3. **The viability V and water content η of cuttings of different apple and pear varieties evaluated at the cryopreservation stages**

Species	Variety	Cutting viability after cooling to					
		−10 °C		−30 ° C		−196 ° C	
		$M \pm m$, %	η_{1min},%	$M \pm m$, %	η_{2min},%	$M \pm m$, %	Δη, %
Apple	Radost	92.0±3.7	40	52.0±3.7	19	36.0±5.1[a]	6
	Teremok	90.0±3.2[a]	42	48.0±3.7	22	22.0±8.0	12
	Belyy Naliv	70.0±4.5[b]	41	52.0±3.7[a]	19	8.0±3.7[b]	16
	Katya	46.0±7.5[c]	40	6.0±2.4[b]	21	0.0±0.0[c]	21
	Sprint	40.0±7.1	42	0.0±0.0[c]	29	0.0±0.0	-
	Amylet	38.0±7.3	43	0.0±0.0	28	0.0±0.0	-
	Edera	26.0±8.4	40	0.0±0.0	23	0.0±0.0	-
Pear	Velyka Litnia	94.0±4.0	41	80.0±7.1	28	74.0±8.7[a]	2
	Uliublena Klapa	90.0±3.2	40	82.0±5.8	28	28.0±7.3[b]	18
	Osinnia Vdala	94.0±2.4	40	92.0±2.0[a]	25	86.0±4.0[a]	2
	Zelena Mliiska	88.0±3.7	40	72.0±8.6[b]	21	8.0±3.7[c]	19
	Bere Kiyvska	86.0±4.0[a]	43	4.0±2.4[c]	22	0.0±0.0[d]	22
	Horodyshchenska	46.0±7.5[b]	40	6.0±2.4	28	0.0±0.0	28

Footnote, see Table 1.

48% in gooseberry, and 70% in raspberry (Table 1). That is, the species difference of berry cuttings has approximately twice as much impact on the viability of frozen-thawed specimens as the variety one does. This is attributed to heterogeneity of the bioobject, namely, to the amount of free intracellular water Δη.

A similar dependence was observed for different varieties of drupaceous plants (Table 2). There are many-fold differences in the viability of frozen-thawed cuttings, depending on the variety. The exposure length at −10 and −30 °C was determined by monitoring the water content in specimens set in accordance with the maximum permissible value for each species of cuttings: 30–37% for cherry and sweet cherry and 35–45% for plum and apricot.

The cause of discrepancies in the viability is associated with intracellular crystallyzation, which is confirmed by numerical values of free intracellular water Δη calculated for these specimens. Significant differences for different varieties are likely to depend on sugar concentrations in the intracellular environment.

Similarly, the water contents in specimens were optimized in accordance with the maximum permissible value of 40% and 19–28% at −10 and −30 °C, respectively, obtained for apple and pear. The results show (Table 3) that there are many-fold differences in the viability of frozen-thawed cuttings and that the viability depends on the free intracellular water amount Δη estimated for each variety.

Analysis of the cryopreservation efficiency for different varieties of fruit and berry crops shows that within one species the average values vary for different varieties: by up to 90% in blackcurrant, 24% in redcurrant, 79% in cherry, 54% in apple, and 82% in pear. Such significant differences in the values obtained for cuttings of different species and varieties are accounted for influence of individual characteristics of a bioobject reflected in the amounts of free, unbound water.

Our review of the literature shows that many factors influence the results of the solution of cryobiological challenges. Experimental studies of mechanisms of all these phenomena are extremely difficult. Meanwhile, the problems of analysis and optimization of these processes must be solved. For these purposes, experimentally statistical methods are successfully applied for building descriptive mathematical models of objects and investigating relationships between the response of a system to changes in the parameters of interest.

No feedback during research is also an important obstacle to the use of existing methods of multi-factor optimization, which is manifested in the possibility of constructing graphical presentations depicting relationships between parameters of interest, both in the course of experiments and analysis of data. Thus, opportunities to trace causality mechanisms, which are a basis for constructing analytical models, are lost. At the same time, an essential shortcoming of conventional methods of research optimization in cryobiology is their specificity, that is, the suitability for certain areas of research is a solution to biological or physical problems. To study a cryobiological object, there is a need for an analytical mathematical model to increase reproducibility and comparability of data obtained. This allows us to accelerate the process of solving cryobiological problems associated with large usage of a bioobject and hereto related problems both of ethical and of economic nature.

The probability of generating fully functional offspring from frozen-thawed cuttings of fruit and berry crops is affected by their initial state and the effectiveness of a cryopreservation method. Basing on the study conducted, we propose a mathematical model (expressions 2–10) for evaluation of the viability of cuttings of different varieties at several stages of cryopreservation. Analysis of the post-cryopreservation viability of fruit and berry cuttings showed that the results depended on the variety as well as on cryopreservation and culturing methods. The model presented makes it possible to compile regulations for monitoring and optimization of cryopreservation of cuttings of different species and varieties of fruit and berry crops.

Thus,

1. The mathematical model providing the possibility of quantification of optimal cryopreservation parameters for different varieties of fruit and berry crops was developed.
2. The minimum values of the intracellular water amounts η_{1min} and η_{2min} minimizing the probabilities of plasmolysis and intracellular ice formation, respectively, were determined with due account for the heterogeneity of a bioobject.
3. The values of free and bound water quantitatively determining the viability of cuttings were estimated with due account for the individual characteristics of different fruit and berry varieties.

Acknowledgements. The authors are grateful to researchers of the Plant Production Institute named after VYa Yuriev of NAAS for their assistance in carrying out these experiments.

REFERENCES

1. *Popov A. S., Popova E. V., Nikishina T. V., Vysotskays O. N.* Cryobank of plant genetic resources in Russian Academy of Sciences. *Int. J. Refriger.* 2006, 29 (3), 403–410. https://doi.org/10.1016/j.ijrefrig.2005.07.011
2. *Maleckij S. I., Melent'eva S. A., Tatur I. S., Judanova S. S., Maleckaja E. I.* Hybrid power conservation in apozygotic progeny of sugar beet (*Beta vulgaris* L.). *Scientific support for the beet industry: Proceedings of the International scientific-practical conference dedicated to the 85th anniversary of RUE "Experimental scientific station for sugar beets": Nesvizh. 2013.* (In Russian).
3. *Novikova T. I.* Use of biotechnological approaches for the conservation of plant biodiversity. *Rastitelnyy mir Aziatskoy Rossii*. 2013, 2 (12), 119–128. (In Russian).
4. *Sakai A., Nishiyama J.* Cryopreservation of winter vegetative buds of hardy fruit trees in liquid nitrogen. *Hort. Sci*. 1978, 13 (1), 225.
5. *Engelmann F.* Cryopreservation of embryos: an overview. *Meth. Mol. Biol.* 2011, V. 710, P. 155–184. https://doi.org/10.1007/978-1-61737-988-8_13
6. *Teixeira da Silva J. A., Engelmann F.* Cryopreservation of oil palm (Elaeis guineensis Jacq.). *Cryobiology*. 2017, V. 77, P. 82–88. https://doi.org/10.1016/j.cryobiol.2017.04.007
7. *Nair D. S., Reghunath B. R., Soni K. B., Alex S.* Cryopreservation of Encapsulated Axillary Buds of Clitoria ternatea (L.). *Cryo Lett*. 2019, 40 (1), 28–35.

8. *Hao Y. J., Liu Q. L., Deng X. X.* Effect of cryopreservation on apple genetic resources at morphological, chromosomal, and molecular levels. *Cryobiology*. 2001, 43 (1), 46–53. https://doi.org/10.1006/cryo.2001.2339
9. *Wu Y., Zhao Y., Engelmann F., Zhou M., Zhang D., Chen S.* Cryopreservation of apple dormant buds and shoot tips. *Cryo Lett*. 2001, 22 (6), 375–380.
10. *Shevchenko N. O., Ivchenko T. V., Mozgovska A. V., Miroshnychenko T. M., Bashtan N. O.* Survival of Sweet Potato Meristems Under Different Cryopreservation Regimens. *Probl. Cryobiol. Cryomed*. 2018, 28 (2), 156. https://doi.org/10.15407/cryo28.02.156
11. *Pegg D. E.* Principles of cryopreservation. *Meth. Mol. Biol.* 2015, V. 1257, P. 3–19. https://doi.org/10.1007/978-1-4939-2193-5_1
12. *Goldstein G., Nobel P. S.* Changes in osmotic pressure and mucilage during low-temperature acclimation of Opuntia ficus-indica. *Plant Physiol.* 1991, 97 (3), 954–961. https://doi.org/10.1104/pp.97.3.954
13. *Kabylbekova B. Zh., Koval'chuk I. Ju., Turdiev T. T., Muhitdinova Z. R.* The effect of cryoprotectants and preprocessing methods on viability of apple buds during cryopreservation. *Vestnik nauki Kazahskogo agrotehnicheskogo universiteta im. S. Sejfullina (mezhdisciplinarnyj)*. 2017, 3 (94), 9–17. (In Russian).
14. *Verzhuk V. G., Pavlov A. V., Tikhonova O. A., Borzhyh N. V., Dorokhov D. S.* Evaluation of the viability genoplasmy of fruit crops after cryopreservation at vapor of liquid nitrogen — 183–185 °C. *Faktory eksperimentalnoy evolyutsii organizmov*. 2013, V. 13, P. 27–30. (In Russian).
15. *Sakai A.* Development of cryopreservation techniques. Cryopreservation of tropical plant germplasm. Current research progress and application. *JIRCAS, Tsukuba & IPGRI, Rome*. 2000, P. 1–7.
16. *Towill L. E., Forsline P. L.* Cryopreservation of sour cherry (*Prunus cerasus* L.) using a dormant vegetative bud method. *Cryo Lett.* 1999, 20 (4), 215–225.
17. *Yi J. Y., Lee G. A., Lee S. Y., Chung J. W., Shin S.* Cryopreservation of Winter-dormant Apple Buds Using Two-step Freezing. *Plant Breed. Biotechnol*. 2013, 1 (3), 283–289. https://doi.org/10.9787/PBB.2013.1.3.283
18. *Gorbunov L. V., Shyianova T. P., Riabchun V. K.* Optimization of dehydration conditions of fruit and berry cuttings. *Henetychni Resursy Roslyn*. 2008, V. 5, P. 182–187. (In Ukrainian).
19. *Pavlov1 A. V., Verzhuk V. G., Orlova1 S. Yu., Radchenko O. Ye., Yerastenkova M. V., Dodonova A. Sh., Gavrilkova Ye. A., Sitnikov M. N., Filipenko G. I., Murashev S. V.* Cryopreservation as a Method to Preserve Some Fruit and Berry Crops and Wild Medicinal Plants. *Probl. Cryobiol. Cryomed*. 2019, 29 (1), 044–057. https://doi.org/10.15407/cryo29.01.044
20. *Liu Y., Wang X., Liu L.* Analysis of genetic variation in surviving apple shoots following cryopreservation by vitrification. *Plant Sci.* 2004, 166 (3), 677–685. https://doi.org/10.1016/j.plantsci.2003.11.003
21. *Rey H. Y., Faloci M., Medina R., Dolce N., Engelmann F., Mroginski L.* Cryopreservation of *Arachis pintoi (leguminosae)* somatic embryos. *Cryo Lett*. 2013, 34 (6), 571–582.
22. *Devi S. D., Kumaria S., Das M. C.* Development of Cryopreservation Protocol for *Aquilaria malaccensis Lam.*, a Recalcitrant Seeded Tropical Tree Species. *Cryo Lett*. 2019, 40 (1), 18–27.
23. *Gorbunov L. V.* Reproducibility results for cryopreservation of different variety cuttings of pomefruit trees. *Problemy Kriobiologii.* 2009, 19 (4), 473–480. (In Russian).
24. *Gorbunov L. V., Petrov I. V.* Interrelation between type and cultivar of berry-like crop and cuttings cryopreservation results. *Biotechnol. acta.* 2013, 6 (2), 121–127. (In Russian).
25. *Gorbunov L. V., Riabchun V. K., Shyianova T. P., Salina A. S.* Method of long-term preservation of fruit and berry cuttings. *Ukraine patent UA 47845. 2010 feb 25*.

МАТЕМАТИЧНА МОДЕЛЬ ОПИСУ ЖИТТЄЗДАТНОСТІ ЖИВЦІВ ПЛОДОВО-ЯГІДНИХ КУЛЬТУР ПІСЛЯ КРІОКОНСЕРВУВАННЯ

Л. В. Горбунов[1]
І. В. Петров[2]
О. В. Звягінцева[1]

[1]Національний технічний університет «Харківський політехнічний інститут», Україна
[2]Інститут проблем кріобіології і кріомедицини НАН України, Харків

E-mail: embiotech@icloud.com

Розроблено математичну модель, що спрощує процес визначення оптимальних параметрів, які забезпечують максимальну життєздатність деконсервованих живців плодово-ягідних культур. Визначено величини мінімальної кількості внутрішньоклітинної води — η_{1min}, що мінімізують ймовірність плазмолізу і — η_{2min} утворення внутрішньоклітинного льоду з урахуванням гетерогенності біооб'єкта.

Розраховано величини вільної води — $\Delta\eta$, що утворюють кристали льоду всередині клітини за кріоконсервування різних сортотипів плодово-ягідних культур. Визначено оптимальні умови зневоднення живців (температура T^{i} і час витримки t_2, мінімальна кількість внутрішньоклітинної води — η_{min}), що забезпечують максимальну життєздатність під час їх сушіння й низькотемпературної адаптації до кріоконсервування. Індивідуальні особливості життєздатності деконсервованих живців різних порід кількісно відображено в показниках вільної води $\Delta\eta$. Максимальної життєздатності деконсервованих живців берези і чорної смородини досягнено за умови, коли внутрішньоклітинна вода знаходиться у зв'язаному, вітрифікованому стані $\Delta\eta = 0$. Розраховане $\Delta\eta > 0$ для живців різних сортів яблук, груш, малини призводить до зниження життєздатності, а повна відсутність зв'язаної води — η_c для сливи, абрикоса, винограду унеможливлює отримання життєздатних зразків після низькотемпературного кріоконсервування.

Ключові слова: математична модель, кріоконсервування, живці плодово-ягідних культур, життєздатність.

МАТЕМАТИЧЕСКАЯ МОДЕЛЬ ОПИСАНИЯ ЖИЗНЕСПОСОБНОСТИ ЧЕРЕНКОВ ПЛОДОВО-ЯГОДНЫХ КУЛЬТУР ПОСЛЕ КРИОКОНСЕРВИРОВАНИЯ

Л. В. Горбунов[1]
И. В. Петров[2]
О. В. Звягинцева[1]

[1]Национальный технический университет «Харьковский политехнический институт», Украина
[2]Институт проблем криобиологии и криомедицины НАН Украины, Харьков

E-mail: embiotech@icloud.com

Разработана математическая модель, упрощающая процесс определения оптимальных параметров, обеспечивающих максимальную жизнеспособность деконсервированных черенков плодово-ягодных культур. Определены величины минимального количества внутриклеточной воды — η_{1min}, минимизирующие вероятность плазмолиза и — η_{2min} образования внутриклеточного льда с учетом гетерогенности биообъекта.

Рассчитаны величины свободной воды — $\Delta\eta$, образующие кристаллы льда внутри клетки при криоконсервировании различных сортотипов плодово-ягодных культур. Определены оптимальные условия обезвоживания черенков (температура T^{i} и время выдержки t_2, минимальное количество внутриклеточной воды — η_{min}), обеспечивающие максимальную жизнеспособность при их сушке и низкотемпературной адаптации к криоконсервированию. Индивидуальные особенности жизнеспособности деконсервировванных черенков различных пород количественно отражены в показателях свободной воды $\Delta\eta$. Максимальная жизнеспособность деконсервированных черенков березы и черной смородины достигнута при условии, когда внутриклеточная вода находится в связанном, витрифицированном состоянии $\Delta\eta = 0$. Рассчитанное $\Delta\eta > 0$ для черенков различных сортов яблок, груш, малины приводит к снижению жизнеспособности, а полное отсутствие связанной воды — η_c для сливы, абрикоса, винограда не дает возможности получить жизнеспособные образцы после низкотемпературного криоконсервирования.

Ключевые слова: математическая модель, криоконсервирование, черенки плодово-ягодных культур, жизнеспособность.