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Development of Cryopreservation Technique for Meristems of *Syringa vulgaris* L. Cultivars

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Abstract: Cryopreservation is considered to be one of the most effective methods for long-term storage of plant genetic resources, particularly for ornamental species. However, there is a very little research on cryopreservation of lilacs. In this study, for the first time the cryopreservation protocol (a variation of a pregrowth-dehydration method) was successfully applied to two cultivars of *Syringa vulgaris*: 'Aucubaefolia' and 'Polina Osipenko'. Explants of both cultivars were able to withstand the different steps of the protocol, and high survival and regrowth percentages were obtained after exposure to liquid nitrogen (67–100% and 63–88%, respectively). The current study is mainly focused on the preculture conditions of the applied method. Based on our results, we propose the use of paclobutrazol (PBZ) with the combination of 6-benzylaminopurine (BAP) and thidiazuron (TDZ) in the preculture medium for increasing explant tolerance to subsequent dehydration and freezing. During post-LN recovery, the explants appeared morphologically normal, and after 12–16 weeks after thawing, they were propagated and cultured as normal plantlets. Therefore, the reported method is effective for long-term storage of lilac meristems and could be used to create a cryobank of achievements in lilac breeding.

Keywords: lilac; cryopreservation; preculture; 6-benzylaminopurine; paclobutrazol; thidiazuron

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1. Introduction

Lilacs are popular ornamental shrubs growing in temperate climates. In addition to landscaping and floristry, they are also used in the culinary, perfumery, cosmetics and healthcare industries. Current pharmacological studies found that the extracts and pure compounds isolated and identified from different parts of lilac species possessed a wide range of biological activities, which explains their use in traditional medicine [1,2].

The genus *Syringa* L. belongs to the Oleaceae family and, according to various classifications, consists of 12 [3,4] to 36 [5] species, mainly distributed in Southeast Europe, Japan, China, the Himalayas, etc. Its natural range covers mountainous areas of East Asia and the Balkan-Carpathian region of Europe. The world assortment of the genus *Syringa* representatives is very diverse. Currently, the International Lilac Register and Checklist includes 2844 registered cultivars, and more than 2000 of them belong to *S. vulgaris* L. [6].

At present, the preservation of ornamental germplasm has become increasingly important. As a result of years of breeding activity, thousands of new hybrids and cultivars were obtained. However, the work on the protection and preservation of plant genetic resources was mainly focused on food crops and endangered species [7]. Scientists are calling for a coordinated international effort to preserve not only rare, endangered, extinct and economically valuable but also ornamental plant species [8,9].

Currently, there are increasing efforts to preserve the constantly expanding diversity of *Syringa* hybrids and cultivars. The world's largest lilac collections include only several hundred

cultivars (Royal Botanical Gardens, Burlington, Canada: more than 400 cultivars; Highland Park, Rochester, NY, USA: over 500 cultivars); scientific and commercial plant biotechnology laboratories also maintain only a few hundred genotypes (Laboratory of Plant Biotechnology of MBG RAS, Moscow, Russia: more than 200 genotypes; PICCOPLANT, Oldenburg, Germany: over 400 cultivars). Generally, most of them are in demand on the plant market. However, plants maintained in field collections are often exposed to various biotic and abiotic stresses (pests, diseases, drought, etc.) [10]. These potential problems highlight the importance of application of alternative conservation methods, necessary for conservation of achievements of lilac breeding. They also provide sustainable use and distribution among public and private gardens. As the number of registered cultivars continues to grow, there is a need to develop more effective methods of long-term storage requiring less space and labour for preservation of rare, valuable and currently uncommon lilacs.

Cryopreservation is one of the most effective techniques for long-term storage of plant genetic resources, which requires limited space, requires a low level of maintenance and preserves the genetic stability of regenerated plants [11]. Cryo-methods are presently used for germplasm preservation in many research centres and institutes around the world [12]. Plant cryopreservation techniques were developed for more than 60 years, and now scientists may use various cryo-techniques for plenty of species from several dozen families [13,14]. Plant germplasm can be cryopreserved by various techniques (slow freezing, vitrification, encapsulation–dehydration, desiccation, etc.) [14,15]. Encapsulation/dehydration [16] and vitrification methods [17] are the most commonly used cryogenic methods. High applicability and rapid implementation of the vitrification technique have led to numerous variations of this method [18–22]. Despite the numerous advantages of cryopreservation, only few available viable protocols can guarantee good regeneration rates of the genetic material, therefore limiting application of cryopreservation for long-term germplasm conservation [23].

Syringa was investigated for in vitro propagation and medium-term storage. Most of the studies concerned multiplication of lilac cultivars, mostly of *S. vulgaris* [24–26]. Conditions of the slow growth culture were also developed for lilac, which enabled three-year-long storage without subcultures [26,27]. However, there are only a few established studies on long-term preservation of *Syringa*. Nukai et al. [28] applied a droplet-vitrification method on 'Julia' (*Syringa* × *henryi* C.K.Schneid). The post-thaw regrowth rate was 20–40%, so the authors expressed the need for further research. However, follow-up experiments revealed some issues: explants survived well after freezing, but they had problems with sprouting into shoots [29]. In addition, there are data on the application of the encapsulation/dehydration method for long-term lilac preservation [30] and published cryogenic protocols for other genera of the Oleaceae family [31–35]. This explains why the development of effective and comprehensive cryopreservation technique for *Syringa* remains a critical issue.

Therefore, the aim of the present research was to apply one of the simple cryopreservation methods for *Syringa vulgaris* cultivars 'Polina Osipenko' and 'Aucubaefolia' and develop efficient technique of long-term storage for lilac.

2. Materials and Methods

The experiments were carried out in the Plant Cryopreservation Group, Department of Cell Biology and Biotechnology, Timiryazev Institute of Plant Physiology of Russian Academy of Sciences, Moscow, Russia in 2021–2022.

2.1. Plant Material

In vitro shoot cultures of two *S. vilgaris* cultivars 'Polina Osipenko' and 'Aucubaefolia' were used in our experiments. They were received from the Laboratory of Plant Biotechnology, Tsitsin Main Botanical Garden of Russian Academy of Sciences, Moscow, Russia. 'Polina Osipenko' (originator: L.A. Kolesnikov, Moscow, Russia) is characterised

by medium regeneration ability (multiplication rate 3–7). 'Aucubaefolia' (originator: A. Gouchault, Orleans, France) is a bud-sport of 'President Grevy' with variegated leaves and high regeneration ability (multiplication rate > 7). Before starting the experiment, the cultivars were cultured on MS or QL media solidified with 6.8 $g \cdot L^{-1}$ agar (C.E. Roeper GmbH, Hamburg, Germany) and supplemented with 0.5, 0.8 or 1.0 $mg \cdot L^{-1}$ BAP with the addition of 0.01 $mg \cdot L^{-1}$ IAA. The plantlets were maintained at 25 ± 2 °C, under a 16 h photoperiod with 2–3 klx light intensity (cool white fluorescence light).

2.2. Cryopreservation

Development of the cryopreservation technique is based on step-by-step optimisation from explant preparation for LN to regrowth after thawing and planting. The variation of pregrowth-dehydration cryo-method based on the technique developed by O.N. Vysotskaya [36] was used in the experiments as it required no toxic cryoprotectants or labour-intensive processes. This method relies on preculture and explant pretreatment which are fundamental aspects of cryo-protocols development, because they are aimed at inducing freezing tolerance. It includes several steps: preculture (i), explant excision (ii), pretreatment (iii), dehydration (iv), fast immersion in LN (v), thawing (vi) and regrowth (vii). Most of the study was focused on optimising preculture conditions.

2.2.1. Preculture

During step (i), explants were cultured on the medium with changes in concentrations of basal MS macronutrients (Table 1) and solidified with 12.0 $g \cdot L^{-1}$ agar (Sigma, St. Louis, MO, USA). The medium contained 60 $g \cdot L^{-1}$ sucrose as a carbon source and 2.7 $g \cdot L^{-1}$ calcium gluconate as a calcium source. Growth regulators BAP (Sigma, St. Louis, MO, USA) (0.2 $mg \cdot L^{-1}$) and TDZ (Sigma, St. Louis, MO, USA) (0.02 $mg \cdot L^{-1}$) were tested in combination with PBZ (Sigma, St. Louis, MO, USA) (0.0 or 1.0 $mg \cdot L^{-1}$) as a plant growth inhibitor (Table 2). All the media were adjusted to a pH of 5.8 prior to autoclaving for 20 min at 121 °C.

Table 1. The differences in composition between basal MS medium and medium used at the step (i).

Macronutrients	Standard MS ($mg \cdot L^{-1}$)	Preculture Medium ($mg \cdot L^{-1}$)
NH_4NO_3	370	740
KNO_3	1640	820
$MgSO_4 \cdot 7H_2O$	1900	950
KH_2PO_4	170	340

Table 2. The composition of tested growth regulators in the medium used at the step (i).

Plant Growth Regulators	Variants of Medium			
	I	II	III	IV
BAP	0.2 $mg \cdot L^{-1}$	-	0.2 $mg \cdot L^{-1}$	-
TDZ	-	0.02 $mg \cdot L^{-1}$	-	0.02 $mg \cdot L^{-1}$
PBZ	1.0 $mg \cdot L^{-1}$	-	1.0 $mg \cdot L^{-1}$	-

At step (i), explants were maintained in a controlled environment (22 °C) for a month under a 16 h photoperiod with 1–2 klx light intensity. Before the start of step (ii), explants were hardened for two weeks at 8 °C under a 16 h photoperiod with 0.4–0.5 klx light intensity.

2.2.2. Explant Excision

Shoot tips of 3–6 mm with 3–5 nodes were used as explants for cryopreservation. The explants were excited from plantlets obtained during the preculture stage. Both apical

and basal explant were used for cryopreservation; after excision, they were transferred to pretreatment medium.

2.2.3. Pretreatment

During step (iii), explants were cultured on the same medium as for step (i) except sucrose (273.84 g·L^{-1}) and agar (10.0 g·L^{-1}) content. The medium was adjusted to a pH of 5.8 prior to autoclaving for 20 min at 121 °C. During this stage, explants were hardened in darkness at 0–2 °C for 48 h before dehydration.

2.2.4. Dehydration

At step (iv), explants were dehydrated on aluminium foil strips under the laminar airflow cabinet at room temperature and 40–60% relative humidity until reaching 30–40% loss of weight (for 4 h). After that, strips with explants were placed into cryovials and rapidly immersed in LN.

2.2.5. Thawing

After 1 week of storage in LN, the cryovials were thawed for 1–3 min in an ethanol bath at room temperature. After thawing, standard MS medium solidified with 9.0 g·L^{-1} agar and supplemented with 0.5 mg·L^{-1} BAP was used as a recovery medium for explant regrowth. The pH medium was adjusted to 5.8 prior to autoclaving for 20 min at 121 °C.

2.2.6. Regrowth

The explants were maintained in a controlled environment (22 °C). After thawing, explants were held in darkness for a week, and after the induction period they were transferred onto a fresh medium and cultured under a 16 h photoperiod with 1–2 klx light intensity.

2.3. Experimental Design and Statistical Analysis

At the preculture stage the experiments were conducted in three independent replications. Data on the height of plantlets were recorded. The effect of medium composition on plants during preculture was investigated using analysis of variance (ANOVA) and Tukey's pairwise tests using PAST 4.11c. software. A p-value < 0.05 was considered significant. Microsoft Office Excel 2019 was used for graphical representations of the results.

For the cryopreservation, 9 explants were kept per cryovial, and 3 cryovials were kept for each variant of preculture medium for both cultivars. For each variant, 10 explants were kept as control after the preculture; 6 explants were kept as control after the pretreatment; and 6 explants as control after the dehydration stages. About 100 explants in total were frozen in LN, and two-thirds of them were thawed and analysed for regrowth rates and storage safety; others were stored in the Cryobank of IPP RAS.

The moisture content of explants before freezing was expressed on a fresh weight basis; dry weight was determined every hour for 4 h of drying. After 14 days of recovery, the survival rates were recorded; the regrowth rates were recorded after 28 days of recovery after thawing. The obtained data were analysed by Chi-square test of independence $p < 0.05$ significance level, following arcsine transformation, using Microsoft Office Excel 2019 and PAST 4.11c. software. Standard errors (SE) and confidence intervals (CI) were calculated according to the methods proposed by Plokhinskii [37] and Isachkin and Krjuchkova [38]. Microsoft Office Excel 2019 was used for graphical representations of the results.

3. Results

3.1. Preculture

The different combinations of PGRs were tested for inhibition of shoot growth and adventitious shoot bud initiation (Table 3). The results of the analyses showed no significant differences in height of plantlets between medium I and III (p = 0.83 for cv. 'Aucubaefolia'

and $p = 1.00$ for 'Polina Osipenko') while the difference between media with (I and III) and without PBZ (II ana IV) was significant ($p < 0.01$).

Table 3. Effect of PGRs on height (cm) of the donor plants.

Cultivar	Variants of Medium			
	I	II	III	IV
Aucubaefolia	1.3 ± 0.1	2.7 ± 0.1	0.9 ± 0.2	3.1 ± 0.3
Polina Osipenko	0.8 ± 0.1	1.9 ± 0.1	0.8 ± 0.2	2.6 ± 0.3

values are mean with SE.

The plants cultured on media without PBZ had elongated shoots with large internodes, while plants cultured on media with 1.0 mg·L^{-1} PBZ had shoots with shortened internodes and well-formed buds. Based on the results, only media supplemented with PBZ were selected for further experiments.

3.2. Dehydration of Explants

Most of the cell water was lost during 1–2 h of dehydration. In the following hours, the weight of explants was not significantly changed. Eventually, the explants lost more than 50% of their weight, and their moisture content dropped to 50–60% (Table 4). As for the woody culture precultured and pretreated on media with high sucrose, it was enough for further freezing in LN and successful recovery after thawing.

Table 4. Moisture content (fresh weight basis) (%) of explants after 4 h of dehydration.

Cultivar	BAP + PBZ	TDZ + PBZ
Aucubaefolia	57.1 ± 0.7	66.7 ± 0.9
Polina Osipenko	56.4 ± 0.7	51.9 ± 0.7

values are mean with SE.

There was no significant relationship neither between the cultivars ($\chi^2 = 1.01$; $p = 0.31$) and none between preculture media ($\chi^2 = 0.19$; $p = 0.67$). There were also no significant differences between used media for both cultivars ('Aucubaefolia': $\chi^2 = 1.74$; $p = 0.24$; 'Polina Osipenko': $\chi^2 = 0.51$; $p = 0.57$). Therefore, the moisture content of explants during dehydration was not affected neither by PGRs of preculture media nor by the characteristics of the cultivars.

3.3. Regrowth after Thawing

Phenol exudation was noticed during explant recovery, and this caused the transfer of explants onto fresh recovery medium after the induction period (keeping in the dark for seven days). No contamination was found, even though liquid nitrogen got inside some cryovials.

Each step prior to freezing in LN was tested for recovery. All the explants were successfully regrowth after the preculture (100%), and almost all the explants were recovered after the pretreatment (92%). The control explants (dehydrated but unfrozen) had high survival rates (Table 5). There were no significant relationships between explant survival of different cultivars or from different preculture media, but for cv 'Aucubaefolia', there were considerable differences between recovery of BAP + PBZ precultured and TDZ + PBZ precultured explants ($\chi^2 = 6.70$; $p = 0.01$).

Most of the explants of both cultivars survived after storage in LN and regenerated successfully (67–100% of viable explants) (Table 5, Figure 1). The different hormone compositions were tried out for explant regrowth after thawing. There were no significant differences neither between explant survival of different cultivars ($\chi^2 = 0.20$; $p = 0.64$) nor between survival of explants precultured on different media ($\chi^2 = 3.93$; $p = 0.05$), but for cv

'Aucubaefolia', there were considerable differences between survival rate of BAP + PBZ and TDZ + PBZ precultured explants ($\chi^2 = 15.99$; $p < 0.01$).

Table 5. Effect of PGRs on explant survival rate (%).

Cultivar	Preculture Medium I			Preculture Medium III		
	Pretreated	Dehydrated	Frozen in LN	Pretreated	Dehydrated	Frozen in LN
Aucubaefolia	100	50.0 ± 0.6	66.5 ± 0.9	100	86.0 ± 1.4	100
Polina Osipenko	100	87.5 ± 1.5	85.0 ± 1.5	83.3 ± 1.3	87.5 ± 1.5	85.0 ± 1.4

values are mean with SE.

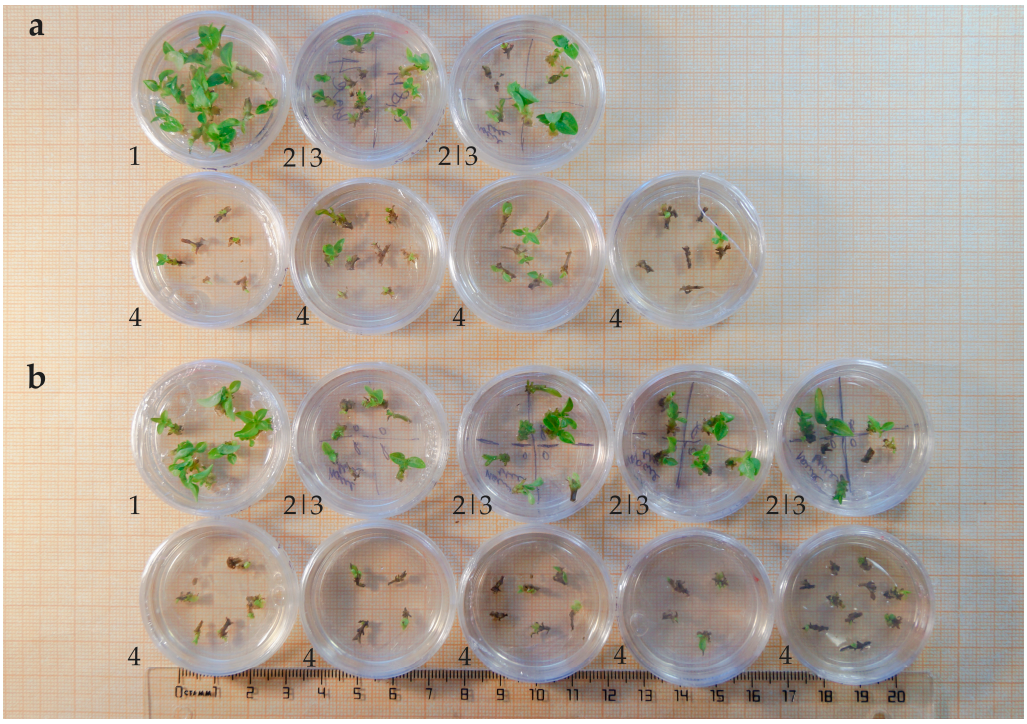
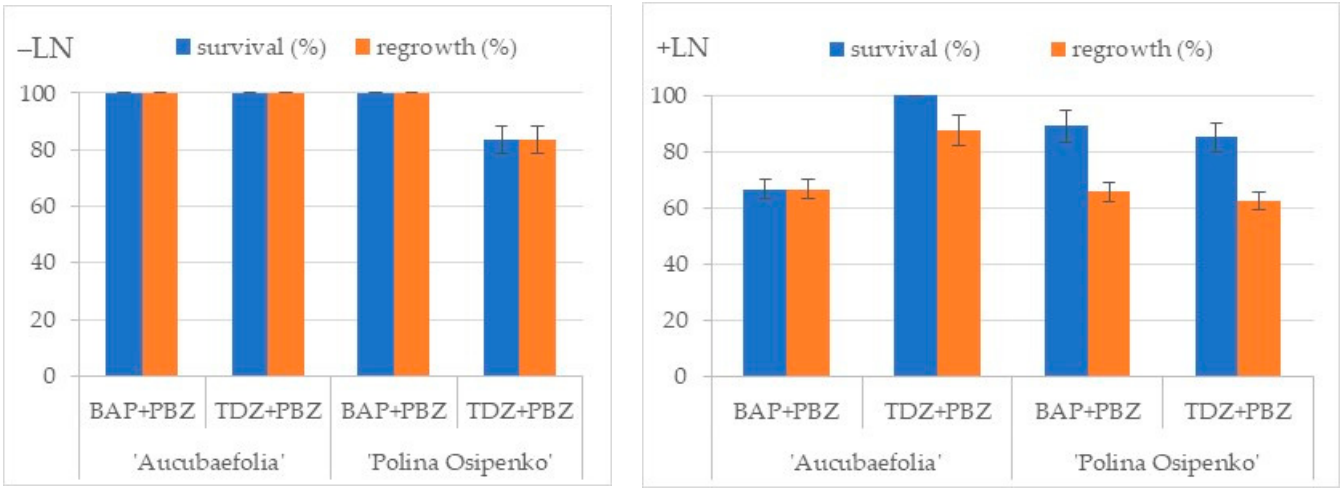


Figure 1. Explant regeneration of (**a**) 'Aucubaefolia' and (**b**) 'Polina Osipenko' after preculture (**1**), pretreatment (**2**), dehydration (**3**) and storage in LN (**4**) at the 25th day of recovery (freezing: 17 February 2022; thawing: 18 February 2022).

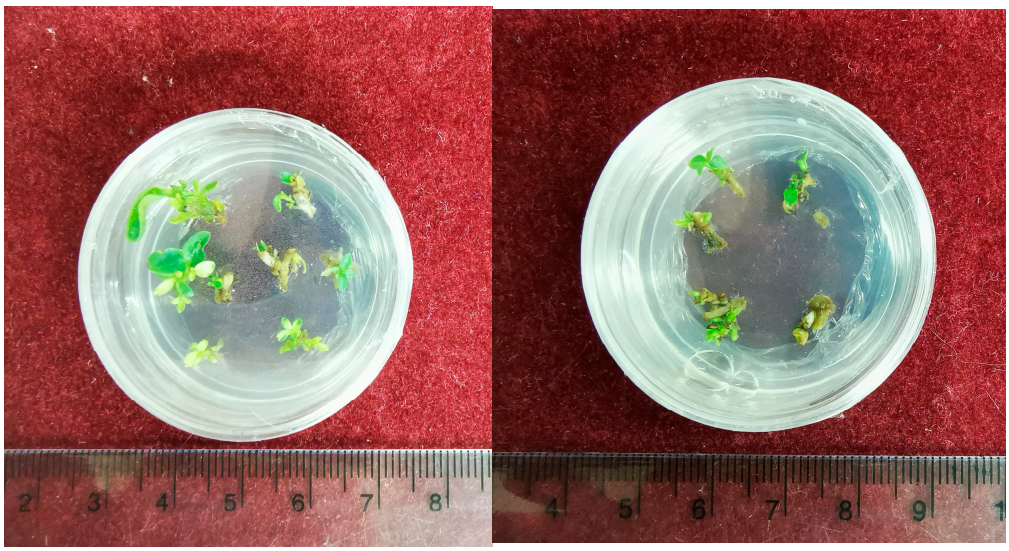
For the regrowth rates after thawing, the Chi-square test of independence showed no significant relationships between cultivars ($\chi^2 = 0.20$; $p = 0.66$) and between preculture media ($\chi^2 = 0.95$; $p = 0.33$). Although the post cryogen regrowth of frozen explants was lower than their survival (70% vs. 86%), it was still very high (Figure 2). Most of the explants produced new leaves (Figure 3) and then sprouted into normal shoots. It must be noticed that 'Aucubaefolia' preserved its characteristic of variegated leaves and demonstrated it along with the explant recovery.

After explants had regrown to normal size (about 1 cm), they were transferred to standard media for lilac cultivation (MS or QL media supplemented with 6.8 $g \cdot L^{-1}$ agar, 0.5 $mg \cdot L^{-1}$ BAP and 0.01 $mg \cdot L^{-1}$ IAA). After 4–6 weeks of the subculture, cryopreserved plantlets were propagated and cultured as normal plants.



(**a**) (**b**)

Figure 2. Survival and regrowth of lilac explants: (**a**) unfrozen (−LN); (**b**) cryopreserved (+LN). The values are mean with CI.



(**a**) (**b**)

Figure 3. Regrowth of explants after 40 days of recovery after thawing (freezing: 17 February 2022; thawing: 18 February 2022): (**a**) 'Aucubaefolia', preculture medium III; (**b**) 'Polina Osipenko', preculture medium III.

4. Discussion

Storage at ultra-low temperatures (LN −196 °C) is based on the reduction in freezable tissue water content through osmotic and/or physical dehydration before immersion in LN. Treatments leading to intracellular solute vitrification are quite drastic and require prerequisite procedures aiming at increasing explant tolerance to subsequent dehydration and freezing. Pretreatment is considered to be one of the most critical steps of commonly used cryopreservation protocols, because some solutions and overexposure may cause chemical toxicity. That is why methods based on cell dehydration prior freezing by treatments with loading and vitrification solutions require plenty of preliminary experiments in order to select the cryoprotectant agent, time and conditions of the cryoprotectant treatments. Meanwhile, the methods based on dehydration by air desiccation require investigations in order to improve the explant physiological state, preculture conditions, etc. The continuous

research, application of other methods or new technological achievements can considerably improve the cryogenic methodologies, allowing the enhancement of the recovery and regrowth of the species [39,40].

Since the application of the droplet-vitrification method on lilac had some issues [28], it was decided to use a cryopreservation method focused more on the increase of plant cryoresistance. The technique developed in IPP RAS was tested in this study [35]. This method was already used in IPP RAS for the long-term storage of strawberries, blackberries, raspberries and rowan [36,41,42].

Plants of temperate regions are able to withstand low temperatures, so cold hardening is usually employed to induce the accumulation of intracellular solutes and increase growth recovery after cryopreservation. Soluble sugars accumulated during cold hardening protect cells against cryo-induced damage by stabilizing membranes during cooling and by reducing freezable water content, thus preventing intracellular ice formation. Some studies have shown the possibility of replacing cold hardening of donor-plants with explants preculture on medium with high concentrations of soluble sugars [43], but cold hardening is still considered essential for successful cryopreservation of most cold-hardy plants. In our study, we used both these methods to induce tolerance to cryopreservation in plants: before the start of the step (ii), the donor-plants were hardened on the medium with 60 $g \cdot L^{-1}$ sucrose at 8 °C for two weeks, and the excited explants were hardened on the medium enriched with sucrose (273.84 $g \cdot L^{-1}$) at 0–2 °C for 48 h. High sucrose in medium significantly increases cold-tolerance [44,45]. Sucrose was also used as osmotic agent, which restricts the water availability to the explant and goes about a development retardant, when added to the medium [46,47].

The applied method was focused on reducing growth processes in donor plants, preparing them for subsequent freezing and obtaining explants of small size but with a maximum number of buds. In order to do this, the mineral composition of the medium was changed; the content of sucrose and agar was increased; and growth regulators and the retardant paclobutrazol were added. Agar was used at higher concentrations due to its ability to reduce growth processes during preculture and vitrification of explants during regrowth [48,49].

The results of our studies showed that the applied method was effective in preparing plants for dehydration and freezing in LN, because not only the recovery after thawing was very high but also the survival and regrowth rates of unfrozen explants reached 100%. Since the increased content of sucrose and agar was due to the technique previously used on other plant genera, the need for using such high concentrations will be investigated further. Moreover, some steps and procedures would be reconsidered in future research in order to optimise the cryogenic technique and obtain simple and effective protocol for cryopreservation of lilacs.

4.1. Effect of PGRs on Micro-Plantlets during Preculture

Preculture is a critical stage in cryopreservation technique. Different pre-treatments, preculture conditions and types of explant affect survival and regrowth after freezing. One way to enlarge the regeneration rate after storage in LN is to use shoot fragments with several nodes as explants. That requires inhibition of gibberellin biosynthesis and reduces elongation of meristematic tissues. For this purpose, scientists usually change medium composition and also used various osmotics and PGRs [50].

In vitro morphogenesis is regulated by optimal balance between phytohormones, mostly auxins and cytokinins [51]. Therefore, PGRs are necessary for normal plant development during in vitro culture. Usually, BAP—the most commonly used cytokinin in plant tissue culture—is used for clonal micropropagation of lilac. However, BAP does not exhibit any inhibiting effect on plant growth (which is needed to obtain optimal explants for effective cryopreservation), so TDZ was also tested in this study. TDZ is a substituted phenyl urea, originally being used as a cotton defoliant. Compared to other PGRs TDZ exhibits both high auxin and cytokinin activity [52]. When used in plant tissue culture, it

initiates adventitious bud and shoot formation and may also inhibit shoot elongation [53]. Moreover, it should be noted that some effects of TDZ on explants can be exhibited after initial treatment (after transfer to media without TDZ) [54]. Since TDZ is more biologically active than BAP and its high concentrations may cause stem thickening and callus formation [55,56], it was used at a much lower concentration (0.02 mg·L^{-1}) than BAP (0.2 mg·L^{-1}).

Both BAP and TDZ induced bud and shoot formation. Studied TDZ concentration did not have enough of an effect on inhibiting shoot growth (the shoot length of plants was 2.8 ± 0.2), so there remained a need to use additional plant growth inhibitors (paclobutrazol). PBZ is one of the members of the triazole family. It inhibits gibberellin synthesis and increases cytokinin levels and consequent reduction in stem elongation [57]. Because of its effects, PBZ can be used for medium-term storage [58,59].

The current research showed that the presence of PBZ in the preculture medium was necessary for obtaining optimal explants for further cryopreservation. The best results were obtained using media I and III (with PBZ); the donor plants cultured on these media produced shortened shoots which were required for excision of optimal explants. The donor plants with elongated shoots obtained from media without PBZ were unsuited for explant excision, which is why only plants precultured on the media supplemented with PBZ were used for further experiments. With this preculture (media I or III), more than 90% of explants withstood pre-treatment; about 80% of explants withstood subsequent dehydration, and over 80% of explants successfully recovered after immersion in LN. Obtaining high survival rate after treatments is a prerequisite in developing and optimising a cryopreservation protocol. Therefore, our results (Table 5) showed that steps prior to immersion to LN had no critical issues.

4.2. Regeneration of the Explants after Thawing

Post-cryogenic recovery show precisely the efficiency of used cryopreservation protocol. The optimisation of each step is carried out according to this indicator. One of the major objectives of our experiments was to establish the effect of preculture conditions on explant recovery after storage in LN.

PGRs are essential for the preculture, survival and regrowth of cryopreserved explants. Depending on used plant hormones, their balance and effects on various processes, the post-LN recovery process may go completely differently. PGRs and their combinations in the recovery medium could be essential for the morphological response in the cryopreserved tissues [60], but we used one of the standard media of lilac propagation to control the explant response to preculture conditions. Both BAP + PBZ and TDZ + PBZ preculture media showed positive effects on the post-LN recovery of the explants; they had high survival (78% and 86%, respectively) and regrowth (66% and 81%, respectively) rates after thawing. TDZ + PBZ preculture affected regrowth of the explants; they regenerated and developed new shoots better compared to the results obtained for BAP + PBZ preculture. The explants precultured on TDZ + PBZ also developed more adventitious buds than explants with BAP + PBZ preculture. Based on the results here, we propose the preculture of plantlets (step i) on the medium supplied with PBZ and the combination of BAP and TDZ. However, the studied PGRs and their effect on post-LN regrowth and morphology of explants are worthy of further investigation, as is the trial of other plant hormones in future experiments with preculture medium.

The studied lilac cultivars responded differently to the applied cryopreservation technique. Cultivar 'Aucubaefolia' showed the best survival (100%) when precultured on the medium with TDZ + PBZ compared to lower 67% survival when precultured on the medium with BAP + PBZ, while cv 'Polina Osipenko' resulted in similar survival and regrowth results for both preculture media (BAP + PBZ: 89% and 66%, respectively; TDZ + PBZ: 85% and 63%, respectively). Cultivar 'Aucubaefolia' showed faster and higher post-cryo-regeneration (83%) and regrowth (77%) than 'Polina Osipenko' (87% and 64%, respectively). It can be assumed that regrowth rate and shoot formation after thawing correlate with regeneration capacity during in vitro culture. 'Aucubaefolia' also displayed

high response to PGRs in the medium; its explants produced more adventitious buds and shoots than 'Polina Osipenko'. Explants of both cultivars from both preculture media (I and III) appeared morphologically normal, and plants were able to be recovered and propagated after 12–16 weeks post-thawing.

According to accepted standards for preservation of plant genetic resources [61,62], for replenishing cryo-collections it is recommended to use protocols with post-cryogenic recovery rates above 20%. So, the reported cryopreservation technique could be used for long-term storage of germplasm of lilac cultivars; it demonstrated higher survival (86% vs. 80%) and regrowth rates (71% vs. 40%) than the droplet-vitrification method published by Nukai et al. [28]. However, further investigations will be necessary to improve the recovery of explants and enhance the efficiency of the cryopreservation protocol for *Syringa*. Moreover, further tests of each step of the cryo-technique will be necessary to optimise and possibly simplify the protocol. This will further allow us to create a cryobank of valuable and rare cultivars and thereby preserve the achievements of lilac breeding.

5. Conclusions

The current study revealed that the applied variation of the pregrowth-dehydration method is effective for cryopreservation of lilac meristems. This study reports the first successful high survival and regrowth rates of explants of *S. vulgaris* cultivars after storage in LN. This finding is an important breakthrough in cryopreservation of lilacs and will lead to further optimising a high efficiency protocol for long-term preservation of *Syringa* meristems.

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Abbreviations

BAP	6-Benzylaminopurine
IAA	Indole-3-acetic acid
IPP RAS	Timiryazev Institute of Plant Physiology of Russian Academy of Science
LN	Liquid Nitrogen
MBG RAS	Tsitsin Main Botanical Garden of Russian Academy of Science
MS	Murashige and Skoog
PBZ	Paclobutrazol
PGRs	plant growth regulators
TDZ	Thidiazuron
QL	Quorin and Lepoivre
SE	standard error
CI	confidence interval

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