

Field performance of cryopreserved seed-derived tomato plants and post-thaw survival of viral-infected meristems

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Abstract: The effectiveness of different cryopreservation techniques of tomato meristems isolated from viral-infected plants 'Irishka' cultivar was determined. The pieces of stem were protected with dimethyl sulfoxide and propylene glycol and cooled in vapour phase of liquid nitrogen ($-170\text{ }^{\circ}\text{C}$). For the vitrification and droplet-vitrification protocols, the meristems were treated with loading solution and dehydrated with different plant vitrification solutions (PVS1 modified, PVS2, 88 % PVS3, PVSN). The samples were placed to sterilized aluminum foil pieces, in 1.2 ml cryovials or in 50 μl aluminum pans for differential scanning calorimetry and were directly immersed into liquid nitrogen. According to the dehydration technique, the meristems were dehydrated with sterile airflow for 120 min. The post-thaw survival rates of meristems (from 34.2 to 78.5 %) were observed only for 50 μl aluminum pans and airflow dehydration. We determined the productivity of plants, obtained from cryopreserved seeds ('Seven', 'Potiron Ecarlate' and 'Druzhba' cultivars). We observed increasing in total and marketable yields for the plants grown from the cryopreserved seeds for all the cultivars. Total number of diseased plants decreased by 33 % for 'Seven', for 'Potiron Ecarlate' it did by 6.7 %, for that of 'Druzhba' the total percentage of sick and healthy plants did not differ after seeds cryopreservation.

Key words: seed cryopreservation; dehydration; meristem cryopreservation; plant vitrification solution; *Solanum lycopersicum* L.; yield

Uspevanje paradižnika v poljskem poskusu, vzgojenega iz zamrznjenih semen in preživetje z virusi okuženih meristemov po odtajanju

Izvleček: V raziskavi je bila določena učinkovitost različnih metod shranjevanja meristemov paradižnika z zamrzovanjem, pridobljenih iz z virusi okužene sorte 'Irishka'. Koščki stebel so bili zaščiteni z dimetil sulfoksidom in propilen glikolom in ohlajeni v parah tekočega dušika ($-170\text{ }^{\circ}\text{C}$). Za vitrifikacijo je bil uporabljen protokol kapljicne vitrifikacije, meristemi so bili obdelani s standardno nosilno raztopino in dehidrirani z različnimi vitrifikacijskimi raztopinami za rastlinska tkiva (modificirana PVS1, PVS2, 88 % PVS3, PVSN). Vzorci so bili potem položeni na koščke sterilizirane aluminijeve folije v 1,2 ml epruvetkah za zamrzovanje ali v 50 μl aluminijastih posodicah za diferencialno vrstično kalorimetrijo, nakar so bili neposredno potopljeni v tekoči dušik. Glede na dehidracijske tehnike so bili vzorci dehidrirani s sterilnim zrakom za 120 min. Preživetje meristemov po odtajanju (od 34,2 do 78,5 %) je bilo opazovano samo za tiste v 50 μl aluminijastih posodicah, ki so bili dehidrirani z zračnim tokom. Določena je bila produktivnost obravnavanih sort, pridobljenih iz semen, shranjenih z zamrzovanjem ('Seven', 'Potiron Ecarlate' in 'Druzhba'). Za vse sorte, ki so bile vzgojene iz semen, shranjenih z zamrzovanjem, je bilo ugotovljeno povečanje celokupnega in tržnega pridelka. Število okuženih rastlin, vzgojenih iz semen po shranjevanju z zamrzovanjem, se je za sorto 'Seven' povečalo za 33 % in za sorto 'Potiron Ecarlate' za 6,7 %. Pri sorti 'Druzhba' se celoten odstotek okuženih in zdravih rastlin ni razlikoval po shranjevanju semen z zamrzovanjem.

Ključne besede: shranjevanje semen in meristemov z zamrzovanjem; dehidracija; raztopine za vitrifikacijo rastlinskih tkiv; *Solanum lycopersicum* L.; pridelek

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1 INTRODUCTION

Conservation of plant genetic resources is important not only for biodiversity preservation, but also for supporting the biotechnology and plant breeding programs. In this regard, the cryopreservation is a powerful tool for a long-term conservation of genetic diversity of plant species and crop improvement, as well it can be considered as an extra strategy even for species with orthodox seeds (Coste et al., 2015). Recently, cryopreservation has also been used to eliminate various pathogens in numerous plant species, so this was coined as cryotherapy (Wang and Valkonen, 2008, 2009; Vieira et al., 2015; Shin et al., 2013). However, there are conflicting findings on the effect of cryopreservation on different crops seeds. The field performance of cryopreserved garlic plantlets under *in vivo* conditions showed the superiority of the morphological traits that increased gradually with the growth improved the net photosynthetic rate, bulb diameter, bulb mass and clove number per bulb (Liu et al., 2019). Cejas et al. (2012) did not observe any phenotypic changes during the early germination stages (0-14 days) of the cryopreserved *Phaseolus vulgaris* L. seeds; but several convincing effects of seeds cryopreservation were revealed at the biochemical level. Arguedas et al. (2018) reported that the significant differences between adult plants derived from cryopreserved and control maize seeds by leaf indices, internodes and ears numbers, plant height and mass of seeds were not observed in field performance. The wild *Solanum lycopersicum* Mill. seeds showed that liquid nitrogen (LN) exposure increased the percentage of seed germination on day 5 but on day 7, the conversion into plantlets and the plant fresh mass differed slightly between non-cryopreserved and cryopreserved samples. Several indicative effects of cryopreservation were recorded at the biochemical level on day 7 of tomato seeds germination (Zevallos et al., 2016). Seeds cryostorage enhanced subsequent plant productivity in terms of growth, but it reduced the seeds production in *Teramnus labialis* (L.f.) Spreng (Acosta et al. 2019). For 9 among 11 species of wild plants, germination of seeds was deteriorated after cryopreservation (Ballesteros and Pence, 2017).

Tomato (*Solanum lycopersicum* L.) is one of vegetables having a world economic importance. Accordingly to the report of «Global Tomato Market 2019 – Robust Consumption Growth in China and India Drives the Global Market» the world tomato sales increased by 6.5 % in 2018 compared to the previous year – up to \$190.4 billion, tomato production reached 188 million tons (+ 3.5% of 2017). However, a large number of viruses that damage the tomato plants can lead to enormous crop losses. *S. lycopersicum* L. plants may be infected by a wide range

of viruses from different families including *Bromoviridae* (*Cucumber mosaic virus* (CMV), *Tomato aspermy virus* (TAV)); *Virgaviridae* (*Tomato mosaic virus* (ToMV)); *Potyvirus* (*Potato virus Y, M* (PVY)), *Secoviridae* (*Tomato ringspot virus* (ToRSV), *Tobacco ringspot virus* (TRSV), *Tomato black ring virus* (TBRV)); *Bunyaviridae* (*Tomato spotted wilt virus* (TSWV)), *Alphaflexiviridae* (*Potato virus X* (PVX), *Pepino mosaic virus* (PepMV)) and *Geminiviridae* (*Tomato yellow leaf curl virus* (TYLCV)). Today, the novel viruses appear and spread rapidly in tomato culture. In Ukraine *Tomato yellow leaf curl virus* (TYLCV), *Tomato Torrado virus* (ToTV) and PepMV, *Tomato brown rugose fruit virus* (TBRFV), CMV, ToMV, *Tobacco mosaic virus* (TMV), TSWV, PVM and PVY have been detected in tomato plants (AIDalain et al., 2014).

Creation of the effective cryopreservation protocol can be vital condition for the virus destruction in the tomato germplasm. The cryopreservation techniques for tomato germplasm were previously described (Kulus, 2019). The different vitrification-based procedures can be identified: encapsulation-dehydration; vitrification; encapsulation-vitrification; dehydration; pregrowth; pregrowth-dehydration and droplet-vitrification (Engelman, 2004). However, the viability of virus-infected samples after cryopreservation is much lower (Wang et al., 2018.). Therefore, the determination of the influence of various cryopreservation techniques on the survival rates of the meristems obtained from viral infected plants is a very topical issue. The previous research demonstrated the transmission of TYLCV and ToMV via seeds (AIDalain et al., 2014, Kil et al., 2016), so their cryopreservation is likely to be able to eliminate these types of viruses.

Our objectives were to: (a) determine effectiveness of different cryopreservation techniques on post-thaw survival of meristems isolated from viral tomato plants of 'Irishka'; (b) study germination, total and marketable yield, number of fruits per plant, mass of one fruit, plant height, number of internodes of cryopreserved seed-derived tomato plants ('Seven', 'Potiron Ecarlate', 'Druzhba').

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL

The *in vitro* tomato culture was obtained from the field growing infected by CMV, ToMV, PVM, TMV, TYLCV plants of 'Irishka' cultivar, which formed sterile flowers. The parts of the stems with meristems were surface sterilized with 30 % commercial bleach (5 % active chlorine) for 25 minutes, then washed 5 times with sterile distilled water for initiation of *in vitro* cultures. Then they

were transferred into glass vials with agar nutrient medium Murashige and Skoog (MS) (Murashige and Skoog, 1962), supplemented with 3 % sucrose without phytohormones. The specimens were cultured at 20 ± 2 °C, with 16 hours of light and 8 hours of darkness under $37 \mu\text{mol}\times\text{m}^{-2}\times\text{s}^{-1}$ light intensity. The explants were propagated by micro-grafting every 30 days.

2.2 CRYOPRESERVATION PROCEDURE IN VAPOUR PHASE OF LIQUID NITROGEN

For cryopreservation of tomato meristems the method described by Grout et al. (1978) was applied for seedlings of *in vitro* grown plants. All the leaves were removed from regenerated plants; the stems were cut and transferred stepwise at 20 min-intervals through increasing the concentrations (5.0, 10.0, 15.0 %) of dimethyl sulfoxide (Me_2SO_4) and propylene glycol (PG). Afterwards the stems were dried with sterile gauze. The stem pieces from the control plants and those treated with cryoprotectants were placed into different types of containers. Containers were made from sealed by heat-pulse welding polyimide-fluoroplastic film PMF-351 («Progress», Russia) of 50 μm , or sterile aluminum foil of 14 μm thickness. Five stems were placed in each container. Cooling was carried out in the vapours phase at a distance of 15 cm above the surface of the LN. The containers were immersed into LN when the temperature in the samples reached -170 °C and held to 30 min. A two-channel sensor measured the temperature change. After that, the containers were thawed by plunging into water at 40 °C for 2 min. Then the stems were three times rinsed in fresh MS without Me_2SO_4 and PG. After rinsing all axillary and apical meristems were dissected from the stems and cultured on filter-paper bridges in glass tubes with liquid MS medium.

2.3 PREPARATION TO VITRIFICATION-BASED CRYOPRESERVATION TECHNIQUES

The apical and axillary meristems up to 1-2 mm with primordia were isolated from three-week *in vitro* cultured plants. The isolated samples were transferred into a liquid MS medium, supplemented with 12 % sucrose and exposed at dark for 24 hours.

2.4 DEHYDRATION

The meristems were sterile airflow-dehydrated (AD) for 120-min and immersed into LN at a needle tip. After-

wards they were warmed in MS medium, supplemented with 10 % sucrose at 25 °C.

2.5 VITRIFICATION

The meristems were treated with loading solution (2 M glycerol and 0.4 M sucrose) for 20 min and then transferred in different plant vitrification solutions (PVS) for 40 min at 22 °C. The dehydrated meristems were put into 1.2 ml cryovials («Corning», USA) or 50 μl hermetic aluminum pans for differential scanning calorimetry (DSC) and were directly immersed into LN for 1 hour. The specimens were warmed in water bath at 40 °C for 2 min. The cryoprotectants were washed out by two consequent transfers of the meristems on filter papers saturated with MS medium, supplemented with 10 % sucrose.

2.6 DROPLET-VITRIFICATION

The meristems were treated with loading solution (2 M glycerol and 0.4 M sucrose) for 20 min and then transferred in different PVS at 22 °C for 40 min. The dehydrated specimens were placed individually into 10 μl droplets of PVS on a piece of previously sterilized aluminum foil (15 \times 20 \times 0.15 mm), which were then directly immersed in LN. The cryopreserved meristems were thawed and the cryoprotectants were washed out by immersion in liquid MS medium, enriched with 12 % sucrose at 24 °C.

2.7 COMPOSITION OF PVS

Modified PVS 1 (22 % glycerol + 13 % PG + 13 % ethylene glycol + 6 % Me_2SO_4 and 0.4 M sucrose); PVS 2 (30 % glycerol + 15 % ethylene glycol + 15 % Me_2SO_4 and 0.4 M sucrose) (Coste et al., 2015); 88 % PVS 3 (44 % glycerol + 44 % sucrose) (Nishizawa et al., 1993, Vilardo et al., 2019); PVS N (34 % sucrose + 15 % glycerol + 14 % ethylene glycol) (Vitsenia et al., 2015).

2.8 POST-THAW CULTURE

The post-thaw meristems were placed in semi-solid MS medium, enriched with 3 % sucrose and stored under dark conditions for a week. They were transferred to the agar MS medium, supplemented with 3 % sucrose, 3 mg l^{-1} gibberellic acid and 0.01 mg l^{-1} indoleacetic acid. Then explants were cultured at 20 ± 2 °C, with 16 hours of light and 8 hours of darkness under $37 \mu\text{mol}\times\text{m}^{-2}\times\text{s}^{-1}$ light in-

tensity. The number of meristems, having a green color for 30 days, determined the survival rate.

To examine the influence of pretreatment steps on the explants the part of meristems was treated with different ways but excluding low-temperature exposure. Non-cooled and not treated with PVS or AD meristems were assumed as the control.

2.9 SEEDS CRYOPRESERVATION

For cryopreservation the tomatoes seeds ('Seven', 'Potiron Ecarlate' and 'Druzhba') were placed into 1.5 ml polypropylene cryovials (FLMedical, Italy) and were directly immersed into liquid nitrogen for 2 days. To thaw, the tubes transferred to air. Seeds were sowed on day 7 after cryopreservation. Substrate to obtain tomato seedlings was potting soil "Rozsada" (Kisson, Ukraine), supplemented with coco coir (Ceres, Sri Lanka).

2.10 CHARACTERISTICS OF TOMATO CULTIVARS

Three cultivars of tomato were obtained from the Institute of Vegetable and Melon Growing of the National Academy of Agrarian Sciences of Ukraine.

The 'Seven' is a breeding cultivar of the Institute of Vegetables and Melons, Ukraine; it is resistant to major diseases. The growing season lasts 107-112 days and this variety is of late maturity with determinate plant type. Fruits are flattened, bright-red colored. The mass of one fruit can reach 350 g.

The 'Druzhba' cv. is a breeding material sourced of the Tiraspol Institute of Vegetables, Moldova; it is especially resistant to late blight. The growing season lasts up to 115 days; it is medium early maturity variety with determinate type of plant. Fruits are round, yellowish-orange in color. The mass of one fruit can gain up to 100 g.

The 'Potiron Ecarlate' is a France native cultivar. It is mid early maturity variety with indeterminate type of plant. Their slightly flat and ribbed shape is reminiscent of pumpkin; the skin is two-tone, of yellow and red color. Each fruit can reach a mass of 600 g.

2.11 FIELD RESEARCH

Tomato seedlings were grown in a greenhouse without heating. The seeds were planted on April 10, 2019. Mass shoots were received after 10-12 days. Seedlings were planted in open ground in the third decade of May. The area of the accounting site was 20 m². The planting scheme was 70 × 35 cm. Caring for the plants consisted

of systematic hoeing of the soil and irrigation (norm 300-500 m³ha⁻¹). During the growing season, morphological description was performed according to the classifier of the *Solanum lycopersicum* species. We recorded the seed germination, total and marketable yield, number of fruits per plant, mass of one fruit, plant height, number of internodes and amount of healthy plants.

2.12 STATISTICAL ANALYSIS

In all experiments, 10-25 meristems and 100 seeds were used per experimental condition and the experiments were replicated 3-5 times. The results were statistically analyzed using Software Past 3. The results are presented as mean and standard deviation. For establishing statistical significance, we used non-parametric Mann-Whitney criterion. The differences were considered significant at $p < 0.05$.

2.13 WEATHER DATA FOR EXPERIMENTAL REGION

Some weather data 2019 year and long-term ones were obtained from the experimental area (Kharkiv region), and were listed in Table 1.

3 RESULTS AND DISCUSSION

3.1 CRYOPRESERVATION OF MERISTEMS

It was shown that regeneration rate made 78 % (from 70 to 83.3 %) for the control group. This parameter did not statistically change for the meristems isolated from stems after cryoprotectant treatment. The post-thaw survival rate was 0 % for two types of containers (Tab. 2). Thus, dissected of meristems from rewarmed stems was not good for *in vitro* grown plants cryopreservation.

For the vitrification-based procedure, preculture of shoot apices with sucrose-enriched medium prior to dehydration with different PVSs has been reported to be effective to improve post-thaw survival of tomato (Kulus, 2019). Meristems were precultured for 24 hour in liquid MS medium, enriched with 12 % sucrose. The results indicated the survival rate of meristems after cultivation in this medium did not differ from the control value (Fig. 1).

Since vitrification solutions contain high concentrations of cryoprotectants such as glycerol, ethylene glycol, propylene glycol or dimethyl sulfoxide, an essential step for successful vitrification is to identify the survival rate

Table 1: Climate conditions of the Ukrainian Eastern forest-steppe

Climate conditions	April	May	June	July	August	September
Rainfall, mm	25.5	58.5	14.0	51.0	7.5	32.0
Rainfall, long-term average, mm	40.8	55.5	65.0	73.3	41.9	48.8
Average daily temperature, °C	10.3	17.9	24.0	21.5	21.5	15.7
Average daily temperature long-term average, °C	9.6	16.5	20.2	21.3	19.8	14.1
Maximum temperature, °C	26.0	30.0	34.0	32.0	33.0	29.0
Long-term maximum temperature, °C	30.0	33.0	38.0	36.5	37.5	31.8
Minimum temperature, °C	-4.0	4.0	10.0	12.0	6.0	-5.0
Long-term minimum temperature, °C	-11.0	-6.8	1.0	6.0	1.5	-6.0
Minimum temperature of soil surface, °C	-4.0	4.0	10.0	12.0	6.0	-5.0

of meristems after PVSs treatment. This index was also determined for the airflow dehydrated meristems. After treatment of meristems with 88 % PVS 3 the growth recovery ranged from 35.5 to 48.5 %, such differences were significantly lower in comparison with the control group. In other variants, no significant differences were observed; re-growth percentages were between 66.6 and 83.3 % (Fig. 1). We observed same decrease in regeneration rate of meristems of the control group. It was most likely related with their reduced viability due to viral infection or their damage during isolation. The decrease in the number of viable meristems after 88 % PVS 3 treatment may be associated with a toxic effect of high concentrations of cryoprotectants or with osmotic responses that lead to damage of samples. A reduced exposure time for meristems in this solution is likely capable to obtain a higher regeneration rate.

Complete death rates were recorded in a week for all the PVSs treated meristems, which were cryopreserved by droplet-vitrification and vitrification in cryovials. The death of the meristems immersed into LN on a piece of aluminium foil can be associated with their damage during the liquid nitrogen boiling or during warming in a nutrient medium through active rehydration. In case of vitrification in cryovials, death was caused by an unbalanced cooling and heating rate, which can lead to the formation of ice crystals.

After cryopreservation in aluminum pans for DSC,

the survival rates of meristems from 34.2 to 78.5 % were observed. The post-thaw survival rates of meristems were 30–40 % for 88 % PVS 3, 70–78.5 % for modified PVS 1, 60–78.5 % for PVS 2 and 55.5–70 % for PVS N, so, the differences between non-cooled and cryopreserved explants were not significant (Fig. 1).

In case of AD the growth recovery of meristems ranged from 63 to 83 %. After cooling at the needle tip, the survival rates did not change significantly (Fig. 1).

It should be noted that all survived but non-cooled meristems formed shoots and regenerated in plants within a month. Despite the high level of post-thaw meristems survival, we could not achieve the formation of the plants-regenerants. During two weeks of the experimental study in the post-thaw conditions, we observed the onset of meristem growth, which stopped but shoots were green. We transferred them to a fresh MS medium but no growth was observed. A month later, the samples remained green but we stopped monitoring them. Selection of phytohormones in the reculture medium or light condition may be necessary.

Thus, we have shown that meristems obtained from the virus-infected 'Irishka' plants had a reduced viability. Cryopreservation of dehydrated with different PVS meristems by droplet-vitrification or vitrification in cryovials did not result in any survival rate. Freezing the pieces of stem under the PG and Me₂SO₄ protection in vapour phase of LN followed by immersion into liquid nitrogen

Table 2: The regeneration rates of tomato meristems after PG, Me₂SO₄ pretreatment and cryopreservation in vapour phase of liquid nitrogen

Variants	Regeneration rate, %		
	Control	PG	Me ₂ SO ₄
Non-frozen	78.31 ± 5.24	76.66 ± 5.47	71.4 ± 4.066
Frozen in aluminium foil containers	0	0	0
Frozen in polyimido fluoroplastic containers	0	0	0

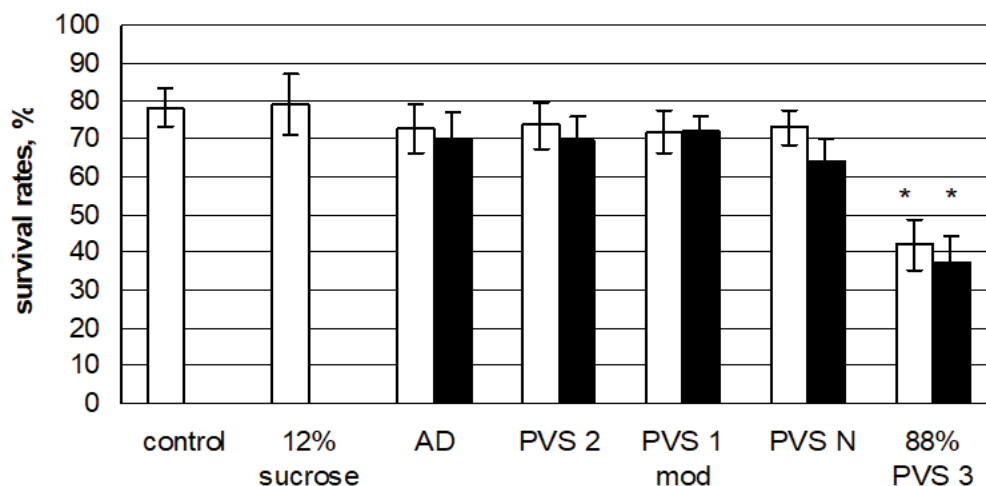


Figure 1: Survival rates of tomato meristems after AD and vitrification in 50 μ l aluminum pans for DSC: \square – non-cooled, \blacksquare – re-warmed. Note: * – differences are significant if compared with the control group, $p < 0.05$

was also ineffective. The use of hermetic aluminum pans for DSC allows us to get a high level of the preserved meristems, independently of PVSs used. Dehydration of the meristems in the airflow and cooling at the needle tip by direct immersion into LN also made it possible to obtain a high survival rate.

3.2 CRYOPRESERVATION OF SEEDS

Cryopreservation of plant materials in LN has been described as a suitable technique to conserve genetic resources of several species. However, the potential effects of LN in subsequent plant growth in field should be studied before large-scale implementation of cryopreserved germplasm banks. In our work, we investigated the effect of LN on the growth and development of plants of three tomato cultivars in field.

For ‘Seven’ all the studied economically valuable features such as marketable yield and general productivity were significantly higher for the plants grown from the cryopreserved seeds. The increase in total and marketable yields compared with the control group was 351 and 268 % respectively. The productivity from each plant was increased almost in five times, because the mass of one fruit and the number of them were higher (Tab. 3).

For ‘Potiron Ecarlate’ the marketable yield increased in 220 %, the mass of one fruit was significantly lower for plants grown from cryopreserved seeds; the number of fruits per plant was significantly higher (Tab. 3).

It was shown that for the ‘Druzhba’ the data of total and marketable yield increased in 27.8 and 71.9 % respectively, number of fruits per plant was significantly higher (Tab. 3).

The height of plants and number of internodes for all the cultivars did not change significantly. However, there was a tendency to an increase in these indices for the plants grown from frozen seeds (Tab. 3).

During the growing season in 2019 in Ukraine, we observed many infected plants with both viral and fungal diseases. It was established that the treatment of seeds with LN led to a decrease in the number of plants infected by viruses. The total number of infected plants grown from the cryopreserved seeds decreased by 33 % for the ‘Seven’, for ‘Potiron Ecarlate’ it did by 6.7 %, for the ‘Druzhba’ the total percentage of sick and healthy plants did not differ (Tab. 3).

Our results indicate that the germination of tomato seeds after cryopreservation did not change compared to the control. The tomato seeds of relatively small size can be cryopreserved without sophisticated pretreatment, required for more differentiated tissues. They are described as both desiccation and liquid nitrogen tolerant. Up-to-date, seeds of several tomato cultivars were successfully stored in LN. Storage periods ranging from 180 to 1,095 days resulted in germination rates of 99 % when water content of 6–7 % fresh mass. If the moisture content was at 8.7 %, the germination rate of rewarmed seeds fell to 84 % (Grout and Crisp 1995). On the other hand, Montoya et al. (2000) found that 69–88 % of cryopreserved seeds remained viable, but did not germinate after storage in LN.

Cryopreservation of tomato meristems in hermetic aluminum pans for DSC, allowed us to obtain a high level of survival rate, but no further recovery was observed. Al-Abdallat et al. (2017) reported the same problem of the divergence between survival and recovery rates. Despite the survival rate of the cryopreserved transgenic

Table 3: Field performance of cryopreserved tomato seeds, 2019

	'Seven'		'Potiron Ecarlate'		'Druzhba'	
	-LN	+LN	-LN	+LN	-LN	+LN
seed germination, %	100	100	100	100	100	100
total yield, kg m ⁻²	1.10	4.97*	3.28	3.49	2.95	3.77*
marketable yield, kg m ⁻²	0.00	2.68*	0.51	1.57*	1.53	2.63*
number of fruits per plant, pcs	5.5	7.0	2.3	6.5*	12.9	18.0*
mass of one fruit, g	50.00	115.65*	181.00	117.11*	50.49	41.15
plant height, cm	66.7	68.3	109.5	114.7	74.7	78.8
number of internodes, pcs	10.2	11.4	16.8	17.7	10.7	12.4
number of healthy plants, %	0	33.33*	13.33	20.0*	6.67	6.67

Note: * – differences were significant compared with non-cryopreserved seeds: -LN – control seeds, +LN – cryopreserved seeds

tomato shoot tips reached even 70 %, however, no further recovery was possible. The authors considered that meticulously plant growth regulators selection, and their concentration optimisation, is required. Additionally, hormonal regulation of tomato explants growth can be altered by the cryopreservation procedure. For example, Grout et al. (1978) reported that viable *S. lycopersicum* explants (cryopreserved shoot tips) produced shoots directly by typical meristem growth when cultured in the presence of gibberellic acid after rewarming. Without gibberellic acid, the surviving explants produced callus and, subsequently, adventitious shoots. On the other hand, non-cryopreserved plant material produced shoots directly without the requirement for addition any plant growth regulators. In our study, we added 3 mg/l gibberellic acid and 0.01 mg/l indoleacetic acid to the reculture medium, but we still did not observe the regrowth of the cryopreserved meristems. Perhaps in order to enhance the uptake of phytohormones, a semi-solid medium, with reduced by half agar concentration, can be applied at the beginning of the recovery culture (Coste et al., 2015).

4 CONCLUSIONS

It was shown that the meristems obtained from virus-infected plants 'Irishka' had a 78 % viability. The effect of 20 % PG, 20 % Me₂SO₄, different PVSs and dehydration by airflow on the regeneration potential of meristems was determined. The significant decrease in the regeneration rate was obtained for meristems treated with 88 % PVS3 (42 % vs 78 %), other variants of pretreatment did not strongly change the meristems regeneration rate.

We determined the possibility of tomato meristems cryopreservation by freezing in vapour phase of LN, droplet-vitrification, as well as vitrification in cryovials and in aluminum pans for DSC. Freezing of stems pieces

in vitro grown plants after PG and Me₂SO₄ treatment in vapour phase of LN was ineffective. Cryopreservation of meristems by droplet-vitrification or vitrification into cryovials did not allow receiving any survival rate. Vitrification in aluminum pans for DSC did not change the meristems survival rate if compared with treated but non-cooled explants. Dehydration of the meristems in the airflow and cooling at the tip of the needle by a direct immersion into LN also made it possible to obtain a high survival rate.

It was shown that all the studied economically valuable features such as marketable and total yield were significantly higher for the plants grown from the cryopreserved seeds of 'Seven', 'Potiron Ecarlate' and 'Druzhba'. The height of plants and number of internodes for all the cultivars did not change significantly; however, there was a tendency to an increase in these indices for the plants grown from the frozen seeds. The total number of infected plants grown from the cryopreserved seeds decreased by 33 % for the 'Seven', for 'Potiron Ecarlate' it did by 6.7 %, for the 'Druzhba'. total percentage of sick and healthy plants did not differ. Thus, the cryotherapy can likely applied for the tomato seeds, but this will demand additional experiments.

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