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Does Cryopreservation Improve the Quality of Tomato Seeds? †

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Abstract: Tomato (*Solanum lycopersicum* L.) is one of the vegetables with world economic importance. However, a large number of viruses that damage tomato plants can lead to enormous crop losses. In our work, we investigated the effect of liquid nitrogen on the growth and development of plants of three tomato cultivars in the field. An increase in the total and marketable yields of the plants grown from cryopreserved seeds was obtained. The height of the plants and the number of internodes for all the cultivars did not change significantly. A decrease in the total number of viral and fungal plants was observed for two cultivars.

Keywords: cryopreservation; liquid nitrogen; seeds; tomato; viruses; yield

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1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the vegetables with 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 USD 190.4 billion [1]. According to the World Processing Tomato Council report, the global tomato processing volume in 2021 was 38.92 million tons, which was 8% more than in 2020 (38.6 million tons). Ukraine ranks sixth in the world in terms of tomato processing [2]. The popularity of tomatoes relates to the fact that they can be eaten in multiple forms, either fresh or processed, some of which are as follows: whole peeled tomatoes, tomato pulp, juice, puree, paste, sauces, ketchup, pickled tomato, tomato powder, flakes, dried tomato fruits and tomato-based foods [3]. A total of 312 species of tomato viruses (including satellite viruses) and viroids were verified. They are classified into 39 genera and 22 families [4]. Viral phytopathogens cause about half of infectious plant diseases. In *Solonaceae* species, viruses that infect tomato plants in Europe, such as tomato torrado virus, tomato chlorosis virus, infectious tomato chlorosis virus, pepino mosaic virus, or brown tomato virus, have caused significant yield and economic losses [5]. According to Salem et al., tomato brown rugose fruit virus is a highly virulent emerging virus, causing disease outbreaks and significant crop losses worldwide [6].

In Ukraine, tomato yellow leaf curlvirus, tomato torrado virus, pepino mosaic virus, tomato brown rugose fruit virus, cucumber mosaic virus, tomato mosaic virus, tobacco mosaic virus, tomatos potted wilt virus, and potato virus Y and M have been detected in tomato plants [7].

Seed germination efficiency is a parameter of significant importance. Rapid and uniform seedling emergence is the basic requirement to increase the crop yield and quality [8]. Improving seed vigor is a primary objective of the seed production industry, to enhance the critical and yield-defining stage of crop establishment [9]. A higher quality of seed results in a shorter time between sowing and seedling emergence [10]. This results in better crop establishment in the field, especially under adverse environmental conditions. The timing, pattern and extent of seedling emergence have a profound impact on the crop yield and market value [10]. It is well known that rapid emergence can lead to an increase in the yield potential by shortening the number of days from sowing to complete growth [10]. A magnetic field, UV radiation, gamma radiation, X-rays, microwaves and termopriming (low or high temperatures) are some of the physical agents that are used for seed priming [11]. It is known that freezing the seeds is a stage of their stratification [12]. Moreover, freezing seeds to ultra-low temperatures of liquid nitrogen (−196 °C) (i.e., cryopreservation) is a necessary condition for their long-term storage [13,14].

There are conflicting data on the effect of cryopreservation on various crop seeds. Cejas et al. [15] did not observe any phenotypic changes during the early germination stages (0–14 days) of the cryopreserved *Phaseolus vulgaris* seeds, but several convincing effects were recorded at the biochemical level; the most significant effect of seed cryostorage was the increase in chlorophyll pigments. Arguedas et al. [16] reported that the significant differences in performance between adult plants derived from cryopreserved and control maize seeds were not observed in the field. The wild *Solanum lycopersicum* Mill. seeds showed that liquid nitrogen exposure increased the percentage of seed germination on the fifth day, but on the seventh day, the number of seedlings and the fresh weight of the plants did not differ significantly between the non-cryopreserved and cryopreserved samples. Seed cryostorage enhances subsequent plant productivity, in terms of growth, but it reduces seed productionin *Teramnus labialis* [17]. For 9 out of 11 species of wild plants, the germination of seeds was decreased after cryopreservation [18]. In addition to long-term storage, the ultra-low temperatures of liquid nitrogen can eradicate viruses. Shoot tip cryotherapy, which was refined based on shoot tip cryopreservation, has been established as a novel method for the efficient eradication of plant pathogens, including viruses, viroides, phytoplasma, and bacteria [19,20]. Recently, cryotherapy has been demonstrated to eradicate seven unrelated groups of viruses and two types of bacteria-like pathogens from several species of economic importance, i.e., *Prunus*, *Musa* spp., *Vitis vinífera*, *Fragaria ananassa*, *Solanum tuberosum*, *Rubus idaeus*, *Ipomea batatas*, *Dioscorea opposite*, and *Allium sativum* [21].

Tomatoyellowleafcurlvirusandtomatomosaicviruscan be transmissible via seeds [7,22]. Therefore, it is interesting to explore the possibility of virus elimination through seeds cryopreservation.

The aim of the study was to determine the possibility of obtaining healthy plants from cryopreserved seeds, and to determine their productivity in the field.

2. Materials and Methods

Tomato seeds of the cultivars Seven, Potiron Ecarlate and Druzhba, with a 9–10% moisture content, were used in this study. For cryopreservation, tomato seeds were placed into 1.8 mL Eppendorf-type polypropylene centrifuge tubes (30 seeds per cryovial), and were directly immersed into liquid nitrogen and held at this temperature during month. For thawing, the cryovials were transferred to room temperature (25 °C) in the dark. Seeds were sowed on day 7 after cryopreservation.

The seeds were planted in a wood box (60 cm × 60 cm × 20 cm depth), with three rows of thirty seeds in each, on 10 April 2019. The substrates used to obtain tomato seedlings were potting soil "Rozsada" (Kisson, Ukraine) and supplemented with coco coir (Ceres, Sri Lanka). Mass shoots were received after 10–12 days. Tomato seedlings were grown in a greenhouse under natural light conditions at 22–25 °C. Seedlings were planted in the open ground on 23 May 2019. The area of the accounting site was 20 m^2. The planting scheme was 70 × 35 cm. Caring for the plants consisted of systematic hoeing of the soil and irrigation (norm 300–500 m^3ha^{-1}). During the growing season, a morphological description was performed, according to the classifier of the species *Solanum lycopersicon* [23]. We recorded the seed germination, total and marketable yields, plant height, number of internodes, and number of healthy plants. At this stage, the number of healthy plants was determined by external characteristics and their ability to form fruits.

The field research was conducted at the Institute of Vegetable and Melon Growing of the National Academy of Agrarian Sciences of Ukraine, located in the eastern part of the Left Bank Forest-Steppe of Ukraine. The general characteristic of the land relief was undulating plain. The type of soil was typical loamy chernozem in the carbonate loess, which was characterized by the following agrochemical parameters of the one soil layer: pH of the salt extract—7.08; the total humus content—2.65%; the content of easily hydrolyzed nitrogen—58.8 mg/kg; mobile phosphorus—44.9 mg/kg; exchange able potassium—34.4 mg/kg. The climate of the study area was temperate continental with unstable humidity and air temperature. The predecessors of tomato plants in the experiments were oats with pea. The level of soil moisture—not less than 70% HB—was maintained with the use of drip irrigation because the humidity in the area was insufficient. On average, 450–600 mm of precipitation falls per year. The temperature regime was characterized by a long period of intensive vegetation of plants. The number of days with an average daily temperature above 15 °C was 95–125 days, and above 10 °C was 150–200 days. The sum of air temperatures during the growing season was 2400 degrees.

Treatments were arranged in a completely randomized design, with three replications of 90 seeds per treatment. All data were statistically analyzed using «PASTv3.11» (Oyvind Hammer, Norway). The results are presented as the mean and standard deviation. The significance of mean differences between experimental and control groups was determined using one-way ANOVA test (Tukey's pairwise) with a 95% probability level.

3. Results and Discussion

Cryopreservation of plant materials in liquid nitrogen has been described as a suitable technique to conserve genetic resources of several species [15]. In our work, we investigated the effect of liquid nitrogen on the growth and development of the plants of three cultivars of tomato in the field. For the Seven cultivar, all the studied economically valuable features (total and marketable yields) were significantly higher for the plants grown from the cryopreserved seeds. The increase in total and marketable yields compared with the control was 351 and 268%, respectively. For the Potiron Ecarlate cultivar, the marketable yield increased by 220%. It was shown that for the Druzhba cultivar, the total and marketable yields increased by 27.8 and 71.9%, respectively (Table 1). The height of the plants and the number of internodes for all the cultivars did not change significantly. However, these indices tended to increase for the plants grown from frozen seeds (Table 1).

Table 1. 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, kgm^{-2}	1.10	4.97 *	3.28	3.49	2.95	3.77 *
marketable yield, kgm^{-2}	0.00	2.68 *	0.51	1.57 *	1.53	2.63 *
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.

During the growing season in 2019 in Ukraine, we observed many damaged plants, with both viral and fungal diseases. It was established that the treatment of seeds with liquid nitrogen led to an increase in the number of healthy plants that had the usual characteristic morphological features of tomato plants, without yellowing and twisting of leaves, browning of fruits, and others. In the future, we plan to determine the viral load in tomato plants using the method of enzyme-linked immunosorbent assay. The total number of diseased plants grown from the seeds treated with liquid nitrogen decreased by 33% for the Seven cultivar, for Potiron Ecarlate, it did by 6.7%, and for the Druzhba cultivar, the total percentage of sick and healthy plants did not differ (Table 1).

Our results indicate that the germination of tomato seed safter cryopreservation did not change compared to the control, and was 100% (Table 1).The relatively small tomato seeds can be cryopreserved without the sophisticated pretreatment required for more differentiated tissues.

4. Conclusions

The results of our experiment, which aimed to determine the field characteristics of plants grown from cryopreserved seeds, showed that the total and marketable yields significantly increased for all the cultivars. For the Seven and Potiron Ecarlate varieties, an increased number of healthy plants were observed. Cryopreservation can likely be carried out not only for the long-term storage of seeds, but also for pre-sowing treatment of tomato seeds.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data obtained has not been previously presented anywhere.

Conflicts of Interest: The authors declare no conflict of interest.

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