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Does the Cryopreservation Improve the Quality of Tomato Seeds? †

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Abstract: Tomato (*Solanum lycopersicum L.*) is one of vegetables having a world economic importance. However, a large number of viruses that damage the 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 field. An increase in the total and marketable yield of plants grown from cryopreserved seeds was obtained. The height of plants and the number of internodes for all 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 world economic importance vegetables. 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. However, a large number of viruses can lead to enormous crop losses. *S. lycopersicum* L. plants may be infected by a wide range of viruses from different families. In Ukraine tomato yellow leaf curl virus, tomato torrado virus, pepino mosaic virus, tomato brown rugose fruit virus, cucumber mosaic virus, tomato mosaic virus, tobacco mosaic virus, tomato spotted wilt virus, potato virus Y and M have been detected into tomato plants [1].

Seed germination efficiency is of great importance in agriculture. The rapid and uniform seedling emergence is the basic requirement to increase the crop yield and quality. Improving seed vigor is a primary objective of the industry seed production to enhance the critical and yield-defining stage of crop establishment [2]. A higher quality of seed results in a shorter time between the sowing and seedling emergence. This results in a better crop establishment in the field, especially under adverse environmental conditions. The timing, pattern and extent of seedling emergence have a profound impact on crop yield and market value. 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 ground [3]. The 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 [4]. It is known, that freezing of seeds is a stage of their stratification.

Moreover, freezing seeds to ultra-low temperatures (i.e., cryopreservation) is a necessary condition for their long-term storage [5,6].

There are conflicting data on the effect of cryopreservation on various crops seeds. Cejas et al. [7] 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. Arguedas et al. [8] reported that the significant differences between adult plants derived from cryopreserved and control maize seeds were not observed in field performance. The wild *Solanum lycopersicum* Mill. seeds showed that liquid nitrogen exposure increased the percentage of seed germination on the 5th day, but on the 7th day, the number of seedlings and fresh weight of 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 the seed production in *Teramnus labialis* [9]. For 9 among 11 species of wild plants, germination of seeds was decreased after cryopreservation [10]. The researchers [1,11] demonstrated the transmission of tomato yellow leaf curl virus and tomato mosaic virus via seeds, so cryopreservation is likely to be able of eliminating the viruses. 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

For cryopreservation the tomatoes seeds (Seven, Potiron Ecarlate and Druzhba cultivars) were placed into 1.8 mL polypropylene centrifuge tubes and were directly immersed into liquid nitrogen on month. For thawing, the cryovials were transferred to air. Seeds were sowed on day 7 after cryopreservation.

Substrate to obtain tomato seedlings were potting soil "Rozsada" (Kisson, Ukraine) supplemented with coco coir (Ceres, Sri Lanka).

The seeds were planted on April 10, 2019. Mass shoots were received after 10–12 days. Tomato seedlings were grown in a greenhouse without heating. Seedlings were planted in the open ground on May 23, 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, morphological description was performed according to the classifier of the species *Solanum lycopersicum*. We recorded the seed germination, total and marketable yield, plant height, number of internodes and amount of helthy plants.

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 character of the land relief is undulating plain. The type of soil is typical loamy chernozem in the carbonate loess, which is 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 and exchangeable potassium—34.4 mg/kg. The climate of the study area is temperate-continental with unstable humidity and air temperature. The predecessors of asparagus 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 of the area is insufficient. On average, 450–600 mm of precipitation falls per year. The temperature regime is characterized by a long period of intensive vegetation of plants. The number of days with an average daily temperature above 15 °C is 95–125 days, above 10 °C—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 100 seeds per treatment. All data were statistically analyzed using «PAST v 3.11» (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. In our work we investigated the effect of liquid nitrogen on the growth and development of plants of three varieties of tomato in field.

For Seven cultivar, all the studied economically valuable features 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 Potiron Ecarlate cultivar, the marketable yield increased in 220%. It was shown that for the Druzhba cultivar the data of total and marketable yields increased in 27.8 and 71.9% respectively (Table 1).

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 (Table 1).

During the growing season in 2019 in Ukraine, we observed lots of damaged plants with both viral and fungal diseases. It was established that the treatment of seeds with liquid nitrogen led to a decrease in the number of plants infected by viruses. The total number of diseased plants grown from the seeds treated with liquid nitrogen decreased by 33% for the Seven variety, for Potiron Ecarlate it did by 6.7%, for the Druzhba cultivar the total percentage of sick and healthy plants did not differ (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, 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 *
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.

Our results indicate that the germination of tomato seeds after cryopreservation did not change compared to the control. The relatively small tomato seeds can be cryopreserved without sophisticated pretreatment, required for more differentiated tissues.

4. Conclusions

The results of our experiment to determine the field characteristics of plants grown from cryopreserved seeds showed that total and marketable yields were significantly increased for all 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 long-term storage of seeds, but also for pre-sowing treatment of tomato seeds.

Author Contributions: N.S., T.I. and conceived and designed the experiments; N.S., T.M., A.M. and G.K. performed the experiments; T.M. and N.B. statistical data processing and analysis; N.S., T.I. wrote the paper. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of Institute for Problems of

Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine (protocol №12, on 24 December 2018).

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

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

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