DOI 10.31489/2022BMG1/32-41

UDC 635.21:57.086.13

N.G. Amantaev*

Karagandy University of the name of academician E.A. Buketov, Karaganda, Kazakhstan
**Corresponding author: nurbolat81@mail.ru*

Cryopreservation methods for *in vitro* potato crops: an overview

Potato is considered as one of the most important agricultural crops in the world. The genetic resources of potatoes (*Solanum tuberosum L. ssp. tuberosum)* and similar cultivated varieties are preserved by storing tubers or plants *in vitro* and cryopreservation. Arrays of worldwide scientific research centers utilize the cryopreservation method to preserve plant genetic resources. It is used especially for those plants that are reproduced by vegetative method; it is impossible to preserve genetic identity of their material when propagating by seeds. Currently, international standards of gene banks have been developed (*FAO, Gene bank Standards for Plant Genetic Resources for Food and Agriculture, 2014*) for the long shelf life of samples of vegetative propagated plants and vegetable crops at ultra-low temperatures. These standards have been discussed by scientists from many countries in the world. It is connected with the fact that no cryopreservation methods are standardized for a particular plant object. Present overview provided information on various methods of cryopreservation of potatoes for long-term storage of the gene pool of vegetative reproduction. The most updated methods included quick–freeze: encapsulation-dehydration, vitrification, slow programmable freezing, encapsulation-vitrification, and droplet-vitrification. All these biotechnological methods made it possible to obtain healthy potato material, which was free from viral and fungal infections. They also facilitate to obtain test tube plants in large quantities, as well as to create large cryocollections of valuable forms of cultivated plant crops.

Keywords: potatoes, collection, cryopreservation, cryopreservation, cryoprotectants, freezing, dehydration, vitrification, droplet.

Introduction

After wheat, potatoes are one of the most important crops in the Republic of Kazakhstan [1]. Potatoes (*Solanum tuberosum*) are a species of perennial tuberous herbaceous plants from the *Solanum* genus (family *Solanaceae*). There are about 140 varieties of potatoes according to the register of breeding achievements and the list of promising varieties of agricultural plants in Kazakhstan (2017).

The Kazakh Research Institute of Potato and Vegetable Growing grew up to 200 samples of potatoes within 3 years (2015–2017), and also annually produce up to 700–1,000 tons of seed potatoes.

The main final indicators for 2015–2017:

– transfer of new varieties — 23 varieties;
– potatoes — 8 varieties;
– area-specific varieties of the Republic of Kazakhstan — 38;
– number of area-specific varieties (included in the register) — 470;
– potato gene pool amounted to 200 samples;
– production of potato seeds of higher reproductions — 1,759 tons.

The yield of crops increased significantly by utilizing organic fertilizers: by 31.64 to 59.89 %.

The target indicators of the strategic development of the Kazakh Research Institute of Potato and Vegetable Growing for the period 2018–2022 were as follows: 2,300 (2018), 2,350 (2019, 2020), 2,380 (2021), 2,400 (2022) tons; and the production of potato seeds of higher reproduction increased and were as follows: 600 (2018), 650 (2019), 750 (2020), 850 (2021), and 910 (2022) tons.

Since potatoes are heterozygous crops, it is difficult to maintain the genetic purity of a cultivated variety through continuous vegetative reproduction [2]. To prevent any loss of potato genetic resources, the method of long-term storage of a plant sample in gene banks, general resource centers and cryo-collections is used in the CIS and foreign countries [3, 4].

Preserving genetic resources in gene banks is of crucial importance due to the high biological value as a breeding sample, as well as for further scientific research in biotechnology, crop production and agriculture. Therefore, in order to preserve the genetic resource of the studied plant samples for long term, the cryopreservation method is the best option.

One of the advantages of cryopreservation at very low temperatures is the ability to significantly slow down or even cease metabolic processes and biological destruction in the cells of living organisms. In this case, the plant material remains genetically stable, resulting in zero genetic changes.

Currently, the major gene banks of the world, such as the International Potato Center *(International Potato Center, CIP),* Argentina — INTA (*Potato Collection INTA-Balcarce*); the Czech Republic — CRI (*Crop Research Institute*) and PRI (*Potato Research Institute, Havlickov Brod*); the Republic of Korea — NAC (*National Agrobiodiversity Center*); Peru — CIP (*International Potato Center*); Leibniz Institute of Plant Genetics and Crop Plant Research *(IPK),* All-Russian Research Institute of Plant Genetics and Crop Plant Research named after N.I. Vavilov (*VIR*), Scientific and Practical Center for Potato Growing and Fruit and Vegetable Growing of the National Academy of Sciences of Belarus (*SPC of NSA for Food in Belarus*) store and preserve potatoes using three storage systems: natural conditions (field collections), *in vitro*, ultra-low temperatures (cryo-collections) [5, 6].

Among the EU countries, the most representative collection of potato germplasm samples is located in Germany at the *Leibniz Institute of Plant Genetics and Crop Plant Research.* Over 1,000 potato samples are preserved in deep freezing conditions at –196 °C [7–9]. In the CIS countries, the cryopreservation methods for plants, including potatoes, are actively developed in Russia and Ukraine [10, 11]. In Kazakhstan, research in the field of cryopreservation of plant germplasm was first started in 2002 at the Institute of Plant Biology and Biotechnology [12, 13].

To date, the gene pool of potatoes in the Kazakh Research Institute of Potato and Vegetable Growing in the Republic of Kazakhstan (www.foodindustry.kz) has 2,250 (2017) samples of the world collection from 40 countries. This includes 445 samples of 46 wild and cultivated potato species, 740 varieties, 14 samples of CIP's inter-specific hybrids, 120 samples of VIR's inter-specific hybrids, and 731 samples of hybrids from the Kazakhstani selection.

According to the Approval on State Registration of Plant Varieties Recommended for Use in the Republic of Kazakhstan, Order No. 434 dated 30.07.2009 (amendment and addition No. 109 dated 05.04.2021), the following varieties are registered in the areas indicated below:

Karaganda region (21 varieties): early ripe — 7, medium-early — 8, medium-ripe — 3, mid-late — 2, late-ripe — 1;

Akmola region (32 varieties): early ripe — 15, medium-early — 10, medium-ripe — 4, mid-late — 3;

Aktobe region (14 varieties): early ripe — 4, medium-early — 5, medium-ripe — 3, mid-late — 2;

Almaty region (72 varieties): early ripe — 21, medium-early — 23, medium-ripe — 22, mid-late — 3, late-ripe — 3;

Atyrau region (3 varieties): early ripe — 1, medium-early — 1, mid-late — 1;

East Kazakhstan region (24 varieties): early ripe — 6, medium-early — 9, medium-ripe — 6, mid-late — 3;

Zhambyl region (10 varieties): early ripe — 2, medium-early — 3, medium-ripe — 5;

West Kazakhstan region (24 varieties): early ripe — 2, mid-early — 2, medium-ripe — 2, mid-late — 4;

Kyzylorda region (11 varieties): early ripe — 4, medium-early — 1, medium-ripe — 6;

Kostanay region (34 varieties): early ripe — 14, medium-early — 7, medium-ripe — 5, mid-late — 6, late-ripe — 2;

Mangystau region (2 varieties): early ripe — 1, medium-early — 1;

Pavlodar region (22 varieties): early ripe — 10, medium-early — 7, medium-ripe — 4, mid-late — 1;

North Kazakhstan region (23 varieties): early ripe — 10, medium-early — 7, medium-ripe — 4, mid-late — 2;

Turkestan region (14 varieties): early ripe — 4, medium-early — 5, medium-ripe — 2, mid-late — 3.

The genetic resources of potatoes in Kazakhstan have scientific and practical value and include the biodiversity of wild species and their unique forms, as well as regionalized varieties of domestic and foreign selection, old varieties of folk selection and unique hybrid material.

The principal owner of the republican gene pool is the Kazakh Research Institute of Potato and Vegetable Growing of the Ministry of Agriculture in the Republic of Kazakhstan (KazRIPVG) [14]. At the Research Institute of Potato and Vegetable Growing of Kazakhstan, the gene pool of potatoes of local and foreign selection is mainly presented by its field collections and is aimed at breeding new promising samples and varieties of potatoes. The biologists-researchers and breeders of our country have a wide variety of source material. The varieties come from local origin, near and far abroad, hybrid forms, wild and simplified cultural species [15].

Maintaining large collections of potatoes grown in the field is an unreliable and time-consuming process for researchers–breeders. This is due to adverse environmental conditions, viral and fungal diseases, insect pests, and other factors, which may lead to the loss of samples of the collection. This loss impoverishes not only the collection of source material but also represents a long-term labor of those researchers–breeders.

The preservation methods preserve the gene pool of field, *in vitro* and cryo-collections complement each other, and only their joint usage can ensure reliable long-term storage of the genetic diversity of potatoes [16].

The purpose of the given scientific overview is to describe the achievements in cryopreservation developed for the potatoes of different types and varieties.

The data were analyzed within the time span of 44 years (from 1977 to 2021) referencing 36 sources.

Results

Recently, researchers from many countries have been studying the possibility of using a fundamentally new method to preserve the gene pool of various plant species based on deep freezing and subsequent storage at low temperatures of cell cultures and meristematic shoot tops [17, 18].

Cryopreservation is the storage of biological material at –196 °C in liquid nitrogen in such a way as to maintain viability and ensure the recovery of plants after re-warming [19]. Potato cryopreservation began in 1977 with two-stage refrigeration procedures and ultrafast freezing methods [20–23].

The literature outlines the methods such as a drip method using dimethyl sulfoxide (DMSO), glass transition, encapsulation / dehydration, encapsulation / vitrification methods and drip vitrification methods.

As per the results of research conducted by scientists and researchers, the usage of the above methods leads to an improvement in the results of cryopreservation of potatoes with the frequency of survival and regeneration of the plant sample taken into account. To add more, these methods are used in research organizations around the world to create cryo-collections of potatoes [24–26].

As it has been shown in recent scientific literature, cryopreservation acts as cryotherapy or as a new method of revitalizing plant material from viral, mycoplasma and bacterial infections [27].

Potato cryopreservation began in 1977 with two-stage freezing procedures and rapid freezing [20–22]. The first protocols were time-consuming due to the need to use programmable freezers and led to low survival rates. Later on, protocols were improved and new methods were developed, such as the dimethyl sulfoxide droplet method (DMSO) [24], vitrification [28], encapsulation/dehydration [25], encapsulation/vitrification (Hirai and Sakai, 1999), and droplet vitrification methods [26]. These protocols have improved potato cryopreservation outcomes in terms of survival and regeneration frequency, and are now regularly used to create collections of cryopreserved potatoes.

Cryopreservation methods

The slow–freeze method is a gradual cooling of plant tissues in special solutions in freezers to –40 °C. Once a certain temperature is reached, a plant sample (plant tissues) is transferred to liquid nitrogen (–196 °C — LN). The cooling rate is of great importance. The cooling rate varies for different types of plant material: 0.1–0.8 degrees per minute. For each plant sample, one needs to select an optimal freezing rate. The current methods of vitrification and encapsulation-dehydration open wide prospects. The advantages of these methods are simplicity, accessibility and no requirements for purchasing any expensive equipment. The method started being used in the works of B. Reed et al. for a number of fruit, berry, and other crops.

In slow freezing cryopreservation, the most commonly used mixture was cryoprotectants, which include 10 % of polyethylene glycol (PEG), 10 % of glucose and 10 % of dimethyl sulfoxide (DMSO) in a dissolved liquid medium. In some cases, slow freezing gives good results [28, 29].

The first studies on potato cryopreservation were conducted by Bajaj [20], who used freezing. Bajaj described that the water content in the cells was reduced by dehydration caused by freezing when using this method. To do this, tuber sprouts and axillary buds were cryo-protected with various solutions of glycerol and/or sucrose. They were then slowly cooled in the LN vapor phase. The researcher obtained a 7 % to 18 % survival rate of tuber sprouts and axillary buds using this protocol. The regeneration rate was up to 21 % for *Norland* and 33 % for *Red Pontiac*. On the other side, the works of L.E. Towill [22] underlined that the explants were slowly cooled at a rate of 0.2–0.3 °C min from −1 to −35 °C followed by immersion in LN.

Henshaw et al. [21, 30–34] applied a practically similar protocol by using 0.3 °C min from −1 to −30 °C with direct cooling in LN. The shoots' regeneration after reheating ranged from 4 % to 85 % depending on the potato species. In general, the described method is complex and time-consuming [35] and also requires special equipment for cryopreservation.

Quick–freeze method. This method involves the direct immersion of biological material in liquid nitrogen without prior cooling. This method is simpler, well reproducible, allowing to effectively store samples of different species represented by woody and herbaceous plants in the form of various types of explants — buds, meristems, embryos, pollen, calluses, and cells.

The quick–freeze method is based on the phenomenon of vitrification, in which intracellular water during freezing passes into the vitreous phase, bypassing the crystallization process as a result of which cellular organelles remain intact. This effect is achieved by using cryoprotectants. A mixture of cryoprotectants is used, which is part of *the Plant Vitrification Solution — a vitrifying solution* (PVS2) (30 % of glycerol, 15 % of DMSO, 15 % of *Polyethylene gluten* (PEG), 0.4M of sucrose), and also includes micro- and macro-elements according to the Murashige-Skuga (MS) nutrient medium prescription [30].

Compared to the previously mentioned freezing method, in the quick–freeze technique, the tips of the shoots were isolated, pre-cultured, and cryo-protected with MS [30] containing sucrose and 10 % of DMSO. Subsequently, each tip of the shoot was directly immersed in the LN at the tip of the hypodermic needle. Reheating was carried out quickly by transferring the needles directly to the MS-medium with benzyl aminopurine (BAP) at 35 °C. Afterwards, the tips of the shoots were removed from the solution and transferred to a regenerative medium. Grout and Henshaw [21] presented one of the first successful protocols for cryopreservation of the tops of potato shoots with a high survival rate of up to 20 % and the ability to regenerate the shoots up to 10 %. An important stage in this method is rapid freezing to turn water directly into amorphous ice. The opposite occurs with rapid reheating so that de-vitrification does not occur when returning to room temperature [21].

The method of *encapsulation–dehydration* is implemented by utilizing the technology of preparing artificial seeds. Apical meristems are encapsulated in an alginate gel, which makes it possible to dehydrate and dry-out the tissues, thus avoiding the formation of ice crystals from inside the cells. After partial dehydration, alginate encapsulated apical meristems are placed in cryotubes and quickly immersed in liquid nitrogen.

The encapsulation-dehydration method is used to produce artificial seeds. The essence of the method is in the fact that the apical meristems of the plant sample are immersed in an alginate gel; this allows dehydration and drying of plant tissues. Therefore, this method prevents the formation of ice crystals inside the cells. Afterwards, the dehydrated and dried in alginate apical meristems are placed in cryotubes and quickly immersed in liquid nitrogen.

This method involves encapsulating micro-plant explants into sodium alginate balls, followed by drying the laminar in the sterile air. The encapsulation-dehydration is used mainly for cryopreservation of apexes of micro-growths of fruit crops. There are also some isolated cases of using this method for cryopreservation of potato apexes [31].

Encapsulation-vitrification methods are a combination of certain elements of vitrification and encapsulation/dehydration. Hirai and Sakai [36] used 14 varieties of potatoes applying the above method in their experiments. The sprouts were grown in a solidified basal medium (MS medium with 0.5 g/L of casaminic acid, 30 g/L of sucrose, 2.5 g/L of Gellan gum) the temperature of 23 °C in a 16–hour photoperiod with a light intensity of 96 mol. For cryopreservation, axillary meristems from nodal segments with 5 leaf buds (approximately 1 mm in size) were isolated and pre-cultured in the main medium with 0.3 M of sucrose, 1 mg/L^{-1} of GA3, 0.01 mg/L^{-1} of BAP and 0.001 mg/L^{-1} of NAA. The meristems were suspended in the MS medium with the addition of 2 % of Na-alginate and 0.4 M of sucrose.

The encapsulated meristems were PVS2-dehydrated at 0 °C for various periods. The balls with PVS2 were shaken (45 rpm) in a water bath. A total of 10–15 balls were suspended in a volume of 1 ml. The PVS2 solution was transferred for cryo-vitalization and was immersed directly in LN. For reheating, the cryovars were placed in a water bath at the temperature of 38 °C for 3 minutes. After removing the PVS2 solution, the reheated beads were washed with 1 ml of 1.2 M sucrose solution for 10 minutes. For regeneration, balls with the tops of the shoots were sown in the basal medium for 1 day. They were then transferred to a basal medium with 0.0005 mg/L^{-1} of GA3. As a result of the research, the authors determined the ability to regenerate all 14 potato samples in the experiment. The rate of shoot formation and the growth rate were much higher and faster when using the encapsulation-vitrification method compared to the encapsulation/dehydration method. The authors observe that the encapsulation-vitrification method is simple (it takes less time to dehydrate the beads with PVS2 than using laminar airflow or silica gel). The advantage of this method is in a large number of explants, which can be cryopreserved at the same time. Therefore, this method is suitable for cryopreservation even on a large scale.

Vitrification method. When utilizing this method, the plant test material is treated with highly concentrated cryoprotectant solutions and quickly immersed in liquid nitrogen. As a result of this method, water is frozen in an amorphous state, which prevents the formation of intracellular ice crystals. The high concentrations of cryoprotectants used in this method can be toxic to the cells to prevent cell damage and death. The duration of treatment should be strictly controlled by the researchers [32].

Benson; Taylor et al. [1] described the process of vitrification in their papers as a way to freeze liquids without crystallization. In this process, the solutions inside the cells are vitrified, being amorphous in a glassy state. They lack organized structures, but they have the mechanical and physical properties of a solid. This method is one of the main and most widely used methods of cryopreservation of plants.

Vitrification is one of the main and most widely used methods of cryopreservation of plants. Most vitrification protocols use the Plant Vitrification Solution *(PVS2).*

This mixture consists of cryoprotectants containing 30 % of glycerol, 15 % of ethylene glycol, 15 % of DMSO in the MS medium with 0.4 M of sucrose added to it.

Sarkar and Naik [18] published the first vitrification protocol for the following potato varieties: *"Kufri Badsha", "Kufri Chandramukhi", "Kufri Lalima", "Kufri Lauvkar", and "Kufri Sindhuri*". In this vitrification method, the apical tips of 0.5–0.7 mm-sized shoots were first isolated from 30-day sprouts and pre-cultured on the medium-density MS liquid filter paper discs, various combinations of sucrose (0.3, 0.5 and 0.7 M) and mannitol (0, 0.2 and 0.4 M) at a 16-hour photo-period at the light intensity of 24 °C.

Kryszczuk et al. [8] applied the vitrification method to four varieties of potatoes ("*Ackersegen", "Blaue Schweden", "Carnea", "Desiree*"). In their works, they used the method of vitrification. This method obtained a significantly higher frequency of regeneration of the tops of shoots (58.0 %) compared to the method of droplet-vitrification with DMSO (13.8 %).

Droplet-vitrification method. This method consists of six stages. The first stage is the preparation of micro plants. The main task of the first stage is micropropagation, while obtaining a sufficient number of apexes to conduct three repetitions of the experiment. The second stage is the isolation of explants. This stage is aimed at obtaining a sufficient number of explants for cryopreservation. The apexes of micro growth shoots are used as explants. To isolate apexes, well-developed ones are chosen for the insulation of apexes. For each sample, cryopreservation is performed in three independent repetitions. At the third stage, the explants are treated with a cryo-protector and osmo- and cryo-protection of explants are performed. At this stage, a two-stage incubation of isolated explants is applied. The fourth stage is for freezing of explants in liquid nitrogen, which comprises the direct application of the liquid nitrogen over the apexes of the shoots. The fifth stage is thawing. This step is necessary to assess the frequency of post-cryogenic sample regeneration. The sixth stage is to study the ability of samples to post-cryogenic recovery (the frequency of post-cryogenic regeneration is assessed) [33].

In many methods of cryopreservation, a paramount stage is the pre-treatment of plant tissues. The pre-treatment is used to ensure that plants withstand the effects of toxic cryoprotectants and the freezing process itself. The tissues can be pre-treated by either chemical treatment using osmotic or penetrating cryoprotectants, cold acclimatization of test-tube plants, or by drying in the air stream or using drying agents. The main task of pre-treatment is to dehydrate the cells and stabilize cell membranes.

Dimethyl sulfoxide (DMSO) is most commonly used for chemical pre-treatment of plant cells and tissues. DMSO is used both for pre-cultivation and during cryopreservation. *Hardening of test-tube plants* is used to pre-treat many plant species of temperate climates for further cryopreservation. The duration of the hardening time varies from one week to several months. For the preliminary adaptation of shoots *in vitro*, a low favorable temperature (4, 5 °C) is used constantly during the entire hardening period, as well as changing temperature mode during the day [34, 35].

The DMSO droplet-vitrification is a simple freezing standard that is currently applied to a wide range of potato varieties. This method is quick and simple, and the process is inexpensive (Schäfer-Menuhr, 1996). Schäfer-Menuhr [24], Keller and Dreiling [9] believed that the problem was still the low rate of regeneration of some potato genotypes. In this regard, new additional and alternative studies are needed to be conducted to improve the results for samples of the studied plant material, which shows a low ability to regenerate.

The droplet-vitrification method (DMSO) is an optimization of the quick–freeze method [24]. The expression "droplet" refers to droplets of cryoprotectant on the aluminum foil where the shoots' tips are placed for freezing with each tip of the shoot in the liquid medium. An innovative idea of using aluminum foil came from Kartha (1982) who cryopreserved the tips of cassava shoots on the foil using the freezing method. Schäfer-Menuhr et al. [24] adopted the idea of the droplet-vitrification method for the tips of potato shoots. Foil is a good carrier for the rapid transfer of a large number of shoot tips simultaneously to and from the LN

compared to the usage of hypodermic needles, where only one shoot tip can be transferred at a time. In addition, aluminum is a good thermal conductor, which is important for both rapid cooling and re-warming of explants. They used 20×7×0.03 mm foil so that two foils fit well into one cryovalent.

In the original droplet freezing protocol, the tips of the shoots were isolated from the sprouts of 10 cm long, and the explants of 2, 3 mm long and 0.5–1 mm wide were then incubated in the MS medium with 30 $g{\cdot}l^{-1}$ of sucrose, 0.5 $mg{\cdot}l^{-1}$ of ribosidazeatin, 0.2 $mg{\cdot}l^{-1}$ of GA3, and 0.5 $mg{\cdot}l^{-1}$ of IAA (which is medium composition after Towill [22] during the night time. The average survival of cryopreserved samples was 80 % with 40 % of the regenerative capacity of plants [24]. It was analyzed the effects of phytohormones (0.5 $mg{\cdot}l^{-1}$ of ribosidazeatin, 0.2 $mg{\cdot}l^{-1}$ of GA3, and 0.5 $mg{\cdot}l^{-1}$ of IAA) in the droplets of agarose on the plant regeneration for 10 potato samples.

The original protocol, according to Schäfer-Menuhr [24] without any phytohormones in agarose droplets, showed a lower rate of plant regeneration compared to the use of agarose droplets containing phytohormones [20]. The plant regeneration ranged from 2.5 % to 22 % with an average value of 13.2 %. By applying the droplet-vitrification method, Kryszczuk et al. [8] compared the original DMSO drip protocol with slightly modified versions for potato varieties (*Ackersegen, Blaue Schweden, Carnea and Désirée*). In these studies, there was no survival at all during autoclaving, and DMSO regeneration was used instead of the sterilized DMSO filter. The use of solid media during regeneration increased the regeneration rate by an average of 15 % compared to the original protocol using a liquid medium (average regeneration rate of 13.8 %). The best results in plant regeneration were obtained by an average of 29.6 % in the plant regeneration owing to the use of cold pre-culture of potato donor plants (day/night temperature at 21/8 °C, an 8-hour photoperiod, 7 days) until the tops of the shoots could be isolated. An improvement in regeneration results was found from 34.6 % to 45.2 % when a variable temperature was applied to the pre-culture donor plants *in vitro* (day/night temperature at 22/8 °C, an 8-hour photoperiod for 7 days) before isolating the shoot tops.

To date, an average regeneration rate of 46 % has been achieved. This collection includes specimens previously kept in the German Collection of Microorganisms and Cell Cultures (DSMZ, Germany) and the Institute of Plant Growing of the Federal Agricultural Research Centre (FAL, Braunschweig, Germany), which were subsequently integrated into the IPK collection [9].

Methods of cryoplastic plates. The developed cryoplastic methods adapted the modified method of vitrification to ensure the stability and greater resistance of the explants to sudden changes in temperature. The concept of the methods is based on aluminum microplates containing several oval wells. These methods facilitate cryopreservation and re-warming procedures and minimize the risk of any mechanical damage or losing tiny explants [36].

Conclusions

Thus, cryopreservation is becoming an increasingly common method for long-term storage of plant genetic resources. It allows for the reliable preservation of the gene pool of potato varieties, hybrids and breeding clones of potatoes along with field collections. Each of the preservation methods of potato samples has its advantages and drawbacks. Therefore, improvements have been made in the development and successful application of the methods in cryopreservation of potatoes with different varieties, species, and hybrids.

In this regard, improvements have been made in the development and successful application of methods in the field of cryopreservation of potatoes with different varieties, species, and hybrids. There are many options for cryopreservation as there are many varieties, species and hybrids of potatoes to be cryopreserved, and these methods effectively complement each other. The extensive experience gained as a result of the cryobanks of potatoes in IPK, CIP, NAC RDA, CAES HRO and NIAS indicate storage, principles and viability after cryopreservation, etc. Numerous studies have been done to examine precisely the influence of various factors affecting cryopreservation (the physiological state of donor plants and shoot tips), including specific cryogenic factors (for example, the type of cryoprotectants and the rate of cooling and reheating).

Therefore, it is necessary to support potato varieties, species and hybrids “at-risk” that have an increased likelihood of being lost.

Thus, the methods of cryopreservation and their modification for potatoes and various types of vegetable crops have been developed. Applying these methods will create a cryogenic collection of the potato gene pool in Kazakhstan. The creation of large cryo-collections with a high level of regeneration of potato and vegetable crops presently remains to be of vital importance.

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Н.Г. Амантаев

Картоптың *in vitro* дақылдарына арналған криоконсервациялау әдістері

Шолу

Картоп — бүкіл әлемдегі ең маңызды ауылшаруашылық дақылдарының бірі. Картоптың генетикалық ресурстары (*Solanum tuberosum l. ssp. tuberosum*) және онымен байланысты дақыл түрлері түйнек және криоконсервациялау арқылы сақталады. Әлемнің көптеген дамыған елдерінде криоконсервация өсімдіктердің генетикалық ресурстарын сақтау үшін кеңінен қолданылады. Бұл, әсіресе, тек вегетативті жолмен таралатын дақылдарға қатысты, себебі тұқыммен көбейту кезінде материалдың генетикалық сәйкестігін сақтау мүмкін емес. Қазіргі уақытта төмен температура жағдайында вегетативті түрде таралатын дақылдардың үлгілерін ұзақ уақыт сақтау үшін генбанктердің халықаралық стандарттары (*FAO, Genebank Standards for Plant Genetic Resources for Food and Agriculture, 2014*) әзірленді, соңғы уақытта олар әлем ғалымдарының белсенді талқылау сатысында тұр. Себебі криоконсервациялау әдістерінің ешқайсысы белгілі бір өсімдік объектісі үшін бірыңғай жүйеленген емес. Бұл шолуда вегетативті көбею генофондын ұзақ мерзімді сақтау үшін картопты криоконсервациялаудың әртүрлі әдістері туралы ақпарат берілген. Ең заманауи криоконсервациялау әдістері: инкапсуляция–дегидратация, витрификация, баяулап кезең бойынша мұздату, инкапсуляция-витрификация, дроплет-витрификация. Барлық осы биотехнологиялық әдістер вирустық және саңырауқұлақ инфекцияларынан тазартылған картоп материалын алуға, көп мөлшерде пробиркалық өсімдіктер алуға, зертханалық жағдайда жыл бойы жұмыс істеуге және белгілі бір мерзімде өсімдіктердің шығарылымын жоспарлауға, пробиркалық өсімдіктерін ұзақ уақыт сақтауға және құнды формалардың «банкін» құруға мүмкіндік береді. т. б.

Кілт сөздер: картоп, коллекция, криоконсервация, криосақтау, криопротекторлар, мұздату, дегидрация, витрификация, дроплет.

Н.Г. Амантаев

Методы криоконсервации для культур *in vitro* картофеля
Обзор

Картофель — одна из наиглавнейших сельскохозяйственных культур во всем мире. Генетические ресурсы картофеля (*Solanum tuberosum l. ssp. tuberosum*) и родственных культурных видов сохраняются путем хранения клубней, растений *in vitro* и криоконсервации. Во многих научных исследовательских центрах мира используется метод криоконсервации для сохранения генетических ресурсов растений. Особенно это касается растений, которые размножаются вегетативным путем, для которых невозможно сохранить генетическую идентичность материала при размножении семенами. В данное время разработаны международные стандарты генбанков (*FAO, Genebank Standards for Plant Genetic Resources for Food and Agriculture, 2014*) для длительного срока хранения образцов-материалов вегетативно размножаемых растений и овощных культур в условиях ультранизких температур, в последнее время эти стандарты находятся на стадии обсуждения учеными многих стран мира. Это связано с тем, что ни один из методов криоконсервации не стандартизован для конкретного растительного объекта. В настоящем обзоре представлена информация о различных методах криоконсервирования картофеля для долгосрочного хранения генофонда вегетативного размножения. К самым современным относят методы быстрого замораживания: инкапсуляция-дегидратация, витрификация, медленного программируемого замораживания, инкапсуляция-витрификация, дроплет-витрификация. Все перечисленные выше биотехнологические методы позволяют получать оздоровленный материал картофеля, свободный от вирусной и грибной инфекций, получать в больших количествах пробирочные растения, создавать большие криоколлекции ценных форм культивируемых растительных культур.

Ключевые слова: картофель, коллекция, криоконсервация, криосохранение, криопротекторы, замораживание, дегидрация, витрификация, дроплет.

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