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THE IMPORTANCE OF ”*IN VITRO*” PLANT GENETIC RESOURCES CONSERVATION - POTATO (*SOLANUM TUBEROSUM* L.)

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Abstract: The traditional method for the conservation of vegetatively propagated species is to maintain field collections that can include a large number of genotypes, representing a wide range of genetic diversity. ”*In vitro*” conservation of plant genetic resources, with the help of modern biotechnologies, can be a potential option for the improvement of agricultural crops. Slow Growth Storage (SGS) technique is a valid approach to conserve ”*in vitro*” various vegetatively propagated species by controlling plantlets growth and development, saving storage space, labor and reducing costs. In potatoes, to eradicate viruses and obtain a healthy initial material, we have at our disposal the technique of sampling and ”*in vitro*” cultivation of meristems. In this study, 6 potato genotypes were tested using the DAS ELISA technique to establish the health status (presence/absence of potato viruses). In four of these genotypes, healthy clones were identified, which were micropropagated ”*in vitro*” and then introduced into the germplasm collection.

Keywords: potato, tissue culture, *in vitro* germplasm conservation, virus eradication, meristems.

INTRODUCTION

Plant genetic resources constitute a universal heritage of overwhelming importance for all mankind. The interest in obtaining superior agricultural productions, both quantitatively and qualitatively, but also the concern for maintaining stability in terms of food safety have contributed over time to the conservation of plant genetic resources.

Maintaining the available genetic resources is a primary requirement for the success of the breeding activity, but also for other fields, such as biochemistry, genetics, physiology, ecology, etc. The loss of biodiversity and the collapse of ecosystems are among the most important threats that humanity will face in the coming decades, therefore research in the field of plant genetic resources conservation is a national priority.

”*In vitro*” conservation of plant genetic resources, with the help of modern biotechnologies, can be a potential option for the improvement of agricultural crops. In many countries of the world, specific procedures for the ”*in vitro*” cultivation of plant tissues are successfully used with the aim of ” ex-situ” conservation of genetic resources. Slow Growth Storage (SGS) technique is a valid approach to conserve ”*in vitro*” various vegetatively propagated species by controlling seedling growth and development, saving storage space, labor, and reducing costs. Furthermore, SGS extends the time between subcultures and decreases the risk of germplasm loss through handling errors such as contamination issues. SGS is applied taking into account different factors: temperature, light or dark conditions, the composition of the medium, including mineral or sucrose concentrations, and the presence/absence of growth regulators, osmotic agents, and growth inhibitors (Benelli et al., 2022).

The traditional method of conservation of vegetatively propagated species is to maintain field collections that can include a large number of genotypes, representing a wide range of genetic diversity (Shii et al., 1994; Reed, 1999 cited by Benelli et al., 2022). In addition, this method is expensive and carries a constant risk of serious losses due to biotic and abiotic stress. ”*In vitro*” cultures are used for medium-term conservation of plant germplasm. Explants cultured ”*in vitro*” can be stunted in growth and development by culture conditions that affect normal metabolism. The technique is generally referred to as "slow growth conservation" or "minimum growth conservation" due to the use of various physical, chemical, or nutritional parameters that limit plant growth. It can also be called "cold storage" when low temperatures are applied instead of standard growing conditions. Basically, SGS extends the interval between subcultures compared to regular intervals to 3-5 weeks depending on the species, increasing the safety of preservation and minimizing the risk of contamination during the subculture process. Slow-growth conservation is applied in commercial micropropagation laboratories as a strategy for short- and medium-term maintenance of plant material in limited space, providing high-quality products to the market.

The potato is remarkable for the breadth of wild-related species (typically populations maintained as botanical seed) that can be used relatively easily in cultivar improvement. There is also a rich diversity of primitive landraces (clonally maintained as tubers or ”*in vitro*”) and, of course, thousands of cultivars, and gene stocks consolidated over nearly 200 years of modern breeding. In the last 50 years, wild potato relatives have made major contributions in terms of increasing disease resistance, improving yields and quality traits (Jansky et al., 2013).

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It is estimated that 20% of plant species are at risk of extinction (Jansky et al., 2013) and these losses will be accelerated by climate change and habitat destruction. Furthermore, for some plant species, much of their diversity is only available in genebanks (Jarvis et al., 2011). They could also represent the future for the potato, where wild-related species are only found in the fragile ecosystems of the Andes (Hijmans and Spooner, 2001), where a changing climate is already having a substantial impact.

The geographical distribution of wild potato species is concentrated between the borders of 16 countries in the Americas (Mexico, United States, Costa Rica, Guatemala, Honduras, Panama, Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, Paraguay, Peru, Uruguay and Venezuela). For thousands of years, the ecological niches of the Andes have been the natural habitat and center of genetic diversity for both wild potato species and countless native varieties. Long-term evolutionary processes have allowed the accumulation of genetic components that constitute valuable resources for the improvement of potato culture, such as the ability to survive under biotic (diseases, pests) and abiotic (drought, frost, climate change) stress conditions. However, such a valuable aspect as genetic diversity is currently threatened by the phenomenon known as genetic erosion.

In the seed potatoes production system, it is essential to use a healthy and high-quality biological material, which contributes to obtaining superior yields. The implementation of a virus eradication technology and the creation of an ”*in vitro*” collection of virus-free germplasm is an important premise in the production of healthy seed potatoes.

MATERIAL AND METHOD

Currently, the ”*in vitro*” collection of potato germplasm within the Research Laboratory for Plant Tissue Cultures of NIRDPSB Brasov includes over 45 genotypes (Romanian and foreign varieties, local populations, lines with late blight resistance, lines of potato derived from botanical seed, wild species). These genotypes are maintained in the ”*in vitro*” collection as microplants. To achieve the objective of completing the current ”*in vitro*” collection of potato germplasm, tubers belonging to 3 varieties (Neil, Evollete, Nevin) created at Research and Development Station for Potato Targu Secuiesc, Covasna county, and 3 perspective lines (A17b, A20n, Ea23v) belonging to the Scientific-Practical Institute of Horticulture and Food Technologies (ISPHTA) from Chisinau, Republic of Moldova were purchased in 2023.

In potatoes, to eradicate viruses and obtain a healthy initial material, we have at our disposal the technique of sampling and ”*in vitro*” cultivation of meristems. The elimination of viruses starting from meristem culture is based on the principle that they do not infect the apical/axillary meristem, the virus-free plant can be regenerated from a small portion of meristematic tissue by micropropagation. The apical/axillary meristem does not contain viruses, because the virus particles are spread through the vascular system, which is not developed in the meristematic region. The size of the meristematic dome determines the ability of the explant to survive on an artificial nutrient medium. The smaller the size of the meristem, the greater the chances that it will be free of the virus. In the field of plant biotechnologies, especially in the case of vegetatively propagated species, meristem culture is a method that is the basis of the virus eradication process.

In order to take the meristematic tissues, the potato tubers were prepared for sprouting in the laboratory (in the dark and ensuring a temperature of about 20ºC), until the sprouts were 1-1.5 cm long. At this stage, the sprouts were detached from the tuber and disinfected (Fig. 1). The protocol for the sterilization of potato sprouts involved the following steps:

- immersion in 1% sodium hypochlorite (NaClO) solution, for 5 minutes;
- 3 successive rinses in sterile distilled water;
- immersion in ethanol, 3 minutes;
- successive rinses in sterile distilled water for 10 minutes;
- sprouts drying on filter paper.

All operations were carried out in the sterile room, in the hood with laminar airflow.

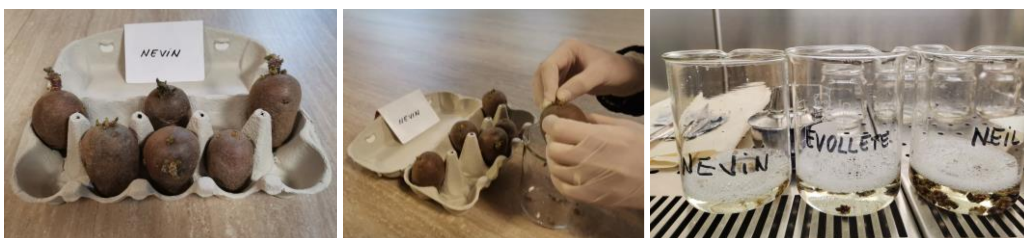

Fig. 1. Preparation of tubers for sprouting, detachment, and sterilization of potato sprouts for the sampling of meristems.

The excision of the meristems (having dimensions between 0.2 - 0.5 mm) was performed under a binocular, in a hood, on a surface that was previously disinfected with 70% alcohol. The inside of the hood is sterilized with a UV lamp. The explant detaches with the first pair of leaf primordia. After excision, the meristems taken were inoculated on the solid culture medium in test tubes (Fig. 2). The culture medium used was MS (Murashige and Skoog, 1962) added with gibberellic acid (GA3), in a concentration of 0.1 mg/l. At an interval of approximately 30 days, the meristems were pasted on a fresh culture MS medium supplemented with 0.5 mg/l α-naphthylacetic acid (NAA).

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Fig. 2. Excision of meristems and their inoculation on the nutrient medium.

After inoculation, the cultures were incubated in the growth chamber (Fig. 3), under controlled conditions of temperature (18–20°C) and light (photoperiod of 16 hours of light and 8 hours of darkness).

Fig. 3. Incubation of cultures in the growth chamber (controlled conditions).

From the meristematic explants, through their ”*in vitro*” inoculation and their growth, respectively through the formation of stems at the base of which new root formation takes place, plants will be born. After approximately 6 months from initiation, plantlets develop from the meristems, which are grown on fresh culture medium. The regenerated microplants are tested by the ELISA technique for final assurance of virus-free plant status. Healthy clones were micropropagated ”*in vitro*” and introduced into the germplasm collection.

RESULTS AND DISCUSSION

Figure 4 presents the results obtained regarding the initiation of cultures from meristems in the 6 new potato genotypes intended for introduction into the ”*in vitro*” germplasm collection. The total number of meristems sampled as well as the number of surviving meristems is highlighted. A total of 126 meristems were excised of which 107 survived.

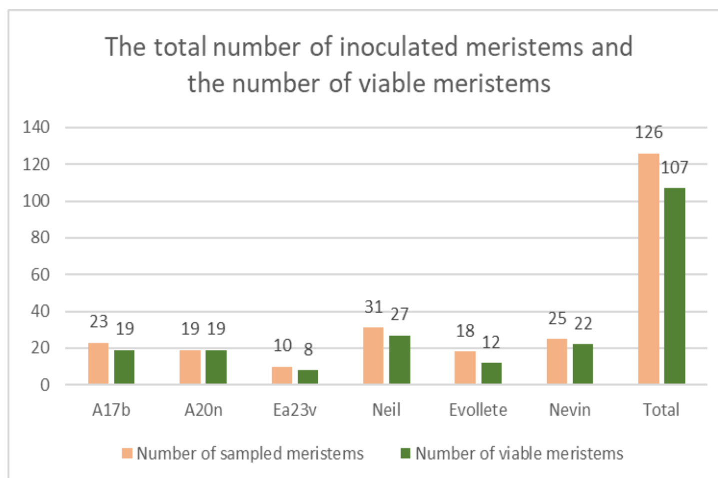

Fig. 4. Results regarding the initiation of meristem cultures in the potato genotypes.

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Table 1.

Influence of genotype on the mean number of viable meristems

Genotypes	Mean number of viable meristems	Difference	Significance
A17b	6.33	0.39	ns
A20n	6.33	0.39	ns
Ea23v	2.67	-3.28	o
Neil	9.00	3.06	*
Evollete	4.00	-1.94	ns
Nevin	7.33	1.39	ns
Mean (Ct)	5.94	-	-

LSD 5% = 2.44; LSD 1% = 3.46; LSD 0.1%= 5.01

From the point of view of statistical calculation (Table 1), the best results in terms of the average number of viable meristems were obtained for the Neil variety (9.00) which registered a significantly positive difference (3.06) compared to the control (mean). The lowest average number of viable meristems was recorded in the variety Ea23v, which obtained a significantly negative difference (-3.28) compared to the control.

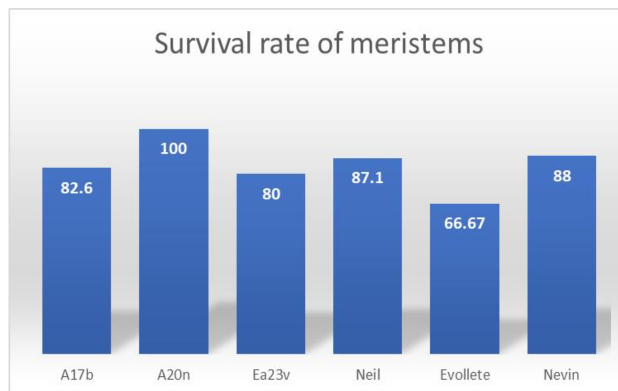

Fig. 5. Results regarding the survival rate of meristems (%).

As can be seen in figure 5, in most potato genotypes not all meristems inoculated on the medium survived, except for the line A20n, in which the survival rate of meristematic explants was 100%. This is due to the size of the excised meristem. The smaller the size of the meristem, the greater the chances that it will be free of the virus, however, the survival chances of the meristem decrease.

Microplants regenerated from viable meristems, were multiplied to obtain a stock of potato plantlets intended for ELISA testing. The biological material was tested for the main potato viruses (PLRV, PVY, PVA, PVX, PVS, and PVM). Kits from Bioreba (Switzerland) were used for virus testing. The DAS ELISA technique was used to carry out the samples, following the protocol established by Clark and Adams (1977). The juice was taken directly from the leaf with the help of a manual homogenizer with balls.

Following the ELISA testing, the results regarding the absence/presence of the most widespread potato viruses were obtained (Table 2).

Table 2.

Results obtained from ELISA testing

Potato genotype	Status
A17b	PVY
A20n	Healthy
Ea23v	Healthy
Neil	PVS, PLRV
Evollete	Healthy
Nevin	Healthy

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Healthy clones (A20n, Ea23v, Evollete, Nevin) were multiplied and then introduced into the ”*in vitro*” germplasm collection. For the genotypes where healthy clones were not obtained, they will be maintained by periodic transfer on fresh medium until spring. In May, the potato plantlets will be transferred to a protected space (greenhouse), in pots with soil to form mini tubers. They will be sprouted, meristems will be excised again and the cycle will repeat until healthy plants (free from viruses) are obtained.

CONCLUSIONS

Potato genotypes conserved in ”*in vitro*” germplasm collection represent a source of genes for disease and pest resistance, abiotic stress tolerance, and quality traits, being increasingly appreciated due to their potential to contribute valuable traits to breeding and research programs all over the world, facilitate the exchange of germplasm between different institutions in the same country or even different countries. They can also be supplied to potato farmers who are interested in growing these genotypes.

Many researchers around the world are sounding the alarm about the vulnerability and limitation of plant genetic resources, due to major threats to global food security. Among these, we can mention: the replacement of local populations and traditional varieties with modern varieties, natural catastrophes (drought, floods, wildfires), the needs of the ever-growing global population, urbanization, industrialization, habitat loss, climate change, etc.

Biodiversity conservation has potential direct economic benefits for many sectors of the economy. The current economic and social crises, as well as global warming, with all the associated negative effects, endanger the maintenance of plant biodiversity.

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AUTHORS CONTRIBUTIONS

Conceptualization, C.M.; methodology, C.M., S.V., and I.P.; data collection, C.M., T.A., P.M.; data validation, C.M., and T.A.; data processing, C.M., T.A., and B.A.; writing original draft preparation, C.M.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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