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IN VITRO PRESERVATION OF POTATO PLANTS

ABSTRACT

Potato plants free of viruses, bacteria and viroid could be maintained *in vitro* for a long time. Proper preparation of potato plants for *in vitro* culture provide its long-term storage in good condition. First step is to establish *in vitro* culture from young greenhouse grown plants in early developmental stage. Explants are maintained and propagated by nodal subculture on hormone-free Murashige and Skoog (MS) medium at temperature 20-22°C. After rooting, for longer preservation plants are maintained at temperature 8-10°C.

Key words: long-term storage, potato collections, *in vitro* culture of potato

INTRODUCTION

Preservation of the biodiversity of wild and cultivated plant species, including the potato crop *Solanum tuberosum* L. and its relatives, is an important task for the collection of plant genetic resources. There are many collections in the world that maintain a large and varied gene pool of *Solanum*. Various methods of storing potato genotypes, such as field or greenhouse propagation, seeds, *in vitro* plants, are used in plant collections. Vegetatively propagated potato is often maintained in field collections. It is a traditional method, requiring a lot of space and work. In addition, field collections are not available throughout the year, and preserved material is exposed to biotic and abiotic stresses during the growing season and during storage, which may lead to its loss. Potato produces true seeds that can be stored for a long time, but the plants obtained from the true seeds sample are not homogeneous, and cannot preserve valuable single genotypes (Engelmann, 2011). Commonly used method to protect potato genotypes in gene banks is *in vitro* cultures. The *in*

vitro culture method ensures the production and rapid multiplication of disease-free material. In this technique the effects of unfavorable environmental factors are eliminated because genotypes are stored under controlled conditions. Maintenance of *in vitro* plants in low temperature reducing growth of plant material and increasing intervals between subcultures (Engelmann, 2011). However, somaclonal variation within the genotype may occur in the long-term stored *in vitro* (Belokurova, 2010; Engelmann, 2011). In Bonin Research Center of IHAR-PIB the method of micro-propagation of *in vitro* potato plants was developed in 1980 (Zaklukiewicz *et al.*, 1995; Sekrecka and Michałowska, 2015). In IHAR-PIB Młochów Research Center this method has been used since 1986 for *in vitro* collection of diploid and tetraploid research materials including parental lines.

MATERIALS AND REAGENTS

- 1) Murashige and Skoog Medium Including Vitamins (Duchefa Biochemie, cat. No P11872.02)
- 2) Plant Agar (Duchefa Biochemie cat. No BO 11493)
- 3) Sucrose Pure P.A. (POCH cat. No 772090110)
- 4) Lysogeny Broth (LB, Luria Bertani) (Sigma Aldrich, cat No L3152)
- 5) Ethanol (CHEMIPU, cat. No 113964200)
- 6) Combitips advanced, (Eppendorf BIOPUR, cat. No 0030089693)
- 7) Distilled water
- 8) Glass test tubes (Roth, cat. No TX22.1)

SOLUTIONS

- 1) MS medium with 3% sucrose and 0.8% agar
- 2) LB medium with $10 \text{ g} \times \text{l}^{-1}$ Tryptone, $5 \text{ g} \times \text{l}^{-1}$ Yeast, Extract $5 \text{ g} \times \text{l}^{-1}$ NaCl (Sigma Aldrich cat. No L3022), autoclaved for 15 minutes at 121°C to sterilize

EQUIPMENT

- 1) Laminar flow cabinet (POLON, cat. No KL-21)
- 2) Multi-pipette (Eppendorf, cat. No 4981 000 019)
- 3) Ph meter (Mera, cat. No HI2020-02)
- 4) Autoclave (HP Medizntechnik, Varioklav, cat. No 50085006)
- 5) Plant growth chamber (22°C , light intensity 8000 Lux)
- 6) Plant growth chamber for long storage ($8\text{-}10^{\circ}\text{C}$, light intensity 1000 Lux)

PROCEDURE

In order to establish *in vitro* plants of selected genotypes presence of common viruses of potato (PVY, PVX, PVM, PVS and PLRV), *Clavibacter michi-*

ganesis, *Ralstonia solanacearum* and potato spindle tuber viroid (PSTVd) infections are checked in plants grown in greenhouse conditions. Forms free of pathogens are established *in vitro* by cutting plant fragments with apical or auxiliary buds and decontaminated with sterilizing agents.

- 1) It is taken 10 fragments with apical or auxiliary buds from about 3 greenhouse grown plants from one genotype. The size of explants is about 1 cm (Photo 1). Generally, younger plants, with more rapidly growing tissues in early developmental stage are the most effective for *in vitro* propagation.

Photo. 1. The potato explants after sterilization.

- 2) Plant explants are immersed in the 70% ethanol for 20-30 s.
- 3) Explants are placed in 2% solution of sodium hypochlorite diluted in water in ratio 1:5 for 4-7 min.
- 4) Explants are washed with sterile distilled water three times for five minutes each time. Steps 1-4 are done with sterile tools and in sterile conditions.
- 5) A successful sterilization is achieved when the explants are decontaminated and remains viable.
- 6) Following the sterilization step, explants are maintained and propagated by nodal subculture on hormone-free MS medium (Murashige and Skoog, 1962) with 3% sucrose and 0.8% agar. Ten *in vitro* plants per genotype are maintained in the collection (Photo 2).

Photo 2. Plantlets maintained in the *in vitro* collection

- 7) Plants are kept in chambers at a temperature of 20-22°C and illuminated 5-8 thousand lux to obtain well-developed and rooted plants in a tube (for about 1 month). Plants in good condition (at least 3 plants) considered as healthy are the starting material for further reproduction in micropropagation.
- 8) The presence or absence of non-pathogenic bacteria is checked on LB (lysogeny broth; Luria – Bertani) medium. Parts of the *in vitro* plantlets are placed in liquid LB media. Opacification of LB medium after 7 days of incubation at room temperature indicates the presence of bacteria in plant material.
- 9) In order to reduce the aging of the stored material, the rooted plants on MS medium at a temperature of 20-22°C are transferred to chambers at a temperature of 8-10°C and illumination of about 1000 lux.

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