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VIRUS ELIMINATION FROM *IN VITRO* POTATO PLANTS

ABSTRACT

The routine and the most effective method of viruses elimination from potato plants is *in vitro* culture of apical shoots combined with thermo- and/or chemotherapy. At times electrotherapy or cryotherapy is also applied. Elimination of potato viruses by thermotherapy required treatment of infected plants with high temperature (35°C - 45°C) for about two weeks. Thermotherapy is useful for elimination of PLRV and PVY from potato plants. In chemotherapy antimetabolite, like ribavirin is used. Chemotherapy is dedicated for elimination of PVM, PVS and PVX. Very often the successful virus elimination require a few cycles of thermo- and/or chemotherapy.

Key words: chemotherapy, potato viruses, ribavirin, thermotherapy

INTRODUCTION

Because potato (*Solanum tuberosum* L.) is a vegetatively-propagated plant, fungal, bacterial and especially viral diseases are easily transmitted through the tubers. Each year the plants propagated from tubers are more and more contaminated with viruses. Viral diseases influence the potato plant vigor and cause the reduction of resistance of potato to other pathogens, which leads to reduction of yield and quality of tubers (Nascimento *et al.*, 2003; Wang *et al.*, 2008; Mahmoud *et al.*, 2009). Hence, it is important that the material used for propagation, especially seed tubers were free from viruses. In routine work contamination with main potato viruses occurring in Poland is assessed by ELISA (PVY, PVX, PLRV, PVM, PVS). The presence of potato spindle tuber viroid (PSTVd) and *Clavibacter michiganensis* is also checked. The presence of quar-

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antine pathogens eliminates plants from further work. Virus-free plans or plants with the lowest concentration of viruses are selected and are established *in vitro* by cutting plant fragments with apical or auxiliary buds and decontaminated with sterilizing agents. Healthy material may be obtained by the viruses elimination from infected plants. The routine methods of viruses elimination from plants are: *in vitro* culture of apical shoots combined with thermo- and/or chemotherapy (Mellor and Stace-Smith, 1970; Mahmoud *et al.*, 2009). In thermotherapy infected plants or explants are treated with the high temperature (35°C - 45° C) for certain period of time (about two weeks) (Kryszczuk, 1999). High temperature activates virus-silencing genes (Downar –Zapolska and Sekrecka, 2017). In chemotherapy antimetabolites like thiouracil, malachite green, azacitidine and salicylic, benzoic, jasmonic and linoleic acids are used. They cause blocking of replication of viral DNA or RNA. Ribavirin is the most frequently used antimetabolite. Depending on the virus(es), thermo- and/or chemotherapy are applied for its/their elimination. Thermotherapy is useful for elimination of PLRV and PVY. Chemotherapy is dedicated for elimination of PVM, PVS and PVX. The most difficult virus to remove from infected plants is PVS. Even very high concentrations of ribavirin (above 15 mg × l^{-1}) do not guarantee the receipt of a virus-free material (Sekrecka *et al.*, 2016). Other methods are searching to remove PVS from infected plants. Usage of electric field pulses (electrotherapy) or low temperature (cryotherapy) on explants is less frequent but promising in order to eliminate viruses from potato (Downar-Zapolska and Sekrecka, 2017).

In vitro culture allows the rapid multiplication of plant material and undergo appropriate treatment plant leading to obtain healthy plants at any time of the year (Nascimento *et al.*, 2003). For better results, usage of at least two of the techniques mentioned above is needed.

MATERIALS AND REAGENTS

1) Agar (Sigma – Aldrich, cat. No A1296)
2) Ethanol 96% (Avantor Performance Materials Poland S.A, POCH, cat. No 396420113)
3) Gibberellic acid (Sigma – Aldrich, cat. No G7645)
4) Glass beakers (Roth)
5) Glass tubes
6) Kinetin (Sigma – Aldrich, cat. No K3378)
7) MS medium according Murashige and Skoog, 1962
8) Ribavirin (Sigma – Aldrich, cat. No R9644)
9) Scalpel blade (Swann-Morton, carbon Steel Surgical blades)
10) Scalpel handles (Weldon Instruments)

EQUIPMENT

1) Autoclave (Prestige Medical, model Extended Plus 2100)
2) Climatic chamber for *in vitro* plants with controlled light and temperature

3) Laminar flow hood II class
4) pH meter
5) Pipette (Eppendorf)
6) Stereoscopic microscope (Motic)
7) Water purification system (Purelab, Elga)

PROCEDURES

Thermotherapy

1) The viruses presence is tested with ELISA within greenhouse grown plants to preselect most healthy plants of the breeding line. Potato plants are free from potato spindle tuber viroid (PSTVd) and *Clavibacter michiganensis* subsp. *sepedonicus* (*Cms*).
2) In order to established *in vitro* plants apical buds from greenhouse grown plants are cut and decontaminated with sterilizing agents in sterile conditions as follow:
   a) Plant materials are immersed in the glass beaker with 70% ethanol for 20-30 s.
   b) Explants are transferred to glass beaker with solution of sodium hypochlorite diluted in water in ratio 1:5 for 4-7 min.
   c) Explants are washed with sterile distilled water three times (in three glass beaker with sterile distilled water) for five minutes each time.
3) After four weeks, *in vitro* plants obtained from explants are cut into segments containing one node each and place in glass tubes with solid MS (Murashige and Skoog, 1962) medium and cultivated at 21°C, under 16 h illumination for four weeks.
4) After that, selected forms (10 plants per genotype) are multiplied in tubes with MS medium supplemented with 3% sucrose and solidified with 0.8% agar and pH 5.7. Plants are cultivated at 21°C with 16 h illumination for four weeks.
5) After four weeks plants in tubes are transferred to chamber with high temperature: at 37°C/35°C (day/night), under 16 h illumination of two weeks. About 2-3 tubes with plants of selected genotypes must be saved at the chamber at 21°C with 16 h illumination.
6) Transfer of shoot tips from temperature treated *in vitro* plants on MS medium (in order to obtain better regeneration of shoot tips 0.04 mg × l^{-1} kinetin and 0.1 mg × l^{-1} gibberellic acid could be added) at 22°C / 20°C (day/night), under 16 h illumination.
7) After four weeks each plant is divided into two. One plant is maintained *in vitro*, second one is used for ELISA testing.

Chemotherapy

1) Multiplication of potato plants selected for virus eradication in tubes (10 plants per genotype) on MS medium with ribavirin (12 mg × l^{-1}).
2) After four weeks shoot tips are isolated and transferred on standard MS medium individually in tubes (in order to obtain better regeneration of shoot tips 0.04 mg × l^{-1} kinetin and 0.1 mg × l^{-1} gibberellic acid could be added) at 22°C/20°C (day/night), with 16 h illumination.
3) After four weeks each plant are divided into two. One plant is maintained *in vitro*, second one is used for ELISA testing.

In order to check effectiveness of methods of viruses elimination in potato, after every cycle of thermotherapy and chemotherapy ELISA is applied. If it is necessary cycle of thermotherapy and chemotherapy could be repeated.

REAGENTS

1) MS medium (basal medium) – combination of macronutrients, micronutrients, vitamins and sucrose mixtures without growth regulators. Prepared according to Murashige and Skoog, 1962.
2) Ribavirin 12 mg × l^{-1} – optimal concentration of ribavirin is between 10 and 15 mg × l^{-1}. Higher concentrations influence normal growth and development of treated plants.

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REFERENCES

Downar-Zapolska J., Sekrecka D. 2017. Methods for elimination of viruses from potato plants – literature review. Ziemniak Polski 3:24-31.

Kryszczuk A. 1999. Termoterapia i kultura merystemów jako jedna z metod uzyskiwania roślin ziemniaka wolnych od wirusów. Biuletyn IHAR 212:151-157 (in Polish).

Mahmoud S.Y.M., Hosseny M.H., Abdel-Ghaffar M.H. 2009. Evaluation of some therapies to eliminate potato Y potyvirus from potato plants. *International Journal of Virology* 5:64-76.

Mellor F.C., Stace-Smith R. 1970. Virus strain differences in eradication of potato virus X and S. Phytopathology 60:1587-1590.

Murashige T., Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-97.

Nascimento C.L., Pio-Ribeiro G., Willadino L., Andrade G.P. 2003. Stock indexing and *potato virus Y* elimination from potato plants cultivated in vitro. Sci. Agric. 60:525-530.

Sekrecka D., Michałowska D., Piskorz J. 2016. Termoterapia i chemioterapia – porównanie skuteczności metod w eliminacji wirusów S i M. Ziemniak Polski 4:10-15.
Wang Q., Cuellar W.J., Rajamäki M-L., Hirata Y., Valkonen J.P.T. 2008. Combined thermotherapy and cryotherapy for efficient virus eradication: relation of virus distribution, subcellular changes, cell survival and viral RNA degradation in shoot tips. Mol. Plant Pathol. 9: 237-250.