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Virus Elimination in Plant Tissue Cultures via Cryotherapy Technique

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Aim of the study: Pathogen-free stocks of plant materials are important for productivity of agricultural crops and ornamental plants. Clonal propagated plants are particularly inclined to accumulate pathogens which are transmitted to new crops in infected cuttings, tubers and other vegetative propagules. Cryotherapy of shoot tips is a new method for pathogen elimination based on cryopreservation techniques. In cryotherapy, plant pathogens such as viruses, phytoplasmas and bacteria are eradicated from shoot tips by exposing them briefly to liquid nitrogen (-196ºC). In this study aimed to indicate one step-freezing methods based on vitrification of cryotheraphy for virus eliminations from plants.

Material and Methods: In the vitrification method, cells and shoot tips must be sufficiently dehydrated by the vitrification solution (which hardly penetrates into the cells during the dehydration process) without causing injury, in order to be able to vitrify upon rapid cooling in liquid nitrogen. Several vitrification solutions have been improved by various resarchers worldwide. On the contrary, the most frequently used solutions are the glycerol-based vitrification solutions described plant vitrification solution PVS2 and PVS3. The PVS2 solution contains 30% (w/v) glycerol, 15% (w/v) ethylene glycol, 15% (w/v) dimethyl sulfoxide (DMSO) and 0.4 M sucrose (pH 5.8). PVS3 consists of 40% (w/v) glycerol and 40% (w/v) sucrose in basal culture medium. After dehydration using PVS2, samples are moved to a cryotube containing fresh cryoprotectant solution, and immersed in LN. Cryopreserved tubes are rewarmed using hot water (40ºC) for 1-2 min, and the vitrification solution is removed from the tube. After removal of the solution, unloading solution is added to a tube, and cryoprotectants are removed from plant tissues for 30 min at 25ºC. After unloading, samples are moved from the cryotube, and recultured.

Results: Virus elimination by cryotherapy of shoot tips from infected plants is a coming out method that can be easily tested with species and genotypes for which cryopreservation protocols are available. Regulations of the method might be need for expanding cryotherapy to additional genotypes and for increasing the percentage of pathogen-free regenerants. In gene banks practising cryopreservation the expertise is easily available and cryotherapy could be adopted in pathogen-eradication schemes for species and genotypes that are going to be cryopreserved.

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