Romanian Journal for Plant Protection, Vol. XII,2019
ISSN 2248 – 129X; ISSN-L 2248 – 129X

DIVERSIFICATION OF *IN VITRO* CULTIVATION METHODS OF TOMATO SEEDLINGS (*Lycopersicum esculentum* Mill.)

Lavinia Diana Nicoleta Barbu [*1,2], *Oana Livadariu*[2], *Oana-Alina Boiu-Sicuia*[1,2]

[1] Research - Development Institute for Plant Protection Bucharest, Romania
[2] Faculty of Biotechnology, University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania

*Correspondence address:
Research - Development Institute for Plant Protection Bucharest
Ion Ionescu de la Brad 8, CP 013813, Bucharest, Romania
Tel. 004-021-2693231 (32, 34)
E-mail: laviniabarbu7@gmail.com

Abstract: In the past 20 years, *in vitro* cultures rate has increased, especially for plants of economic interest. Propagation of cells, tissues and organs cultures can be performed in glass or various plastic vessels. Regardless of the material from which they are made of, these vessels may have different sizes and shapes (flasks, Erlenmayer shape flasks, square dishes, roller bottles, tubes, etc.), but the most important aspect in their use is the absence of toxic trace elements release. Containers intended for *in vitro* cultures should be sterilized to eliminate the possibility of contamination of the plant material or the nutritive substrate. Tomato plants are of high economic interest. When grown in greenhouses or in the field, they are exposed to a number of pests and diseases attack, from seed germination to seedlings and till fruiting. The *in vitro* cultures can provide an alternative to classical methods of seed germination and seedlings production. Through propagation the seedlings are free of pathogens without pesticides exposure as in classical protection methods. This study presents an *in vitro* growth method to obtain first stage tomato seedlings, using plastic food containers of polypropylene. This propagation method showed that the food containers made of polypropylene can be safely used for *in vitro* cultures of tomato seedlings, as the phytopathogenic contamination of both plant material and nutrient substrate was reduced.

Key words: *tomato, propagation, in vitro growth containers*

INTRODUCTION

Tomatoes (*Lycopersicum esculentum* Mill.) are annual plants, belonging to the *Solanaceae* family, specifically grown for their fruits. These can be consumed at both mature ripeness or before, as green tomatoes (mostly used for pickles). The importance of tomato fruits is given both by nutritional qualities (Butnariu et al., 1992) and versatility for consumption, as it is suitable for various modes of preparation, both at household and industrial level. Globally, of the entire quantity of tomatoes being produced annually, 80% is marketed under different processing forms, while only 20% of the quantity is consumed fresh (https://hms.harvard.edu)

In Romania, the tomato crop has a significant share in all production systems. *In vitro* cultures, as integrated part of plant biotechnology, have been successfully applied to plants of economic interest. Applied also on tomatoes, *in vitro* cultures have various advantages such as: maintenance of plants genetic stability, curing and propagation of virus-free plants, propagation of valuable varieties, use of a balanced nutritious substrate, optimization of growth parameters, etc. (Alatar et al., 2017).

The purpose of this study was to evaluate the potential use of economically viable containers as growing vessels for *in vitro* plant propagation. Such containers are those made of polypropylene for food uses. Using them would lower the costs for plant propagation.

MATERIALS AND METHODS

***In vitro* culture containers.** Used polypropylene vessels were purchased from local stores channel. For safety reasons and lack of contamination, it is mandatory that polypropylene containers packaging is intact (Figure 1).

Figure 1. Polypropylene food vessels use for tomato propagation

Biologic material. Tomato seeds Buzau 22 variety were used. This variety has a determinate growth type, with mid-season maturity, being suitable for/in open field conditions.

Seed disinfection. The disinfection procedure was carried on just before sowing. Tomato seeds were disinfected in a three steps procedure of one minute each, with 70% ethanol, 15% sodium hypochlorite and, again, with 70% ethanol (Schultz et al., 1993). Subsequently, five successive rinses were made with sterile distilled water.

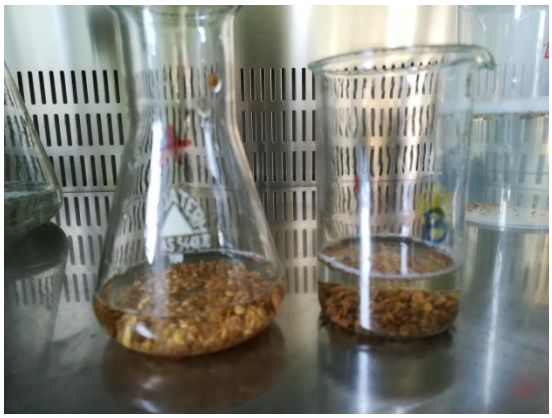

Figure 2. Disinfected tomato seeds in sterile distilled water

Experimental variants. Two experimental variants were tested in order to produce tomato seedlings *in vitro* conditions. These experimental variants were: V1 - polypropylene food containers exposed for 30 minutes under germicidal light (UV-C radiation) and V2 - polypropylene food containers used directly from commercial packaging, without UV exposure.

Experimental conditions. For each experimental variant 70 polypropylene food containers were used. For propagation, Murashige and Skoog medium (Murashige & Skoog, 1962) was used as nutrient substrate. Each growth container, of 350 ml capacity, was filled-in with 30 ml of MS medium. Three seeds, previously disinfected, were placed in every growth

container on the Murashige and Skoog (MS) medium, having 8% agar. To maintain relative humidity, the growth containers were placed in additional polypropylene containers, of 500 ml capacity. The resulting assembly was covered with polyvinyl chloride foil of alimentary use (Figure 3). Cultures were maintained at room temperature from 22 to 25˚C (Der Van & Heuvelink, 2005), in daylight conditions, for two months, in both steps of cultivation.

Figure 3. Tomato seedlings grown in the containers assembly enclosed with polyvinyl chloride foil

After the first month of growth the seedlings were transplanted in new vessel assemblies filled with fresh MS medium, prepared as in the first step. Visual observations were made during the two months growth period to determine the contamination degree of cultures.

RESULTS AND DISCUSSIONS

Tomato phytosanitary status was monitored for two months, during the two *in vitro* growth steps. Some of the contaminating pathogens were identified based on their macroscopic morphology and microscopic features. The visual aspect of the prevalent mold showed velvety texture, with blue to blue-green farinaceous top growth. The microscopic analysis of the dominant mold revealed septate hyaline hyphae, with brush shape conidiophores with conidia. Some of the fungal colonies had simple conidiophores, and other had branched conidiophores with metulae and phialides. Conidia were grouped as chains, at the apex of the conidiophores. Due to the macro- and microscopic characteristics, it was established that the main contaminants encountered in the propagation vessels were caused by *Penicillium* species (Figure 4).

The degree of contamination was determined at the end of each growth step and the microbial contaminated samples were removed. The percentage of contaminated vessels was different among the two variants. A higher contamination was encountered in the growth containers without UV exposure (Figure 5).

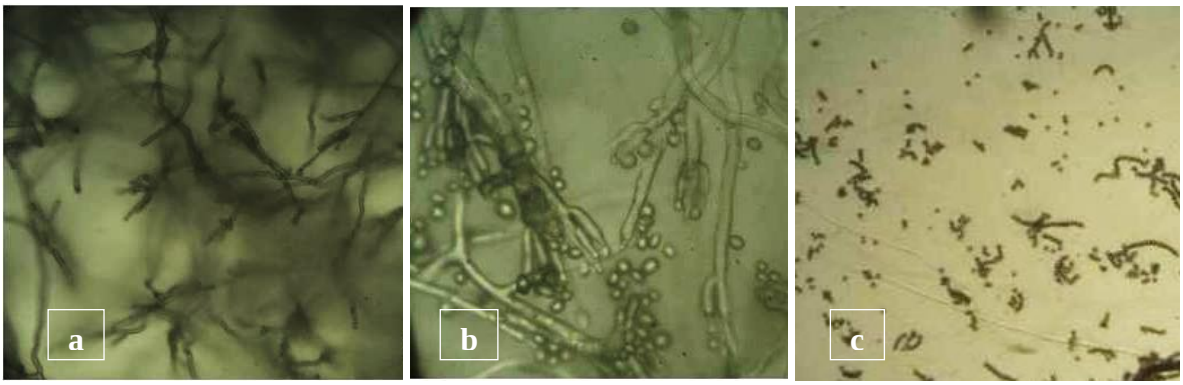

Figure 4. *Penicillium* spp. contaminants of *in vitro* cultures: a) habitus, b) conidiophores with conidia, c) chained conidia

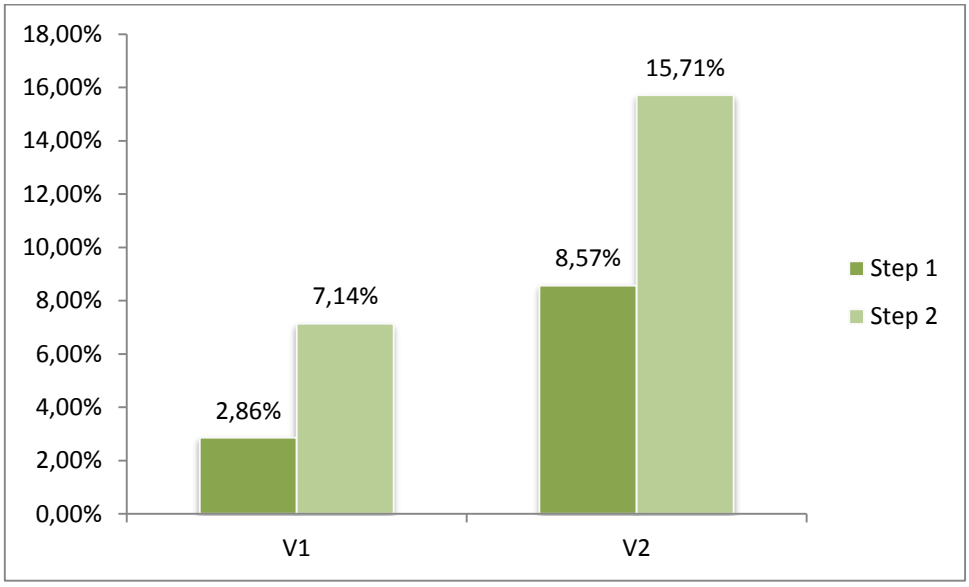

Figure 5. Contamination percentage encountered in the polypropylene food vessels used as propagation containers

Results showed that UV light exposure provided a good prevention method against microbial contamination, due to the germicidal activity of the UV-C light. The percentage of contaminated vessels, in the first step of growth, was less than 3% in the V1 experimental variant.

Considering the low rate of contamination in the UV exposed containers, less than 10% at the end of the second growth step of the seedlings, it can be said that polypropylene food containers, if exposed for 30 minutes in germicidal light (UV-C light), can be used in plants propagation. Disinfection of biological material and use of an autoclaved nutrient substrate remain essential steps to reduce the risk of contamination of *in vitro* cultures.

An important aspect in using polypropylene food containers, beside their lack of toxicity, is their convenient price. The costs of our containers was calculated as less than 10€/100 containers. The polypropylene food containers that we used for *in vitro* plant propagation have proven to be a cost effective option for tomato propagation under *in vitro* conditions.

Comparing current results with those from previous performed studies (unpublished data) it can be said that polypropylene food containers have some advantages over glass containers used for *in vitro* cultures. The benefits of polypropylene containers for propagation are the mechanical reliability and plasticity (i), reduced costs (ii), low weight (iii) and easy handling (iv). Polypropylene food containers could be used for *in vitro* propagation of numerous other plant species, if hygiene rules and exposure of containers to UV-C light are ensured.

CONLUSIONS

This study confirms the possibility of food-grade polypropylene containers use for *in vitro* propagation of tomatoes. These cost effective containers when exposed to germicidal UV light for 30 minutes, maintain a reduced contamination rate (8.57%) within the growth vessels, compared to those without UV-C light exposure.

REFERENCES

ALATAR, A., FAISAL, M., ABDEL-SALAM, E., CANTO, T., SAQUIB, Q., JAVED, S., EL-SHEIKH, M., K HELDHAIRY, A. (2017). Efficient and reproductible *in vitro* regeneration of *Solanum lycopersicum* and assessment genetic uniformity using flow cytometry and SPAR methods. *Saudi Journal of Biological Sciences*, 24, 6, 1430-1436. Doi: 10.1016/j.sjbs.2017.03.008.

BUTNARIU, H., INDREA, D., PETRESCU, C., SAVIȚCHI, P., CHILOM, P., CIOFU, R., POPESCU, V., RADU, G., STAN, N. (1992). Legumicultură, Editura Didactică și Pedagogică, Bucureşti.

DER VAN, P., HEUVELINK, E. (2005). Influence of sub-optimal temperature on tomato growth and yield: a review. *The Journal of Horticultural Science and Biotechnology*, 80, 6, 652-659. Doi: 10.1080/14620316.2005.11511994.

HARVARD HEALTH PUBLISHING, HARVARD HEALTH LETTER, [updated 2013 February; cited 2019, October]. Available from: https://www.health.harvard.edu/diseases-and-conditions/tomatoes-and-stroke-protection.

MURASHIGE, T., SKOOG F. (1962) A revised medium for rapid growth and bioassays with tabacco tissues culture. *Physiologia Plantarum*, 15, 473- 497.

SCHULTZ, B., WANKE, S., DRAEGER, S., AUST, H.J. (1993). Endophytes from herbaceous plants and shrubs: effectiveness of surface sterilization methods. Mycological Ressearch, 97, 12, 1447-1450.