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THE INFLUENCE OF ORANGE JUICE ON MITOSIS AND IN VITRO GROWTH TO *HIBISCUS ESCULENTUS*

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Abstract

The regeneration capacity of the cells of an explant depends on several factors: the nature and origin of the explants, their type, structure and degree of juvenility, cell maturity and physiological state, endogenous phytohormone content, composition of culture medium and culture conditions, etc. Among them, the culture medium is an essential factor for the success of in vitro culture. In its composition, in addition to macro and microelements, vitamins, sucrose, etc., can be added some natural nutritional extracts, such as deproteinated coconut milk or various vegetable and fruit juices, to improve the complexity of the culture medium. This paper presents the results obtained in the laboratory from the in vitro culture of okra (Hibiscus esculentus), on modified Murashige-Skoog culture medium (MS) by adding natural orange juice in three different concentrations: 5, 10 and 20%. It was observed that at the 10% concentration of orange juice were registered the highest values, both in terms of germination, explant growth and mitotic activity. The lowest values from this point of view were obtained at a concentration of 20%. These results suggest the nutritional potential of orange juice added to the MS culture medium to increase the growth rate and in vitro survival of okra via cells competence improvement.

Key words**:** *okra, in vitro, orange juice, culture medium, growth, mitosis*

INTRODUCTION

The beginning of the third millennium is characterized by the strong involvement of bioengineering and biotechnologies in agriculture but also in other fields of activity. Many problems related to the negative impact of pathogens on crops [15, 16, 17] can be solved by modern and sustainable improvement of the plants.
Food security is one of the global challenges of this century. Sustainable crop management has a strategic role, as it is responsible for food security with a special contribution to the overall process of sustainable economic development and environmental protection [8, 14, 19].
Due to the wide range of problems solved by in vitro cultures, they are currently used in agriculture, forestry, pharmacy, food industry, light industry, etc. In agriculture in general and in horticulture in particular, in vitro cultures are used for the multiplication of valuable species, varieties or clones, for the improvement of cultivated species, the conservation of horticultural germoplasme, obtaining secondary metabolites, etc.
On a synthetic nutrient medium can be grown whole plants, organs, organ fragments, tissues but also isolated cells and protoplasts. The plant material used to initiate in vitro cultures is called explant. The explant is actually the portion of the plant that is detaches from the donor plant and is inoculated under sterile conditions, on an artificial culture medium. The explant is the living unit that contains in the cells all genetic information of the mother plant and based on totipotency is able to regenerate one or more plants identical to the donor plant [22].
Unlike traditional multiplication, where it is operated with seeds or large portions of the plant, in vitro multiplication uses small explants, of the order of millimetres or even microscopic (cells, protoplasts) that under normal culture conditions would not be able

to grow resisting to the pathogens and self-synthesizing the necessary nutrients.

Based on the concept of cell totipotency, according to which each cell contains the genetic information necessary to obtain by regeneration a complete plant organism, Murashige and Skoog (1962), managed to develop a culture medium considered basic, which, with small modifications, can be used for almost all types of in vitro cultures [12]. This culture medium is named after them: Murashige and Skoog (MS).

Phytovitrocultures are organized in conditions of strict sterility, similar to the technique of culture of microorganisms, but using different specialized culture medium to induce the plant regeneration. The culture medium represents the physical and chemical support necessary for the growth and development of in vitro explants [1, 21].

Some of the conditions that a culture medium must comply are [12]: to correspond to the nutritional and hormonal requirements of the cultivated species for the phase in which it is found (stabilization, proliferation, callogenesis, rooting, etc.); to ensure optimal conditions for growth and development in terms of osmotic pressure, pH, humidity; be ionically balanced; be easy to prepare and reproducible; be inexpensive and contain as few as possible expensive and inhomogeneous constituents.

The culture mediums used for plants vitroculture generally have a complex structure, being composed of a large number of constituents of diverse nature and with a different role [10]. These constituents can be grouped into:

a. Nutrient constituents: mineral elements: macro and microelements and organic elements: sugars (as a carbon source), amino acids (as a source of organic nitrogen) and vitamins;

b. Constituents with phytoregulatory role of growth and development of explants in vitro: auxins, cytokinins, gibberellins and other substances with stimulating or inhibitory role: abscisic acid, ethylene, colchicine, etc.;

c. Constituents with a role in stabilizing the culture medium: water, solidifying agents, osmotic and pH stabilizers, antioxidants and absorbents.

The culture medium used for static cultures also contains a gelling agent, which usually is the agar. MS culture medium can be modified by adding components or removing other components. From this point of view, various natural extracts can be added, for example; tomato juice, banana, melon, yeast extract, malt extract, etc. [2, 9, 18].

Okra (*Hibiscus esculentus*, synonymous with *Abelmoschus esculentus*) is part of the *Malvaceae* Family. It is an annual plant that grows up to 2 m in height; the leaves are 10–20 cm long and are broad, with 5–7 lobes. The edible fruit is a green capsule (8–20 cm long) and contains many seeds. Okra is grown in warmer tropical and temperate areas for its fibrous green pods, highly valued for their nutritional value, being an excellent source of vitamins, proteins and fibres [6, 10, 11].

Okra is one of the most heat- and drought-resistant vegetables and tolerates the clay soils. These advantages place it in the list of foods with high potential to ensure food security and safety, in the conditions of climate change recorded worldwide [4, 5]. It is desirable to multiply such resistant plants, in the context in which the major global crises, such as climate change or the pandemics, have serious repercussions on food security [13].

In vitro okra behaves very well, many results highlighting the importance of vitrocultivation of this species [6, 7].

MATERIALS AND METHODS

The methods and techniques of the plant biotechnology have several basic elements, such as: complex nutrient medium (culture medium), total asepsis of the culture medium, the plant material to be inoculated, instruments and controlled climate conditions.

The plant material for inoculation consisted of okra seeds which were disinfected with 0.25% sodium hypochlorite for 5 minutes and then rinsed three times with distilled water.

Three variants of MS culture medium (V1-V3) modified by adding fresh orange juice in three different concentrations were made: 5,

10 and 20%. The control variant consisted of conventional MS culture medium, with pH stabilized at 5.5 [12].

The plant material was inoculated into sterile Erlenmeyer glasses containing about 25 ml of agarized MS culture medium. 10 culture pots were made for each variant. The pots were closed with aluminium foil lids and were placed in the growth chamber, under fluorescent lamps with an intensity of 1,000 lux, for 16 hours/day at 25^0C, for 30 days.

The subculturing of each variant was performed on fresh culture medium, with the same composition corresponding to each variant.

Laboratory measurements were performed on the vegetative growth of the neoplants and the mitotic index was calculated by microscopic analysis of the meristematic tissues from the top of the neoformed roots at each variant. For this, the Feulgen-Rossenbech staining method was used and the microscopic preparations were analysed by the squash method [20].

For microscopic determinations was used the Optika digital microscope with LCD display.

Analysis of variance (ANOVA) was used for statistical calculation.

RESULTS AND DISCUSSIONS

A standard culture medium consists of a mixture of macro and microelements (chlorates, nitrates, sulfates, phosphates and iodates of Ca, Mg, K, Na, Fe, Mn, Zn and Br), vitamins, a carbon source, substances organic growth (amino acids, urea and peptones and a source of nitrogen). The addition of various plant extracts is done in order to stimulate cell mitosis and thus determine the improvement of plant growth in vitro.

In our experience, the in vitro germination percentage of okra on MS medium added with orange juice has values between 82.08 (Control) and 90.24 (V2) (Figure 1).

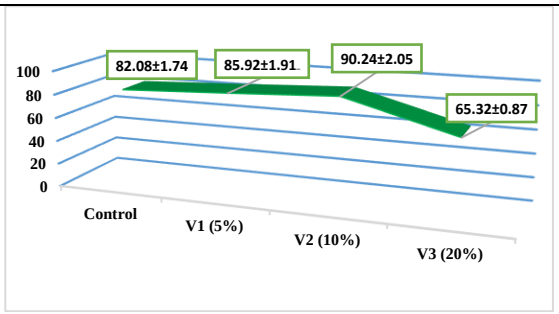

Fig. 1. Germination values (%) of okra on MS culture medium modified by the addition of orange juice in different concentration
Source: Own calculation.

From the point of view of vegetative growth, okra cultivated in vitro registered different values, depending on the concentration of orange juice added in the MS culture medium. Thus, the best results were identified in V2 variant, where the concentration of fresh orange juice added in the MS culture medium was 10% (Figure 2). The values obtained were 35% propagules regenerated after two subcultures (V2); 21% (V1) and 16% (V3). The value recorded by the control variant was 26%. It can observed that variant V2 exceeded the value of the control variant and the lowest values from this point of view were obtained at a 20% concentration (V3).

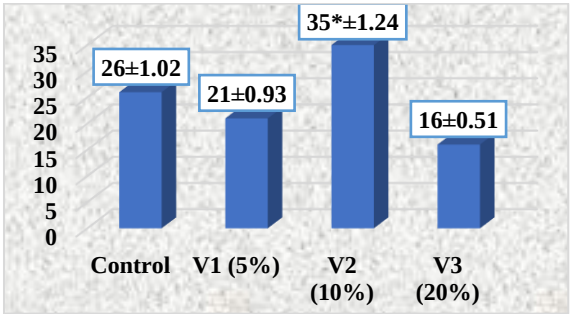

Fig. 2. Percentage of propagules regenerated in vitro after two subcultures to okra on MS culture medium modified by the addition of orange juice in different concentration
*Significant at $p \leq 0.05$ (ANOVA analysis)
Source: Own calculation.

Microscopic analysis of the cross section of the okra meristematic roots shows the presence of aerenchyma cells and air cavities (Figure 3).

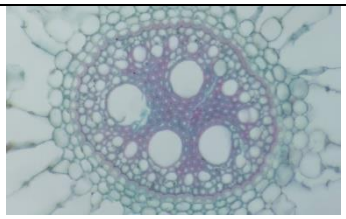

Fig. 3. Meristematic root (cross section) of okra visualised at digital microscope
Source: Own lab survey.

In terms of mitotic index (MI), variant V2 recorded the highest intensity of mitotic division (54.81%), followed by variant V1 (41.28%) and variant V3 (31.05%), which recorded the lowest mitotic activity , compared to the control variant, which recorded a mitotic index of 34.16% (Figure 4).

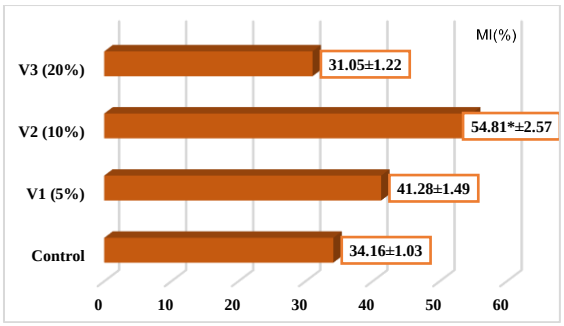

Fig. 4. Mitotic activity registered in meristematic roots of okra on MS culture medium modified by the addition of orange juice in different concentration
*Significant at $p \leq 0.05$ (ANOVA analysis)
Source: Own calculation.

In vitro plant culture pursues several objectives, some of the most important being the following: rapid and mass propagation of some varieties, preservation of genetic material from valuable genotypes, production of virus-free plants.
Any type of explant is characterized by a certain biochemical balance, depending on the age of the donor plant, its physiological stage, the organ from which it was collected, the structure and dimensions of the explant itself, etc. Also, the culture medium and especially its components have a major influence.
Regarding the complex substances that can be part of an in vitro culture medium, in many experiments a series of extracts were tested: protein hydrolysates [2], yeast extract [18], malt extract, tomato juice [9], coconut milk, sea buckthorn fruit extract [3], immature corn endosperm extract, etc. The importance of these types of complex substances is due to their natural origin, as well as the numerous mechanisms of action of the active substances contained, such as: flavonoids, alkaloids, glycosides, saponins, tannins, etc.
Given the importance of in vitro cultures for agriculture, horticulture or medicine, all strategies aimed at improving the use of nutrients in culture medium are justified. Thus, the acceleration of organogenesis processes and the shortening of the in vitro growth time can directly influence the profitability of production. The positive effect of phytogenic compounds on in vitro plant growth must also be evaluated in terms of the positive impact on the environment.

CONCLUSIONS

The culture medium used for inoculation and in vitro growth of explants have, in generally, a complex structure, being composed of a large number of constituents of various nature and with different roles. In terms of phytonutrients, a number of natural extracts can stimulate the growth and survival of plants in vitro.
The results obtained in this experience suggest the stimulating potential of orange juice for in vitro growth of okra. It is possible that the stimulating effect for mitotic division and organogenesis is due to the high content of biologically active polyphenolic compounds from the orange juice, arranged in easily accessible complexes to plant cells.
Orange juice added to the MS culture medium can thus reduce the risk of oxidative stress for in vitro phytocultures. However, further study is needed for clear conclusions.

REFERENCES

[1]Arab, M.M., Yadollahi, A., Eftekhari, M., Ahmadi, H., Akbari, M., Khorami, S.S., 2018, Modeling and Optimizing a New Culture Medium for In Vitro Rooting of G×N15 *Prunus Rootstock* using Artificial Neural Network-Genetic Algorithm. Sci Rep Vol. 8:9977.
[2]Bkhairia, I., Ben Salem, R.B.S, Nasri, R., Jridi, M., Ghorbel, S., Nasri, M., 2016, In-vitro antioxidant and functional properties of protein hydrolysates from golden grey mullet prepared by commercial, microbial

and visceral proteases, J. Food Sci. Technol. Vol. 53(7):2902-2912.
[3]Bonciu, E., Olaru, A.L., Rosculete, E., Rosculete, C.A., 2020, Cytogenetic Study on the Biostimulation Potential of the Aqueous Fruit Extract of *Hippophae rhamnoides* for a Sustainable Agricultural Ecosystem, Plants, Vol. 9(7):843.
[4]Bonciu, E., 2019, The climate change mitigation through agricultural biotechnologies, Annals of the University of Craiova-Agriculture, Montanology, Cadastre Series, 49(1):36-43.
[5]Bonciu, E., 2017, Food processing, a necessity for the modern world in the context of food safety: a Review, Annals of the University of Craiova - Agriculture, Montanology, Cadastre Series, Vol. 47(1):391-398.
[6]Chen, H., Jiao, H., Cheng, Y., Fengyang Wang, F., 2016, In Vitro and In Vivo Immunomodulatory Activity of Okra (*Abelmoschus esculentus* L.) Polysaccharides, Journal of Medicinal Food, Vol. 19(3).
[7]Dhande, G.A., Patil, V.M., Raut, R.V., Rajput, J.C., Ingle, A.G., 2012, Regeneration of okra (*Abelmoschus esculentus* L.) via apical shoot culture system, African Journal of Biotechnology, Vol. 11(86):15226-15230.
[8]Durău, C.C., Sărățeanu, V., Cotuna, O., Paraschivu, M., 2021, Impact of the grassland management planning application on some features of the grassland vegetation from Western Romania – Case Study, Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development, Vol. 21(3):325-331.
[9]Mastuti, R., Munawarti, A., Rosyidah, M., 2017, The Effect of Tomato Juices and Bean Sprout Extracts on Vitro Shoot Regeneration of *Physalis angulata* L., AIP Conference Proceedings, Vol. 1908:040006-1–040006-5.
[10]Mukhtar, T., Hussain M.A., Kayani, M.Z., 2017, Yield responses of 12 okra cultivars to southern root-knot nematode (*Meloidogyne incognita*), Bragantia, Vol. 76(1):108-112.
[11]Mukhtar, T., Arshad, I., Kayani, M.Z., Hussain, M.A., Kayani, S.B., Rahoo, A.M., Ashfaq, M., 2013, Estimation of damage to okra (*Abelmoschus esculentus*) by root-knot disease incited by Meloidogyne incognita, Pakistan Journal of Botany, Vol.45:1023-1027.
[12]Murashige, T., Skoog, F., 1962, A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures, Physiologia Plantarum, Vol. 15:473.
[13]Paraschivu, M., Cotuna, O., 2021, Considerations on COVID 19 impact on Agriculture and Food Security and forward-looking statements, Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development, Vol. 21(1):573-581.
[14]Paraschivu, M., Cotuna, O., Sărățeanu, V., Durău, C.C., Păunescu, R.A., 2021, Microgreens - current status, global market trends and forward statements, Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development, Vol. 21(3):633-639.
[15]Paraschivu, M., Ciobanu, A., Cotuna, O., Paraschivu, M., 2020, Assessment of the bacterium *Erwinia amylovora* attack on several pear varieties (*Pyrus communis L.*) and the influence on fruits sugar content, Agricultural Sciences & Veterinary Medicine University, Bucharest, Scientific Papers. Series B. Horticulture, Vol. LXIV(1):163-168.
[16]Paraschivu M., Cotuna, O., Paraschivu, M., Olaru, L., 2019, Effects of interaction between abiotic stress and pathogens in cereals in the context of climate change: an overview, Annals of the University of Craiova - Agriculture, Montanology, Cadastre Series, Vol. XLIX/2:413-424.
[17]Paraschivu, M., Cotuna, O., Paraschivu, M., Durau, C.C., Damianov, S., 2015, Assesment of *Drechslera tritici repentis (Died.) Shoemaker* attack on winter wheat in different soil and climate conditions in Romania, European Biotecnology Congress the 20th August 2015, Bucharest, Journal of Biotechnology, Vol. 208:S113.
[18]Park, W.T., Arasu, M.V., Al-Dhabi, N.A., Kyung Yeo, S., Jeon, J., Park, J.S., Lee, S.Y., Park, S.U., 2016, Yeast Extract and Silver Nitrate Induce the Expression of Phenylpropanoid Biosynthetic Genes and Induce the Accumulation of Rosmarinic Acid in *Agastache rugosa* Cell Culture, Molecules, Vol. 21:426.
[19]Partal, E., Paraschivu, M., 2020, Results regarding the effect of crop rotation and fertilization on the yield and qualities at wheat and maize in South of Romania, Agricultural Sciences & Veterinary Medicine University, Bucharest, Scientific Papers. Series A. Agronomy, Vol. LXIII, no.2:184-189.
[20]Rosculete, C.A., Bonciu, E., Rosculete, E., Olaru, L.A., 2019, Determination of the Environmental Pollution Potential of Some Herbicides by the Assessment of Cytotoxic and Genotoxic Effects on *Allium cepa*, Int. J. Environ. Res. Public Health, 16(1):75.
[21]Vis, M.A.M., Ito, K., Hofmann, S., 2020, Impact of Culture Medium on Cellular Interactions in in vitro Co-culture Systems, Front. Bioeng. Biotechnol. Vol. 8:911.
[22]Zhou, H.C., Li, M., Zhao, X., Fan, X.C., Guo, A.G., 2010, Plant regeneration from in vitro leaves of the peach rootstock 'Nemaguard' (*Prunus persica x P. davidiana*) Plant Cell Tissue and Organ Culture, Vol. 101(11):79-87.