THE CURRENT RESEARCH OF GARLIC IN VITRO PROPAGATION

STADIUL ACTUAL AL CERCETĂRILOR PRIVIND MICROMULTIPLICAREA „IN VITRO" LA USTUROI

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Abstract: *Garlic (Allium sativum L.) is a diploid vegetable plant (2n = 16), monocotyledonous, belonging to the Alliaceae family of the genus Allium, being the second most widespread species in its family, after onions.*

Because garlic does not produce seeds, it is propagated vegetatively by bulbs. The disadvantage of this type of propagation is the transmission of pathogens, viruses and mycoplasmas, with the final result being reduced plant vitality, decreased production and crop quality.

In vitro micromultiplication is the method of vegetative propagation with the highest level of propagation through which healthy, disease-free, juvenile plants are obtained. Over time, a lot of research has aimed to streamline this method of multiplication by discovering new disinfectants, new stimulants and diversifying the techniques used.

Keywords: methanol, acetaldehyde. sulphur dioxide, dimethyl dicarbonate, yeasts

Rezumat: *(Allium sativum L.) este o plantă legumicolă diploidă (2n=16), monocotiledonată, aparținând familiei Alliaceae din genul Allium, fiind a doua cea mai răspândită specie din familia sa, după ceapă.*

Deoarece usturoiul nu produce semințe, înmulțirea se face pe cale vegetativă, prin bulbi. Dezavantajul acestui tip de înmulțire îl constituie transmiterea agenților patogeni, a virozelor și micoplasmelor, având ca rezultat final reducerea vitalității plantelor, diminuarea producției și a calității recoltei.

Micromultiplicarea „in vitro" este metoda de înmulțire vegetativă cu cel mai mare nivel de propagare prin care se obțin plante sănătoase, libere de boli cu caractere juvenile. De-a lungul timpului, foarte multe cercetări au avut drept scop eficientizarea acestei metode de înmulțire prin identificarea de noi agenți dezinfectanți, a noi substanțe stimulatoare și diversificarea tehnicilor folosite.

Cuvinte cheie: in vitro, micropropagare, usturoi

INTRODUCTION

For centuries, garlic has been used all over the world, being one of the first documented examples of plants used in food as well as for treating diseases and

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maintaining health. Garlic is grown for bulbs and for young leaves, eaten fresh. The bulbs are eaten raw, dried or pickled, and are used to season and flavor a wide range of foods. Having a particularly intense aroma, it is used in small quantities, especially when raw. The leaves are eaten fresh or cooked, with a milder aroma than the bulbs (Stan and Munteanu, 2001).

Garlic is considered the most powerful natural antibiotic, prevents cardiovascular disease, is an adjuvant food in diabetes, increases the level of antioxidants and helps eliminate toxins. The active ingredients have antiseptic, bactericidal, antispasmodic, diuretic, aphrodisiac, expectorant, vasodilator, anti-influenza and cancer prevention properties (Haynes, 2016).

The center of origin of garlic in Central Asia, as confirmed by the discovery of fertile clones of garlic in the Tian Shan region of Kyrgyzstan and supported by biochemical research and molecular biology (Manjunathagowda *et al.*, 2017). Today, garlic is grown all over the world, where its ecological requirements are met. The region with the highest diversity of varieties is Eurasia and the lowest center of diversity is in western North America (Brewster, 2008).

In conditions like the centers of origin, garlic is a perennial or biennial plant, and in the conditions of our country it is an annual plant, with vegetative propagation, through bulbs. The bulb consists of a small disc-shaped stem and a bud covered by a tuberous leaf that is the pulp of the bulb. Each clove is covered with a protective leaf, white or purple, typical of the variety. The number of cloves in a bulb is at most equal to the number of leaves and can vary from 4 to 60 (Stan and Munteanu, 2001). Garlic bulbs planted in autumn go through an inactive period and require 6-8 weeks of low temperatures after planting. During autumn and winter, the bulbs develop their root system and initiate the growth of the top. The development of the leaves is accelerated by the short days, reaching a height of 30-40 cm or more, depending on the variety. If the garlic needs low temperatures after planting, heat and increase the length of the day are necessary for the formation of bulbs (Brewster, 2008).

Garlic bulbs cannot be stored for more than one season without losing viability, and it is necessary to cultivate them every year. The major disadvantage of vegetative propagation methods is its vulnerability to attack by diseases, pests or natural disasters, as well as plant degeneration. The plants propagated vegetative, once infected virally, the pathogen passes from one generation to another, so that, in a few years, the entire population can be infected. Symptoms are difficult to detect, and no physical or chemical treatment can eradicate viruses from infected plants. The only effective method of obtaining virus-free plants is *in vitro* multiplication.

MATERIAL AND METHOD

The tissues culture is based on the concept of totipotency of higher plant cells, formulated by Hamberlandt in 1902. Micropropagation is the growth of organs, organ fragments, tissues or plant cells on artificial media, under conditions of total asepsis

and controlled environmental factors. The advantages of *in vitro* multiplication are high productivity, maintaining genotypes that are difficult or impossible to multiply by traditional methods, preserving genetic stocks for long time (Constantinovici, 1998).

The aim of micropropagation is to produce many plants capable of surviving in natural environmental conditions, and any of the methods used involves going through steps that ensure the success of the whole process. Each of these steps requires a change in culture media and culture conditions (fig. 1).

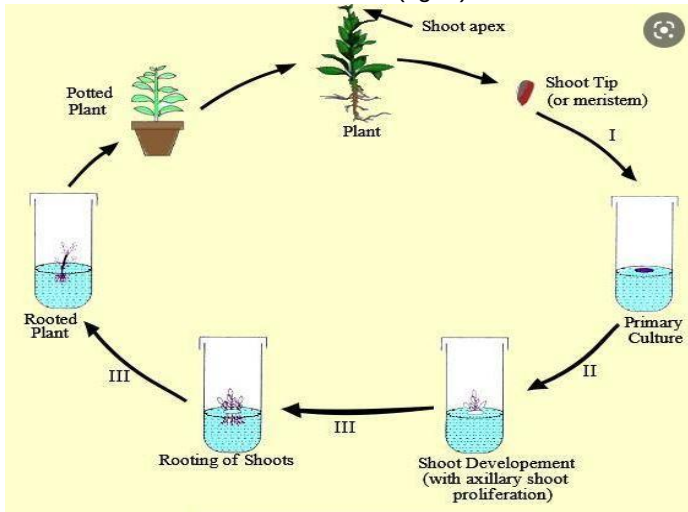


Fig. 1 Stages of the *in vitro* micromultiplication process (source: https://bit.ly/31RUdWZ)

RESULTS AND DISCUSSIONS

The processes of organogenesis and cell differentiation are influenced by physical, biological and chemical factors. Cells, fragments of tissues or organs isolated from the mother plant and placed *in vitro* conditions need a sterile, nutritious and balanced culture medium. It contains a mixture of double-distilled water, macro elements (N, P, K, Mg, Ca, Na, Cl), microelements (B, Zn, Mn, I, Al, Fe), vitamins, growth regulators, a source of nitrogen and a source of energy. The composition of culture media differs from one author to another, the most used being the media Murashige & Skoog, Gamborg, Nitsh, Linsmaier & Skoog.

Hormonal balance plays a key role in initiating, maintaining crop viability and regenerative capacity. The combined action of auxins and cytokinins has been shown to be more effective than individual phytohormones and their type, combination and concentration are essential for the regeneration and multiplication processes. The auxin / cytokinin ratio determines the direction of inoculum evolution: if the auxin / cytokinin ratio is supraunitary, a rhizogenic reaction will be obtained, if the ratio is subunitary, a caulogenous reaction will be obtained, and near to unit ratio will be obtained callus formation (Cachiță-Cosma *et al.*, 2004).

The success of micropropagation also depends on the ensuring the special and controlled conditions in the growth chamber: the relative humidity of the air must be higher than 50%, the temperature around 220-240C, the photoperiod of 16 h light hours and 8 h dark with an intensity of 2500-3000 lux (fig. 2).

Fragments of organs or tissues used to initiate culture are called explants. Cells in their structure, aseptically inoculated on nutrient media and incubated under controlled conditions lose their specialized structure and are stimulated to proliferate.

Fig. 2 Growth room appearance (original)

The explant becomes the inoculum that must survive independently and resume its functions that would have had according to its nature and origin in the body of the mother plant (Cachiță-Cosma *et al.*, 2004). The explant can be taken from any part of the plant: growth tip, meristem, leaves, stems, roots, as well as from the reproductive organs. It is generally recommended to initiate an *in vitro* culture from explants taken from plants as young as possible or from the youngest areas of the organs (fig. 3).

Another condition on which the success of *in vitro* cultures is established of aseptic measures, starting with the sterilization of rooms and surfaces, culture vessels, plant material, water and culture media, as well as the correctness of the procedures (Cachiță-Cosma *et al.*, 2004).

Numerous studies have been done over time on the development of garlic micromultiplication protocols. These refer to the composition of the culture media, the type of explant used as well as the influence of the genotype. The first experiments on garlic tissue cultures were performed in 1970 and Bhojwanii (1980) was the first who test different combinations of growth regulators for the regeneration of garlic by direct organogenesis.

The composition of the culture medium is a major determinant of plant growth *in vitro* and, for this purpose, several types of culture media are used:

Murashige & Skoog (MS), Gamborg (B5), Linsmaier & Skoog (LS), etc. The selection and combinations of media are important parameters for the optimization of *in vitro* regeneration protocols.

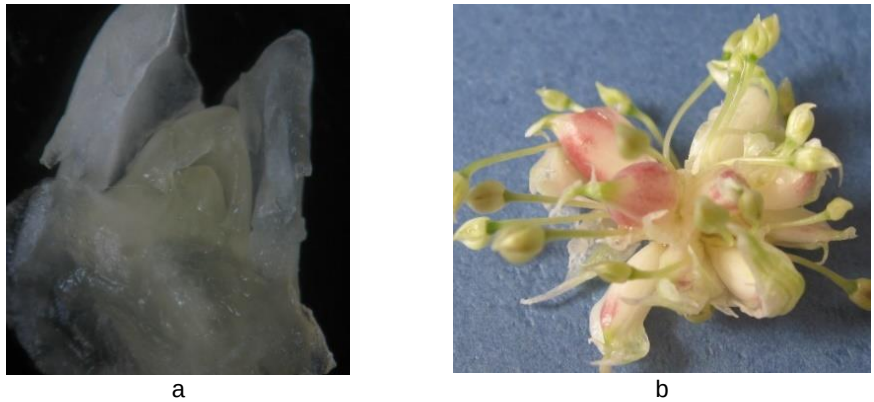
Growth regulators are organic substances with physiological actions like phytohormones, being synthetic substances used in very high dilutions. They stimulate the multiplication, growth, and differentiation of cells, being metabolized more slowly than endogenous hormones, the action of growth regulators being longer lasting and stronger effects. The growth regulators are auxins, cytokines, gibberellins, ethylene and abscisic acid, the most used being the first two (Cachiță-Cosma *et al.*, 2004).

The action of auxins consists in the growth of cells in length, the stimulation of cell division, the permeability of plasma cells and the stimulation of rhizogenesis (Constantinovici, 1998). The most used auxins are: 3-indolylacetic acid (AIA), 3-indolylbutyric acid (AIB), 1-naphthylacetic acid (ANA) and 2,4-dichlorophenoxyacetic acid (2,4-D).

Cytokinins stimulate the formation of stems and buds, maintain cell viability, promote cell differentiation and multiplication, having a role in preventing senescence (Constantinovici, 1998). The most used cytokines are: 6-furfurylaminopurine (Kinetin), benzyladenine (BA), 2-isopentyladenine (2iP) and zeatin (Z).

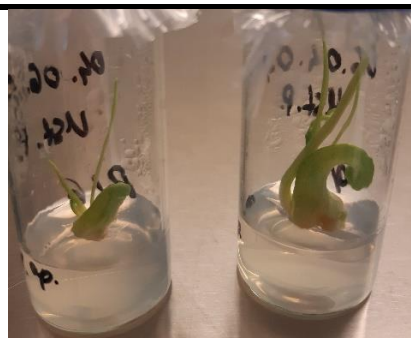
Garlic micromultiplication can be done using the following types of biological material: root fragments, apical meristems, shoot tips, aerial bulbs and fragments of the basal disc of the bulb.

Research reports has shown that apical meristems are the best source of explants because they can divide actively throughout life. Meristematic tissues are the youngest in the body of plants and are also called dividing or formative tissues because their cells divide continuously, and the rate of division is faster in meristematic tissue than that of viral cells. As a result, apical meristems are juvenile and virus-free (fig. 4).



a b

Fig. 3 Types of garlic explant: a) apical meristem; b) flower bulbs (original)



a

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Fig. 4 Micropropagation of garlic by apical meristem: a) shoot multiplication; b) *in vitro* bulbils

Table 1

Research reports on virus-free garlic seed production by meristem culture

Reference	Results
Bhojwanii (1980)	The lower amount of auxin and the higher amount of cytokinin favored the proliferation of multiple shoots.
Kahane *et al.* (1992)	Successful initiation of multiple shoots by the exclusive use of 0.5 mg / l benzyladenine (BA).
Seabrook (1993)	Benzyladenine (BA) or isopentyladenine (2iP) causes the same results in the multiplication phase.
Haque *et al.* (1998)	The best percentage of rooting was in the case of using the Murashige & Skoog (MS) medium without growth regulators and for the formation of micro bulbils is necessary to increase the concentration of sucrose.
Barandiaran *et al.* (1999)	Decreasing the number of subcultures by using the same culture medium for all stages of seedling development.
Devi, Khar and Lawande (2007)	The culture medium with the highest amount of cytokinin determined the highest multiplication rate, with the formation of roots and microbubbles in the shortest time.
Taşkin *et al.* (2013)	They demonstrated by RT-PCR testing that the plants obtained by micromultiplication were free of OYDV and LYSV viruses.
Mehta *et al.* (2013)	Murashige & Skoog (MS) medium with 1 mg / l kinetin is best suited for multiplication, and for rooting the same type of medium supplemented with 2 mg / l indolyl butyric acid (IBA).
Metwally *et al.* (2012)	The best results on root formation were obtained in the Murashige & Skoog (MS) medium without growth regulators.
Fiserova *et al*. (2016)	The addition of ABA (abscisic acid) in MS medium promotes the formation of bulbs only in case of poorly growing cultivars; in those that grow well it shows an inhibiting effect. Low regeneration capability can be improved by exogenous paclobutrazol.
Gemachu (2021)	The maximum number of shoots was observed on the medium supplemented with 2 mg / l benzyladenine (BA) and 0.25 mg / l naphthyl acetic acid (ANA). For rooting shoots, Murashige & Skoog (MS) medium with 1 mg / l naphthyl acetic acid (ANA) determined 100% root formation.
Mirghiș (1992)	The results showed the dependence of the *in vitro* reaction capacity of the meristems on the genotype-environment interaction, on the size of the meristematic tissue, on its physiological state and on the

	endogenous phytohormonal level. The basic Murashige & Skoog (MS) environment favored the development of vigorous seedlings with roots and a higher rate of multiplication, and the phytohormonal balance had a significant influence on the ability to multiply.

CONCLUSIONS

1. Garlic is one of the most important and cultivated vegetable plants belonging to the genus Allium, being used for food and medicinal purposes for thousands of years.

2. Being a vegetative propagated species, garlic is infected with pathogens (bacteria, fungi, viruses) that are easily transmitted to offspring, resulting in decreased product quality.

3. In vitro micropropagation is the growth of plant tissues or cells on artificial media, under conditions of total asepsis and controlled environmental factors.

4. A key role for the success of tissue culture technology is played by the composition of nutrient media that must ensure the regeneration and development of seedlings in sterile conditions.

5. Explants are very small fragments of plants that can come from the vegetative organs: growth tip, meristem, leaves, stems, roots, as well as from the reproductive organs: anthers, pollen, eggs, embryo, seeds, spores.

6. Research conducted nationally and internationally has shown suitability for garlic micromultiplication using a wide variety of culture media and a variety of types of explants.

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