

## Conference Paper

# An Analysis of the Micropropagation and Peculiarities of Eggplant (*Solanum melongena* L.) Cultivation Technology

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**ORCID:**Olga Pyshnaya: <http://orcid.org/0000-0001-9744-2443>**Abstract**

This review is devoted to biotechnological methods and approaches used in eggplant selection. Using in-vitro techniques, micropropagation makes it possible to obtain *Solanum melongena* L. plants identical to the original ones. This article discusses various aspects of eggplant microcloning; draws conclusions about the successful induction of shoots, which is an important stage required for the regeneration of the callus; and describes the methodological issues of origin, explant treatment, cultivation features, composition of nutrient media, growth regulators, growing conditions and rooting algorithms, and adaptation of the resulting regenerant plants. The microclonal reproduction of *Solanum melongena* L. by organogenesis from cotyledon and hypocotyl explants is described. After explants were introduced into an in-vitro culture, significant differences in the nature of growth and development of the tissues in the nutrient media were observed on the 6-8th day. A comparative analysis of the stimulating effect of growth regulators revealed that thidiazuron with a concentration of 0.02 mg/l had the highest efficiency; its effect on the regenerative capacity of the explant depended on the concentration in the medium. The dependence of all stages of microclonal reproduction on the genotype is shown.

**Keywords:** *Solanum melongena* L., eggplant, nightshade, microcloning, biotechnology

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## 1. Introduction

Eggplant, or dark-fruited nightshade (lat. *Solanum melongena* L.) is a species of perennial herbaceous plants of the Solanaceae family, numbering about 90 genera and 2300 plant species growing in various ecologically zones [1]. *S. melongena* L. presumably descended from the wild species *S. incanum* domesticated in India and southeast China; in India, you can find wild ancestors of eggplant [2]. Vavilov believed that the culture originated in the Indo-Chinese center [3].

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Eggplant is often used in cooking; it is also popular with nutritionists [4]. Their attention is drawn to the polyphenolic compounds contained in eggplant and chlorogenic acid, which prevents the development of type II diabetes. The nutritional value of eggplant is due to the content of calcium, iron, potassium, phosphorus, fiber and B vitamins [5]. The combination of biochemical indicators determines the importance of its use, but, unlike other members of the family, in particular, tomato, eggplant is quite demanding.

Eggplant has a large share of the gross harvest of vegetables. Its wide distribution is due to its high yield, biological value, ability to grow in various ecological zones [1]. According to 2019 data, the world eggplant production volume is 51.313 million tons. The top ten includes growing eggplant are China (over 32 million tons), India (12.55 million tons), Egypt (1.2 million tons), Iran (677.7 thousand tons), Indonesia (509.7 thousand tons), Italy (317.6 thousand tons), Turkey (854 thousand tons), Japan (306 thousand tons), Spain (238.7 thousand tons), Philippines (235.6 thousand tons). Over the past decade, production has practically doubled [6]. In Russia, the volume is 47.2 thousand tons. The key regions are Krasnodar and Stavropol Territories, Rostov, Volgograd, Saratov and Astrakhan Regions, which together account for over 75% of eggplant production in Russia [2].

At present, a large number of eggplant varieties, that meet the requirements of agricultural production and amateur gardening, have been created [4]. Growing eggplant outdoors is effective only in the southern regions, since it is very thermophilic. In the temperate climate zone, eggplant is grown only in greenhouses [6]. Some varieties of eggplant are very demanding on moisture and light; almost all varieties do not tolerate transplanting, as they are very sensitive to the root system damage; their cultivation in Russia is possible only by the seedling method [1].

## 2. Features of the Microclonal Reproduction of *Solanum melongena* L.

The main disadvantage of seed reproduction is the genetic heterogeneity of the planting material, as well as the duration of the juvenile period [7]. Vegetative propagation, which contributes to the preservation of the genotype of the mother plant and the reduced juvenile period, is impossible for eggplant [8]. The combination of these problems determines the need to use the micropropagation method. It is based on the totipotency of plant cells - their ability to give rise to an integral plant organism under the influence of exogenous processes. The *in vitro* method can be used to obtain plants that are genetically identical to the original specimen [8].

In recent years, interest in microclonal reproduction of eggplant has decreased due to increasing possibilities of genetic modification of plants [9].

According to Magioli C. and Mansur E., the efficiency of eggplant organogenesis depends on the type of explants used and combinations of plant growth regulators [9].

Micropropagation has several advantages:

- due to the use of meristem culture, the plants are not infected with the virus. For the Solanaceae family, susceptibility to viral diseases is a problem: Russian and foreign scientists have described a number of cases when a significant number of valuable varieties of tomato, potato and pepper died due to viral diseases. It was possible to restore these valuable varieties only with the help of a healthy in vitro cultured meristem obtained from infectious plants [10; 11];

- the multiplication factor increases [4];

- there is an acceleration of the transition of plants from the juvenile to the reproductive phase of development, which makes it possible to use the warm season more fully, which is very important for temperate climatic zones [1];

- it becomes possible to accurately control the environmental factors: temperature, humidity, length of day, spectral composition and light intensity, composition and amount of nutrients used, etc. [12].

O. Matushkina adds the ability to reduce one-time labor costs (due to the fact that work is carried out more evenly throughout the year), as well as saving territories for the cultivation of planting materials - especially in those regions where there is a need to use greenhouses [13]. D. Ualieva believes that micropropagation requires manual labor, experienced and qualified employees. The economy of territories is covered by complex biotechnological processes that require reagents and special equipment. Among the most important advantages of microcloning, Ualieva mentions a significant reduction in the timing of selection, which can eventually become more targeted [14].

V. Pivovarov and N. Shmykova described the stages of microclonal reproduction of eggplant (based on the results of experimental tests on Snezhny and Solaris varieties), characterized by the following features of cultivation in the in vitro system [4]:

- During the isolation phase, it is often difficult to obtain an initial sterile culture. For this, antibiotics (tetracycline, benzylpenicillin, etc.) with a concentration of 100-200 mg / l are added to the nutrient medium.

- The most commonly used medium is Murasig and Skoog (MS) [15], which contain mineral salts, biologically active substances and growth stimulants (auxins, cytokinins). As an oxidation inhibitor, ascorbic acid (1 mg / l), glutathione (4-5 mg / l), dithiothrietol

(1-3 mg / l), diethyldithiocarbamate (2-5 mg / l) are used. This stage usually lasts 1-2 months; during this period, meristematic tissues grow and primary shoots develop [15].

### 3. Characteristics of the Development of Explants in Nutrient Media.

R. Butenko and N. Kataeva [16] describe features of the development of explants in various nutrient media and their dependence on the origin (Tables 1-2):

- In explants of cotyledons, dense dark and light green callus with numerous buds developed, the rapid development of micro-rosettes and good roots was observed;
- Hypocotyl explants had a white callus with a medium bud development rate. Shoots developed in the upper part of the hypocotyl, but the roots developed more actively;
- The explants of young leaves were characterized by dense green callus with leaf primordia, and micro-rosettes developed on some explants, but root formation was slowed down.

It was found that micropropagation aimed at obtaining a significant number of meristematic clones weakens shoots [4].

The ratio and concentration of auxins and cytokinins in the nutrient medium is of great importance. Of the cytokinins, BAP is often used (1-10 mg/l). Among auxins, p-indoleacetic acid (IAA) and 1-naphthylacetic acid (NAA) at concentrations of up to 0.5 mg/l are optimal.

Shoot induction is an essential step for the successful regeneration of transformed plant tissues. P. Foo published results on the ability of BAP and kinetin to induce callus regeneration in cotyledon explants [17]. On the other hand, kinetin at a concentration of 2.0 mg/l successfully induced shoots with an average of  $1.50 \pm 0.22$  shoots per explant, while BAP did not induce any shoot formation. This study showed that kinetin alone is sufficient to induce shoot formation in eggplant varieties.

Scientists conducted a comparative analysis of the stimulating effect of growth regulators, which indicates the greatest effect of thidiazuron. For the regeneration media, MS is most effective in combination with BAP and reduced TDZ concentration. A high concentration of TDZ causes vitrification, but significantly reduces the percentage of rooted shoots. Shoots obtained from the buds of cotyledon callus at the same concentration of stimulants rooted more actively than buds and microsockets developed from the loose callus of the hypocotyl. Shoots formed from young eggplant leaves developed less actively [16]. V. Verba et al. emphasize that the problem of vitrification

of shoots can be solved by the multiple transplantation of their upper part in ½ MC medium [1].

Cytokinins can accumulate in plant tissues at a concentration above the required physiological level, which leads to the formation of morphologically altered plants. Cytokinins are commonly used in plant tissue culture to induce cell growth, as well as to stimulate the shoot development; due to this, they are actively involved in various physiological processes of plants. This includes propagation of shoots, formation of root tubers, induction of callus, and aging of leaves [17]. E. Kotko points out that the proliferation of axillary meristems, the formation of vitrified shoots and a decrease in the ability to root due to the underdevelopment of root primordia are possible [14]. According to N. Kataeva and R. Butenko, the negative effect of cytokinins can be overcome by minimizing their content in nutrient media, or by alternating cultivation cycles in the media with low and high phytohormone levels [16].

#### 4. Rooting of the Resulting Shoots to the Specified Conditions.

N. Shmykova offers one of two ways to root microshoots:

- for 2-24 hours incubation in a sterile solution of auxin at a concentration of 20-50 mg/l, and cultivation in the agar medium without phytohormones or in a suitable soil substrate [19];
- within 20-30 days, cultivation of microshoots in a nutrient medium at a low concentration of auxin (1-5 mg / l) [4].

At this stage, the concentration of mineral salts in the MS medium is reduced by 2-4 times [16], and the amount of sugar is reduced to 0.5-1%; there are only auxins, while cytokinins are completely excluded. BCI, IAA or NAA are used to activate rooting

However, in 1989, N. Savinova proposed a hydroponic method, which simplifies both rooting and plant adaptation to natural conditions. Thus, for eggplant, it is recommended to add activated carbon to the liquid medium, or to shade the bottom of culture vessels with dark non-absorbent paper [20].

#### 5. Growing Planting Materials in a Greenhouse and Preparing them for Transplanting As Seedlings.

According to Mallaya, the same algorithm is used for eggplant. Plants with two or three leaves and a well-developed root system are considered planting materials. They are

removed from test tubes or flasks with tweezers. To avoid injury, ENT forceps with rubber tips at the bent ends are used. The roots are washed from agar, and then planted in pick boxes or peat pots, which are previously sterilized at 85 - 90° C (1-2 h) with a soil substrate. For eggplant, it is recommended to use a substrate of peat soil and sand (3: 1). Regenerants are grown in greenhouses at a temperature of 20-22 °C, a humidity of 65-90% and an illumination of no more than 5000 lux. 20-30 days after planting; they are fed with a complex mineral fertilizer or a solution of Murashige and Skoog [20].

TABLE 1: Characteristics of the development of eggplant explants in the induction media 4 weeks after the introduction into the culture according to R. Butenko [16]

Growth regulators *, mg / l	Cotyledons (divided)		Hypocotyl (parts)			Young leaves
	along	across	upper	average	bottom	
0.02 TDZ	green callus, buds, shoots	green callus, buds, shoots	shoots and loose white callus, shoots	white loose callus	white loose callus	green callus, kidneys,
0.2 TDZ	green callus, buds, shoots	green callus, buds, shoots	shoots and loose white callus, shoots	white loose callus	white loose callus, shoots	green callus, shoots, minor rhizogenesis
0.2 BAP	green callus, buds, shoots	green callus, buds, shoots	white loose callus, shoots	white loose callus	white loose callus, slight rhizogenesis	green callus, minor rhizogenesis
2.0 BAP	green callus, buds, shoots	green callus, buds, shoots	shoots and loose white callus, shoots	weak white callus	white loose callus	green callus, shoots
2.0 NUK	rhizogenesis, white-green loose callus	rhizogenesis, white-green loose callus	powerful white-green callus	powerful white-green callus	powerful white-green callus, rhizogenesis	Dense green callus
0.2 KIN	yellowing, faint white loose callus	weak white loose callus, rhizogenesis	white loose callus	white loose callus	white loose callus	green callus, minor rhizogenesis
2.0 KIN	yellowing and death of explants	yellowing and death of explants	yellowing and death of explants	yellowing and death of explants	yellowing and death of explants	yellowing and death of explants

Burgutin spoke out against washing agar just from eggplant. Unlike tomato, the root system of eggplant tolerates transplanting worse. Agar promotes the rooting of the plant in the soil, as it contains some nutrients. It is recommended to transplant the eggplant plant into the soil together with agar to prevent injury to the root system, deepening so that only developed leaves remain above the soil surface. Thus, this recommendation must be taken into account because it represents a hybrid option between the hydroponics and growing in soil. It provides optimal root moisture [21].

TABLE 2: The number of buds and shoots of eggplant in explants 4 weeks after the introduction into in vitro culture according to R. Butenko [16]

Growth regulator *, mg / l	Explant type					
	Cotyledon		Hypocotyl		Leaves	
	shoots, pcs.	Buds, pcs.	shoots, pcs.	Buds, pcs.	shoots, pcs.	Buds, pcs.
0,02 TDZ	13,9 ±3,0	39,9 ± 4,0	8,0 ±2,8	17,0 ±5,1	5,0 ±1,8	12,3 ±3,4
0,2 TDZ	-	17,2 ±3,7	2,3 ±1,5	9,1 ± 3,3	1,4 ±0,8	7,1 ± 1,3
2,0 NAA	-	3,0 ±3,4	0,2 ±0,2	0,5 ±0,9	-	-
0,2 BAP	1-6 ±1,2	6,0 ± 1,3	7,0 ±3,3	4,0 ± 1,1	4,3 ±1,6	2,0 ± 0,6
2,0 BAP	-	6,7 ±3,3	3,5 ±2,9	5,5 ± 3,3	2,3 ±1,2	3,5 ± 1,2
0,2 KIN	-	1,2 ± 1,5	4,6 ± 1,3	-	2,4 ± 0,8	-
2,0 KIN	-	-	-	-	-	-

\*TDZ – tidiazuron;  
 NAA – naphthylacetic acid;  
 BAP – benzyl aminopurine;  
 KIN – 6-furfurylaminopurine.

An additional complication in eggplant microcloning is growth retardation after transplantation into soil. This is directly related to the disruption of the stomatal apparatus and the loss of a huge amount of water [7]. G. Vural and E. Ari suggested spraying leaves with a 50% aqueous solution of glycerin during the entire acclimatization period. This will prevent dehydration of plants and increase their survival rate [7]. N. Dubenok believed that the spraying reduces further adaptation of plants. He recommended a simpler method - growing eggplant regenerants in tunnel shelters, in conditions of “artificial fog”, or in boxes covered with glass or foil [22]. 10-14 days after, the cover is removed; the pots or boxes are placed in normal, non-sterile conditions [22]. To confirm the value of humidity, V. Borodychev emphasizes one more feature of microcloned eggplant seedlings. Under in vitro conditions, the structure and growth of the root can be changed; as a result, the absorption of water and mineral salts from the soil worsens [8, 23]. Artificial mycorrhization of plants is considered expedient, given its positive role in supplying plants with mineral and organic nutrients, water, biologically active substances, as well as in protecting plants from pathogens [23].

## 6. Conclusion

Increasing the competitiveness of agricultural products while reducing resource consumption and costs is possible by accelerating the selection process. The latest biotechnological approaches are important. Clonal micropropagation is an important tool used

in solving many applied problems in eggplant selection; however, there are no methods for this. One of the reasons may be the dependence of all stages of microclonal reproduction on a specific genotype. That is why optimized methods and an individual approach are required for individual groups of varieties.

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