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RESEARCH ARTICLE

Micropropagation of the medicinal plant *Physalis alkekengi*

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ABSTRACT

Background: The theoretical and practical issues of clonal micropropagation of the medicinal plant *Physalis alkekengi* L. are considered in the article. For the 1st time, on the basis of the conducted research, the micropropagation of the medicinal plant *P. alkekengi in vitro* was carried out. **Aims and Objectives:** The organs and tissues of the medicinal plant *P. alkekengi* L. were the objects of the current study. **Materials and Methods:** Sterilization of media, plant material, and work was performed under aseptic conditions. Plant explants were propagated *in vitro* in a medium. Murashige and Skoog medium was used as the main medium for micropropagation. **Results:** It was found that the size of the explant from 0.6 to 1.0 cm is optimal for micropropagation. **Conclusion:** For the 1st time on the basis of microclonal propagation, the *in vitro* micropropagation of the medicinal plant *P. alkekengi* was carried out. In addition, the effect of the concentration of the antifungal drug nystatin on plant growth and development was studied.

KEY WORDS: *Physalis alkekengi* L.; Medicinal Plant; Nodal Explant; Clonal Micropropagation; Virus; Tissue; Explant; *In Vitro*

INTRODUCTION

The aim of the research was to study the clonal micropropagation of ornamental and medicinal plants *Physalis alkekengi* L., biotechnological approach of cultivating plants from the explant and obtaining planting material.

It is known that many plants reproduce by dividing the bushes, cuttings, and seeds. However, these methods have several disadvantages. In this regard, an effective method of micropropagation of various vegetable crops and plants has been developed.[1,2] It should be noted that clonal micropropagation can be done in different ways, for example, suppression of apical dominance and development of axillary buds; the formation of microtubers and microbulbs; receiving callus tissue; and microcloning using explant tissues.

Among the above methods, the most promising is the method of microcloning with the help of explant tissues. Most of the higher plants have an intermediate type of growth, in which there are additional meristematic tissues in the axils of the leaves that can be formulated in the shoot, identical to the main.[3,4] In this regard, for the 1st time, we have developed a method of clonal micropropagation of medicinal plants *P. alkekengi.*

P. alkekengi is a medicinal plant from the family of the nightshade. It is used for therapeutic purposes such as, anti-inflammatory, diuretic, antidiabetic, and antitumor. Decoction or water infusion of fruits is taken with urolithiasis, cystitis, hepatitis, bronchitis, intermittent fever, edema, ascites, rheumatism, gout, and bruises. Fresh fruits and juice of the plant are used for dermatoses, respiratory

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diseases, gonorrhea, dysentery, and hypertension [Figure 1]. Decoction of roots is recommended by folk medicine as an antitussive and analgesic.[5-8]

It should be noted that with clonal micropropagation of plants, the leading factor of a plant is the selection of the size of the explant. This is due to the fact that the smaller the size of the explant, the smaller the regenerative capacity of the plant explant and vice versa. In an explant of large size, the possibility of viruses and other pathogens appearing in its cells increases, which prevents regeneration. In this regard, in clonal micropropagation *in vitro*, the process of explant development was carried out by controlling the size, physiological age of the plant, the composition of the nutrient media, as well as the effect of the antifungal preparation nystatin on the growth and development of the plant.

MATERIALS AND METHODS

The objects of the study were the organs and tissues of the medicinal plant *P. alkekengi* L.

Research studies were conducted at the center for advanced technologies of the republic of Uzbekistan in the cell engineering laboratory.

In the experimental part of the work, common methods of biotechnology research were used as the main research method.[3,4,9]

Sterilization of media, plant material, and work was performed under aseptic conditions. Plant explants were propagated *in vitro* in a medium. Murashige and Skoog medium was used as the main medium for micropropagation.[4,10]

P. alkekengi was cut into parts [roots, stems, and leaves, Figure 2a and b] in sterile conditions. The explants were cultivated at a temperature of 24–25°C during the day and at night under illumination of 5–6 klx (Kilolux is a unit of measurement of illumination). The growth of the stem and roots began to develop on the 10th day after planting on a medium, and the plants were fully formed in 10–11 days.

Transplantation of *P. alkekengi* in the Soil

After the appearance of the roots, stem, and leaves in the plants, they were planted in sterile soil and the conditions of a moist chamber were created. Then, the development of plants was observed. Watering plants were carried out as the drying of the soil.

RESULTS

For the mass reproduction of test-tube *P. alkekengi*, periodical microclonal propagation was carried out. It should be noted that

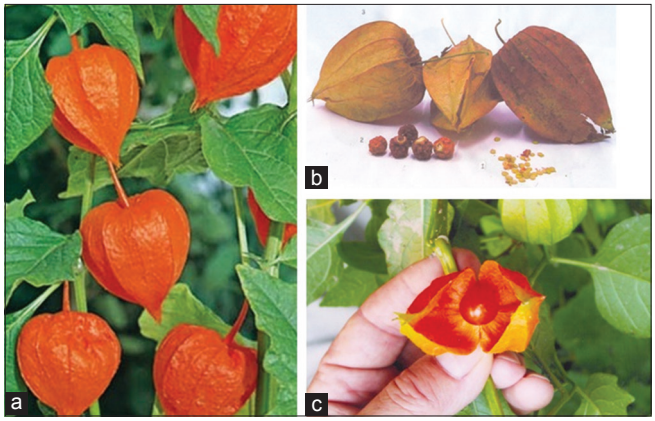

Figure 1: Photograph of *Physalis alkekengi*; (a) appearance of the plant, (b) fruit and calyx, and (c) calyx, fruits, and seeds

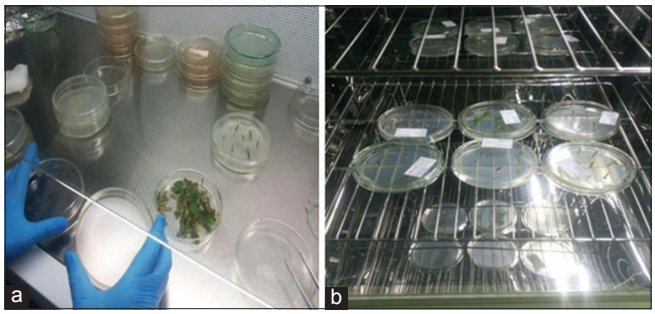

Figure 2: (a and b) Planting explants in a medium

in clonal micropropagation of plants, one of the main parameters is the selection of the size of the explant. This is due to the fact that in micropropagation, where large sizes of explants are used, the probability of infection in an *in vitro* culture also increases. In this regard, we have selected the optimal sizes of the explant, and at the same time, the composition and concentration of the antifungal preparation nystatin were selected.

It should be noted that the optimal size of the explant depends on the species characteristics of the donor plant, as well as on the properties of the organ that serves as the source of explants, and it is necessary to ensure the sterility of the explant. For the purpose of mass propagation, stem and root explants of 0.4; 0.6; 0.8; and 1.0 cm of the *P. alkekengi* were used.

On the explants of roots with a size of 0.2 cm, shoots were not observed. Regeneration of root explants was observed on the stem segments measuring 0.8–1.0 cm for 10–11 days. Regeneration of plants, where stem sections of length 0.8 were used; 1.0 cm, occurred faster (on the 10th day), but at the same time, growth of microorganisms was observed around the explant. On a nutrient medium, places free from segments of plant stems, and no growth of microorganisms was observed. The best results were observed on stem explants of 0.8–1.0 cm in size [Table 1, Medium No. 2 and No. 3].

At the same time, in this work, we selected the optimal concentration of the antifungal preparation nystatin on the growth and development of the explant of the plant

Table 1: Selection of the size of the *Physalis alkekengi* explants (M±m; ***n***=5) (Medium without hormones)

Length of root explant (cm)	Rooting		Length of stem explant (cm)	Length of stems		Length of leaves	
	Days	Length (cm)		Days	Length (cm)	Days	Length (cm)
Medium No. 1 control (without nystatin)							
0.2	2–3	-	0.2	8–9	-	8–9	-
0.4	4–5	-	0.4	8–9	-	8–9	-
0.6	6–7	-	0.6	8–9	0.3±0.04	8–9	0.5±0.06
0.8	7–8	-	0.8	8–9	0.5±0.06	8–9	1.0±0.08
1.0	7–8	-	1.0	8–9	0.5±0.06	8–9	1.0±0.08
Medium No. 2 control (with nystatin 500,000 U/L)							
0.2	2–3	-	0.2	8–9	-	8–9	-
0.4	4–5	-	0.4	8–9	3.0±0.08	8–9	0.5±0.06
0.6	6–7	-	0.6	8–9	3.5±0.12	8–9	2.0±0.14
0.8	7–8	-	0.8	8–9	3.5±0.12	8–9	2.5±0.32
1.0	7–8	-	1.0	8–9	6.0±0.16	8–9	2.5±0.32
Medium No. 3 (with nystatin 1,000,000 U/L)							
0.2	2–3	-	0.2	8–9	-	8–9	-
0.4	4–5	-	0.4	8–9	4.0±0.08	8–9	1.5±0.16
0.6	6–7	-	0.6	8–9	6.5±0.32	8–9	2.8±0.18
0.8	7–8	-	0.8	8–9	6.5±0.32	8–9	2.8±0.18
1.0	7–8	-	1.0	8–9	8.0±0.34	8–9	3.5±0.36
Medium No. 4 (with nystatin 1,500,000 U/L)							
0.2	2–3	-	0.2	8–9	-	8–9	-
0.4	4–5	-	0.4	8–9	-	8–9	-
0.6	6–7	-	0.6	8–9	0.2±0.02	8–9	0.5±0.06
0.8	7–8	-	0.8	8–9	0.4±0.03	8–9	0.8±0.06
1.0	7–8	-	1.0	8–9	0.4±0.03	8–9	0.8±0.06

P. alkekengi. The data obtained are presented in Table 1. The table shows that in the control medium, after 8–9 days (explant size 0.5–1.0 cm), the length of the stem was from 0.3 to 0.5 cm, and the leaf length was from 0.5 to 1.0 cm. It should be noted that nystatin is antifungal medications. In this regard, during clonal micropropagation of plant explant, we used Medium No. 2 with nystatin (500,000 U/L) and the following results were obtained [Table 1].

From the obtained data, it became known that after 10 days the seedlings of the plant, where the concentration of nystatin was 500,000 U/L, the stem was 3.0–6.0 cm, while the size of the leaves reached from 0.5 to 2.5 cm. The obtained data shows that the nystatin medium more favorably influences to the growth and development of the stem and leaves of the plant [Figure 3a-d].

Based on the data obtained, at the next stage of the study, we prepared Medium No. 3, where the concentration of nystatin was 1,000,000 U/L [Table 1]. The data obtained show that the medium with nystatin has a positive effect on the growth and development of plant explants. At the same time, after 10 days, the growth of the stem reached 8.0 cm, and the length of the leaves was 3.5 cm. Comparing the obtained data with the data of the explant control (in the control, the length of the stem was

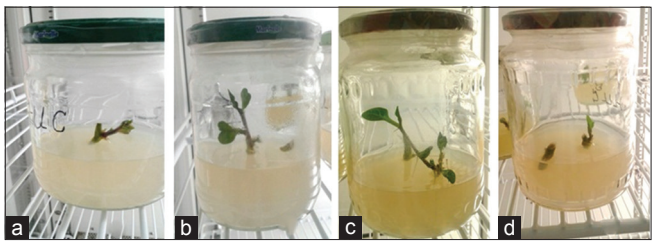

Figure 3: Effect of nystatin on the *Physalis alkekengi* growth and development (a) Control, (b) Medium No. 2 (with nystatin 500,000 units/1), (c) Medium No. 3 (with nystatin 1,000,000 units/1), and (d) Medium No. 4 (with nystatin 1,500,000 units/1)

0.3–0.5 cm, and the leaves were 0.5–1.0 cm), the efficiency of plant growth and development in Medium No. 3 is increased several times. Therefore, we prepared Medium No. 4, where the concentration of nystatin was 1,500,000 units/l. The data obtained are shown in Table 1. The table shows an increase in the concentration of nystatin adversely affects the growth and development of stems and leaves. At the same time, nutrient Medium No. 4 with a high concentration of nystatin inhibits the growth and development of the plant.

It should be noted that after the appearance of the roots, stem, and leaves in plants *in vitro*, they were planted in sterile soil [Figure 4]

Figure 4: *Physalis alkekengi* in sterile soil

and created the conditions of a wet chamber and then, observed the development of plants. Watering plants were carried out as the drying of the soil. At the same time, the microcloned plant *P. alkekengi* began to develop intensively in sterile soil.

Thus, we have studied the effect of the antifungal drug nystatin and selected the optimal size of explants on the *in vitro* growth and development of the plant *P. alkekengi*.

DISCUSSION

Considering the advantages of microcloning over other traditional methods, in this work, we used the clonal micropropagation method for mass propagation of test-tube plants of *P. alkekengi*. It should be noted that in the clonal micropropagation of plants, one of the main parameters is the selection of the size of the explant. This is due to the fact that in micropropagation, the size of the explant is of great importance. In this regard, we have selected the optimal sizes of the explant and found that stem explants with dimensions of 0.8–1.0 cm are the optimal size for microclonal propagation of the plant *P. alkekengi*.

Similar experiments on microclonal propagation of medicinal plants were conducted by scientific researchers G. Jahirhussain, S. Parvathi, V. Tamilselvan, V. Muniappan, K. Deepa, and R. Veerappan in India (Research Department of Botany, Government Arts College). The micropropagation is carried out from the nodal explants of *Physalis minima* Linn. Bod and kinetin (KIN). Benzyladenine was found to be a number of shoots from nodal explants when compared to KIN.

Strength and Limitations

This method has several advantages over existing traditional methods of reproduction since high multiplication factor (10^5–10^6) obtaining a genetically homogeneous material, freeing the plant from viruses, reducing the duration of the selection process, the ability to automate the process, cultivation, mass production of health, and planting material from plants.

In the experiment, we observed some deficiencies in the *in vitro* growth of the *P. alkekengi*: The plant stems grew quickly and strongly, but the leaves grew more slowly. We think that in the following experiments, these deficiencies can be corrected with the use of growth hormones for plants.

CONCLUSION

For the 1st time, on the basis of the performed studies, the *in vitro* micropropagation of the medicinal plant *P. alkekengi* was carried out. It was found that the size of the explant from 0.6 to 1.0 cm is optimal for micropropagation. In addition, the effect of the concentration of the antifungal drug nystatin on plant growth and development was studied. The possibility of transplanting microclonal plants into sterile soil was shown.

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