

In vitro clonal microreproduction of ginger

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The purpose. To develop a method of clonal microreproduction of ginger for deriving quality planting stock.

Methods. Laboratory, biotechnological, statistical. **Results.** Germination of gemmas on roots in conditions of thermostat at temperature $28\pm 2^{\circ}\text{C}$ and moisture content of 90% has been observed for 4 weeks of cultivation. Yield of gemmas has made 161,8%. Application of 0,1% of solution of sublimate with exposure of 45 – 50 min ensures 50,0 – 63,9% of aseptic gemmas of ginger at different biotypes. Altitude of primary shoots in a month of cultivation averaged 2,2 cm, amount of roots — 4,5 pieces for 1,9-cm gemma. Addition into nutrient medium of BAP raises sprout-formation up to 7,7 pieces/shoot for 1 passage. Formation of roots was observed on 10 – 14 day of cultivation in all probed biotypes irrespective of presence of auxins in nutrient medium. In conditions of growing of microplants of ginger *in vitro* survival of seedling made 72 – 98%. **Conclusions.** At stimulation of sprout-formation on ginger hands in thermostat at temperature $28\pm 2^{\circ}\text{C}$ and moisture content of 90% the yield of gemmas has made 161,8%. Application of 0,1% of solution of sublimate with exposure of 45 – 50 min enables to gain 50,0 – 63,9% of aseptic gemmas of ginger at different biotypes. The yield of viable gemmas makes 61,4 – 81,9%. Application of BAP (3 mg/l) ensures high multiplication ratio of ginger — up to 7,7 pieces/shoot for 1 passage. Culture shoots of ginger *in vitro* do not demand add-on of auxins into nutrient medium for stimulation rhizogenesis. Culture seedling of ginger *in vitro* has high level of survival in conditions of open ground — 72 – 98%. That enables to use a method of cloning *in vitro* for deriving quality planting stock. The developed method of clonal microreproductions of ginger enables to obtain from one *in vitro* explant up to a thousand rooted plants.

Key words: ginger, gemmas, shoots, *in vitro*.

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Ginger is one of the medicinal plants that is in demand in many countries around the world and has about 150 species [1]. Ginger is used globally for the production of an extract of oleosin and essential oils, as well as for direct consumption of rhizomes for culinary purposes. Ginger is known as a national anti-inflammatory drug in Egyptian, Indian and Chinese cultures, for example, for the treatment of Echinococcus granulosus infection [2, 3]. The main countries of ginger cultivation are India, China, Jamaica, Taiwan and many other countries of the world. Indian scientists noted that the replacement of conventional methods on the use of tissue culture can increase the productivity of yield crops [4]. Also, it should be noted that the demand for pure ginger seedlings is difficult to satisfy by using conventional (traditional) reproduction methods due to the ineffectiveness of production and transmission of infections [5]. Since the planting material should be plentiful, about 2.5 t/ha and ginger breeding is carried out vegetatively by the division of rhizomes [6]. However, this method is labor-consuming, since in many countries it is not possible to store ginger for a long period of time and it goes for processing and export [7]. The application of the clonal micropropagation method is necessary for generating a large number of ginger seedlings [8]. Indian researchers use BAP and NAA at different concentrations to stimulate organogenesis and rhizogenesis in ginger [9]. Recent studies of ginger *in vitro* have shown that the average number of shoots using the direct organogenesis method is 6.6 shoots, compared with the method of indirect somatic embryogenesis - 3.8 shoots. This allows effectively creating a planting material [10, 11]. Acceleration of reproduction *Zingiber offic.* using tissue culture method is achieved using growth regulators, but their concentration should be collected experimentally.

Purpose. Develop a method of clonal micropropagation of ginger to obtain high-quality planting material.

Methods. The source material for the study was rhizomes, buds and shoots ginger. To stimulate buds formation on ginger rhizomes, the thermostat were used at a temperature of $28 \pm 2^{\circ}\text{C}$ and a humidity of 90 %.

Cultivations of rhizome segments were carried out in plastic cuvettes for 1–2 months.

The resulting buds in the size of 0.5–2.0 cm were cut with a scalpel with a part of the rhizome up to 0.5 cm and washed in a soap solution of 72 % (30 min.). To free from the soap film, the buds were washed with distilled water 3–4 times. Prepared buds were immersed in a flask with a solution of ethanol (C₂N₅OH), a mass fraction of 76%, for 5 min. In order to obtain an aseptic ginger in vitro, a 0.1% solution of mercuric chloride was used at different exposure. Then it was washed three times with dH₂O at an interval of 15–20 min. [12].

A part of the cut rhizome was cut off and the aseptic buds of ginger was planted on the nutrition medium of Murashige and Skoog (MS) without hormones.

For stimulation of shoot formation aseptic buds were planted on a modified MS of different composition:

- MS I - MS without hormones;
- MS II - MS + 1.0 mg/L 6-benzylaminopurine (BAP) + 30 g/L sucrose;
- MS III - MS + 3.0 mg/L BAP + 30 g/L sucrose.

This culture does not require stimulation of rhizogenesis by exogenous auxins and forms a powerful root system through endogenous auxins.

The countings were carried out six passages at an interval 8–10 weeks of cultivation.

The analysis of the results was carried out using software Excel 2007.

Results. Under the conditions of cultivation of segments of ginger rhizomes (181 pcs.) in a thermostat at a temperature of 28 ± 2 °C and a humidity of 90 %, 293 buds were obtained at an average height of 2.0 cm and a diameter of 1.4 cm. Optimal ginger buds should not exceed 3 cm in length for ease of releasing from infection.

The research has shown that the most effective sterilizing agent for ginger buds is a 0.1 % solution of mercuric chloride with an exposure of 45–50 minutes, which provided 61.4–81.9 % of viable buds with a sterility index of 50.0–63.9 % in different biotypes.

After a month of cultivation, 54.9 % of the introduced buds formed shoots and roots in the MS I without hormones. The height of shoots averaged 2.2 cm with 4.5 the number of lateral roots per one bud, 1.9 cm in length (Fig. 1). A part of the buds formed only 0.6 lateral roots per one buds.



a) b)
Fig. 1 Aseptic culture (a) and organogenesis (b) of ginger

The sprouted ginger buds were used as primary explants to develop the method of cloning ginger in vitro.

The viable buds of ginger purposed for further cloning were transferred to MS supplements with cytokinins and sucrose. During the year, six passages of cloning ginger shoots with an 8–10 week cultivation exposition have been conducted.

The first and second cultivation passages were carried out on a nutrient medium of MS I without hormones, which allowed to obtain 1.0–8.0 new shoots from one explant, with an average number of shoots per bud 2.6 (Table 1).

Addition of BAP at a concentration of 1.0 mg/L to MS resulted in an increase in the reproduction index on average up to 3.1 pcs/shoots. Increasing the concentration of BAP in medium MS III to 3.0 mg/L, the reproduction index of shoots was an average of 5.3 pcs. per one shoots, located within LSD₀₅. Individual biotypes of ginger have increased the number of newly formed buds from 1.0 in the control version to 3.6–7.7 in MS III, which coincides with the indicated parameters of shoots formation in foreign literature.

Table 1. Clonal micropropagation of ginger in vitro

Biotype number	Reproduction index, pcs/shoots		
	MS I	MS II	MS III
No. 1	3.0	4.4	5.4
No.2	8.0	3.5	6.3
No. 3	2.0	1.5	5.5
No. 4	2.0	3.0	5.6
No.5	2.0	3.0	4.8
No. 6	2.0	3.5	3.9
No. 7	1.0	3.0	4.7
No. 8	2.0	1.0	5.7
No. 9	2.0	1.5	3.6
No. 10	6.0	1.2	5.1
No. 11	2.0	3.0	4.6
No. 12	1.0	3.0	3.6
No. 13	2.0	7.5	7.7
No. 14	2.0	4.0	6.4
No. 15	2.0	3.0	7.0
Average	2.6	3.1	5.3
LSD ₀₅	0.5	0.4	0.3

The developed ginger shoots had a dark green color, the leaves are deployed lanceolate with gloss, with the flavor and taste characteristic of the given crop. The base of the shoots had a globular structure of light green color on which the formation of additional shoots and roots was observed (Fig. 2).

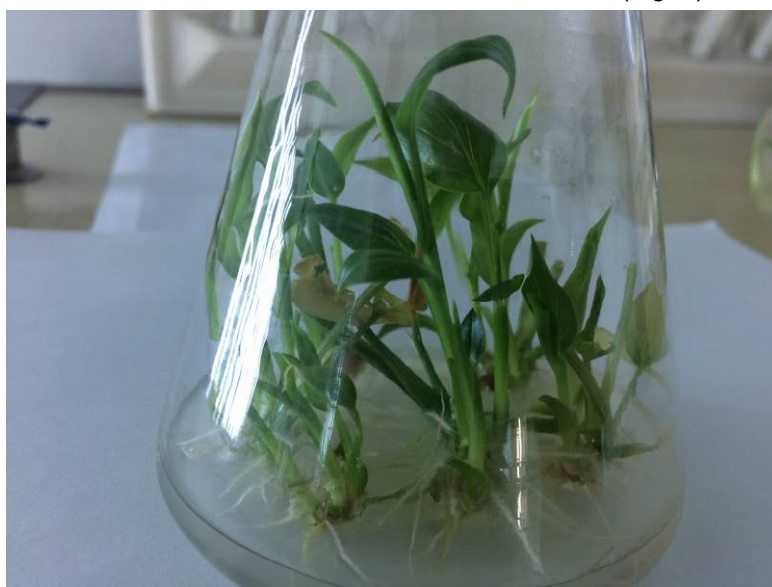


Fig. 2 Clonal micropropagation of ginger on MS III

The formation of roots started on 10–14 days of cultivation in all studied biotypes. Ginger shoots formed a powerful root system of 3–4 to 25–30 roots per one shoots with the length of 1.5–8.0 cm in white and light green with additional roots (Fig. 3).



Fig. 3. Appearance of roots ginger

Obtained 1000 plants with a powerful root system during five cultivation passages from one ginger bud introduced *in vitro*.

4000 cloned seedlings were handed over to study the conditions of adaptation and cultivation at the Veselyi Podil Experimental and Breeding Station and the Yaltushkiiv Experimental and Breeding Station. Seedling survival index was 72-98 %. At the end of the growing season, ginger plants formed from 3 to 7 new shoots.

Conclusions

Stimulation of buds formation on ginger rhizomes in a thermostat at a temperature of 28 ± 2 °C and a humidity of 90 % provides a yield of buds of 161.8 %. For 50.0–63.9 % aseptic ginger it is *in vitro* appropriate to use 0.1 % solution of mercuric chloride with an exposure of 45–50 min., resulting is 61.4–81.9 % of viable buds. The use of BAP (3.0 mg/L) provides a high multiplication factor of ginger up to 7.7 pcs/shoots per passage. *In vitro* ginger shoots do not require the addition of auxins to the nutrient medium to stimulate rhizogeny. Cultivated *in vitro* ginger seedlings have a high survival index in open ground (72–98 %) which allows using the *in vitro* cloning method to produce high-quality planting material. To obtain *in vitro* from one explant up to a 1000 of rooted plants the developed method of clonal micropropagation of ginger makes it possible.

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