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OBTAINING IN VITRO CALLUS BIOMASS OF AMARANTH VARIETY "LERA"

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Aim. To obtain callus biomass of the amaranth variety "Lera" *in vitro* and determine the optimal concentration of growth regulators in the nutrient medium for maximum callus formation.

Methods. The study was conducted using in vitro cell culture. Cultivation was performed on a modified Murashige-Skoog medium supplemented with benzylaminopurine and naphthylacetic acid at various concentrations. Experimental conditions: photoperiod 16/8 h (light/darkness), temperature 20±1 °C, illumination 2000 lux, relative humidity 70%. The total cultivation duration was 56 days.

Results. The seed sterilization method used resulted in a 52% yield of viable explants. Callusogenesis was studied on media containing 3 mg/L BAP in combination with NAA at concentrations ranging from 0.25 to 1.25 mg/L. A total of 61 explants were introduced into each medium. On the 28th day, the explants were subcultured onto a fresh medium of identical composition. The frequency of callus induction was 15–38% on the 28th day and 50–77% on the 56th day, depending on the medium composition.

Conclusions. The optimal medium for obtaining callus culture of the amaranth variety "Lera" is Murashige-Skoog medium supplemented with 3 mg/L BAP and 0.5 mg/L NAA, which provides the highest frequency of callus induction.

Keywords: *Amaranthus hypochondriacus*, cell culture method, callus biomass, frequency of callus induction.

Amaranthus spp. are significant plants with great importance in the food, cosmetic, and pharmaceutical industries. Amaranthus is known for its valuable nutritional properties, including proteins, fats, fibers, and minerals [1], and it contains various metabolites such as flavonoids, carotenoids, squalane, and betalains [2, 3].

The *Amaranthus hypochondriacus* variety "Lera" is an early-ripening cultivar. It has a red inflorescence measuring 50–55 cm, green stems and leaves with red veins, and white grains, while the plant itself reaches a height of 200–220 cm [4]. The ethanol extract of *A. hypochondriacus* leaves and seeds contains numerous metabolites, including flavonoids, polyphenolic compounds, and essential amino acids [5].

Plant biomass obtained through cell culture methods provides a continuous and reliable source of metabolites year-round. The efficiency of *in vitro* callus formation depends on the plant genotype, the composition of the culture medium, and the presence of appropriate concentrations of growth regulators [1].

To obtain callus biomass of *Amaranthus,* a modified Murashige-Skoog (MS) medium supplemented with 3.0 mg/L 6-benzylaminopurine (BAP) and 0.5 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) or 1-naphthaleneacetic acid (NAA) has been used. Studies have shown that this

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combination can promote callus formation and growth within 20 days, though the callus induction rate varies by variety, ranging from 50% to 95% [1].

For *Amaranthus tricolor* callus biomass production, it is recommended to use MS medium with either 1 mg/L BAP and 10 mg/L NAA or 1.0 mg/L BAP and 0.5 mg/L 2,4-D [6].

Aim. To obtain callus biomass of amaranth from seeds of the "Lera" variety using the *in vitro* cell culture method and to determine the optimal concentration and ratio of growth regulators in a modified MS medium for maximum biomass production.

Methods. The object of the study was environmentally friendly amaranth seeds of the "Lera" variety from "Amarant Bio" (Cherkasy, Ukraine). To improve germination, the seeds were soaked in water for 72 hours. They were then sterilized with a 30% hydrogen peroxide solution for 20 minutes and washed three times with sterilized distilled water.

The sterilized seeds were transferred to Petri dishes containing a hormone-free MS medium and incubated under the following conditions: photoperiod 16/8 h (light/dark), temperature 20±1 °C, illumination 2000 lux, and relative humidity 70%. After 9 days, sprouts measuring 1.3±0.2 cm were obtained and transferred to a modified MS medium.

The modified MS medium was supplemented with BAP and NAA at various concentrations. Glucose (30 g/L) served as the carbon source. Cultivation was conducted for 28 days under the same conditions: photoperiod 16/8 h (light/dark), temperature 20±1 °C, illumination 2000 lux, and relative humidity 70%. After this period, subculturing was performed using a nutrient medium of identical composition.

The frequency of callus induction was determined as the ratio of explants with callus to the total number of explants. All experiments were performed in triplicate. The results were statistically analyzed using Microsoft Excel 2010 software, with the arithmetic mean and standard error calculated.[1, 7].

Results and Discussion. The yield of viable explants was 52%, with a sterilization efficiency of 100% when using the proposed seed sterilization method.

Aseptically grown whole amaranth sprouts were used for further cultivation. Cultivation was conducted on MS medium supplemented with BAP in combination with varying concentrations of NAA. Variants of the MS nutrient medium with different concentrations of growth regulators are presented in Table 1. A total of 61 explants were introduced into each medium.

Cultivation was conducted for 28 days. Some explants exhibited thickening, particularly noticeable in the apical meristem, along with an increase in callus mass. Infected explants were discarded and excluded from further experiments. During cultivation on different nutrient media, variations in the intensity of callus formation were observed. The callus formation frequency of *Amaranthus hypochondriacus* cultivated on the studied modified nutrient media (MS1– MS4) on the 28^{th} day of cultivation is presented in Fig. 1.

On the 28^{th} day of cultivation, the explants were subcultured onto a fresh modified medium of identical composition. Cultivation was continued for another 28 days. Infected explants were discarded and excluded from further experiments. The frequency of callus induction of *Amaranthus hypochondriacus* cultivated on the studied modified nutrient media (MS1– MS4) on the 56^{th} day of cultivation is presented in Fig. 2.

The lowest frequency of callus induction was observed in the callus culture of *Amaranthus hypochondriacus* cultivated on the nutrient medium MS1, supplemented with 3.0 mg/L BAP and 0.25 mg/L NAA. It was found that the optimal medium among those tested was MS2 (3.0 mg/L BAP and 0.5 mg/L NAA), which showed the highest frequency of callus induction on both the 28^{th} and 56^{th} days.

Table 1. **Composition of modified MS medium for cultivation of *Amaranthus hypochondriacus***

Modified MS medium	Concentration of growth regulators, mg/ L	
	BAP	NAA
MS1	3	0.25
MS2	3	0.50
MS3	3	1
MS4	3	1.25

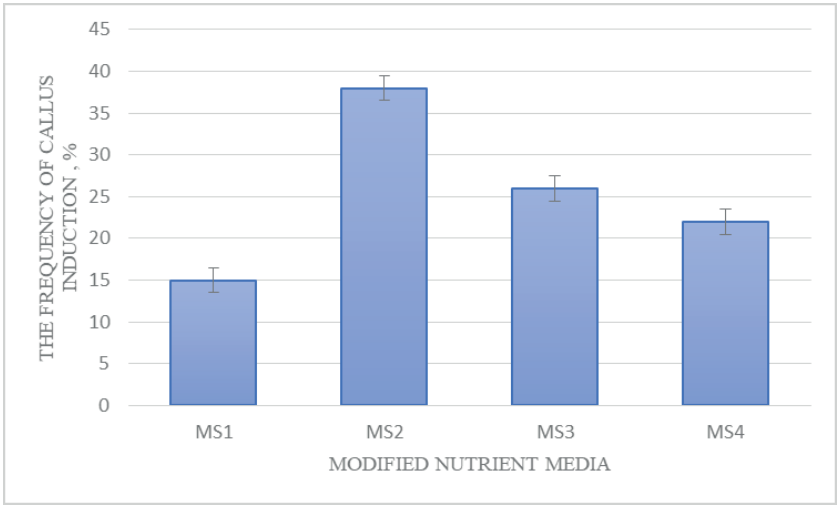

Fig. 1. **The frequency of callus induction of Amaranthus hypochondriacus on the 28th day of cultivation on modified nutrient media MS1–MS4 (mean ± standard error, *n* = 3)**

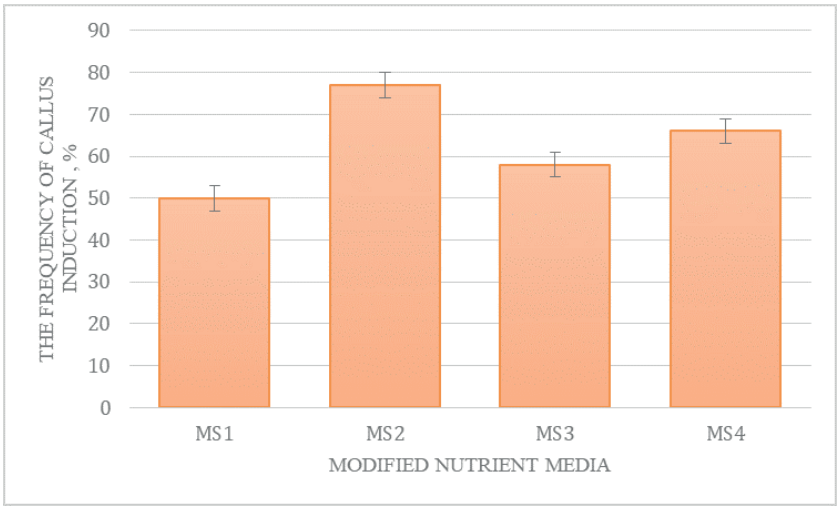

Fig. 2. **The frequency of callus induction of *Amaranthus hypochondriacus* on the 56th day of cultivation on modified nutrient media MS1–MS4 (mean ± standard error, *n* = 3)**

Conclusions. It was established that the optimal nutrient medium for cultivating the callus culture of *Amaranthus hypochondriacus* is Murashige–Skoog, supplemented with 3 mg/L BAP and 0.5 mg/L NAA, at a temperature of 20±1°C, illumination of 2000 lux, and relative humidity of 70%, on which the highest frequency of callus induction was observed.

Authors' Contribution

K. I. Hutsko — goal setting, conducting research stages: preparation of nutrient medium, seed germination, introduction of explants into the medium, subculturing, analysis of results, writing the thesis. R.O. Petrina– methodology search, control of cultivation conditions, analysis of results, writing the thesis.

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