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PCR ANALYSES OF FIRST-GENERATION PLANTS OF *AMARANTHUS CAUDATUS* L. AFTER «FLORAL-DIP» GENETIC TRANSFORMATION

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Amaranth is the C₄-plant which used in various industries and is a promising object for genetic transformation. After «floral-dip» transformation of Helios and Karmin cultivars of *Amaranthus caudatus* L. using *Agrobacterium rhizogenes* A4 strain and *A. tumefaciens* GV3101 strain, transformed plants of the first generation were obtained. Gene vectors pCB125 and pCB131 contained a *bar* gene imparting resistance to commercial herbicide Basta. Seeds of Helios and Karmin cultivars of amaranth were obtained. The T-DNA transmission of strains A4 (pCB131) and GV3101 (pCB125) was evaluated by herbicide selection of amaranth plants. Tolerant plants were obtained for both cultivars after spraying with the herbicide. The percentage of *A. × caudatus* cv. Helios (vector pCB125) plants tolerant to the effect of the herbicide (160 mg/l) was 4.05 %, cv. Karmin (vector pCB125) — was 2.4 %. The percentage of tolerant *A. × caudatus* cv. Karmin (vector pCB131) plants was 0.6 %. The transgene presence of pCB125 gene vector (*A. tumefaciens* strain GV3101) and pCB131 vector (*A. rhizogenes* A4 strain) in amaranth tissue was analyzed. Integration of the *bar* gene and *nos* terminator in plants was confirmed by PCR analysis. The percentage of *bar*-positive plants for cv. Helios (gene vector pCB125) was 0.6 % of the total initial quantity of plants (12 positive plants of 2,000), and for cv. Karmin (gene vector pCB131) was 0.1 % of the total initial quantity of plants (2 plants of 2,000). The percentage of *nos* terminator-positive plants for cv. Helios was 0.15 % of the total initial quantity of plants (3 plants of 2,000).

Key words: *Amaranthus caudatus* L., *Agrobacterium*, transformation, genetically modified plants.

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Amaranth plants are used in the food industry, medicine, cosmetics and agriculture. They serve as a source of biologically active substances, the most valuable of which are squalene and amarantin. Squalene has anti-cancer and wound healing properties while amarantin has an antioxidant activity [1, 2]. The seeds of the *Amaranthus* species are rich in methionin and lysine amino acids. Biologically valuable substances can be obtained by using biotechnological methods. Plants that synthesize new alien substances may potentially be obtained through genetic transformation by using an *Agrobacterium* vector. However, there are relatively few works devoted to the transformation of amaranth. Transgenic roots were obtained for *Amaranthus tricolor* L. [3] and *A. spinosus* L. [4].

Transgenic plants were obtained for *A. hypochondriacus* L. and *A. tricolor* [5, 6], *A. retroflexus* L., *A. viridis* L., *A. cruentus* L. (*A. paniculatus* L.) [7]. No additional information is available about the transformation of *A. caudatus*, which varieties are also used in agriculture [8]. In our previous work, we obtained transgenic plants of *Amaranthus* × *caudatus* cv. Kremovii rannii, Karmin, Helios, Rushnichok; *A. caudatus* × *A. paniculatus* L. — cv. Sterkch with incorporated *gus* and *bar* genes of the pCBV19 gene vector of *A. tumefaciens* [8, 9]. In this article, the results of the transformation of amaranth varieties with other genetic constructs and other bacterial strains are considered.

The aim of this work was to analyze the presence of *nos*-terminator sequence and *bar* transgene from *A. tumefaciens* GV3101 (pCB125) and *A. rhizogenes* A4 (pCB131) strains in tissue of the first generation of transgenic amaranth plants.

Materials and methods

The cultivars Helios and Karmin of the *Amaranthus* × *caudatus* were used in the work. The seeds were obtained from the M.M. Grishko National Botanical Garden, NAS of Ukraine.

Karmin is an early ripening cultivar. The average height of plants is 190–200 cm and the inflorescence color is purple. The green-purple leaves are oval-ovate. The maturity of seeds occurs 100–110 days from the beginning of the growing season. The colour of seeds is milk-white and the average weight of 1,000 pc. is 0.8 g. The grain yield is 80–90 kg/ha, and the biomass — 1,100–1,110 kg/ha. The cultivar was included in the State Register of Varieties of Ukraine in 2000. The originator of the variety, patent applicant and owner is the M.M. Grishko National Botanical Garden, NAS of Ukraine [10].

Helios is an early ripening cultivar. The growing season is 105 days. Plant height is 150–170 cm. The leaves are light green with orange streaks. The inflorescence has a light golden color in the ripening phase. The seed is milk-white. Grain yield is 15–30 kg/ha, and biomass — 1,500 kg/ha. When tested in the Kyiv region, this variety showed the highest content of oil and squalene compared to other local amaranth cultivars. The cultivar was included in the State Register of Varieties of Ukraine in 2010 [11].

These cultivars were chosen because they are early ripening, mature in the steppe and forest-steppe climatic zones of Ukraine, and have compact inflorescences mainly only at the top of the stems, which facilitates the iso-

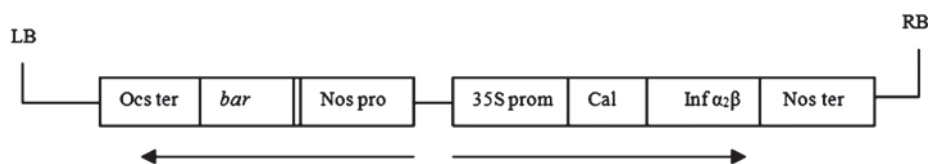


Fig. 1. Schematic representation of the T-DNA site of the pCB125 vector. LB — left border sequence, RB — right border sequence, Nos pro — nopaline synthase promoter, Nos ter — nopaline synthase terminator, 35S prom — promoter of cauliflower mosaic virus gene (CaMV), *bar* — *bar* gene (phosphinothricin acetate transferase gene), Ocs ter — octopine synthase terminator, *inf α₂β* — human interferon *α₂β* gene, Cal — fused with plant (*N. plumbagenifolia* L.) calreticulin apoplastic targeting signal

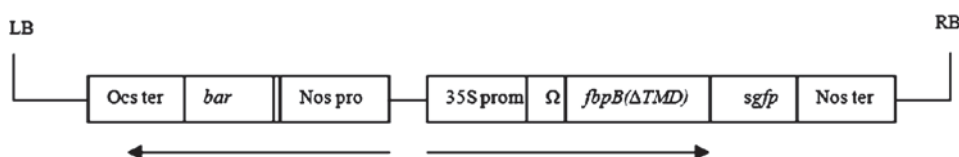


Fig. 2. Schematic representation of the T-DNA site of the pCB131 vector. LB — left border sequence, RB — right border sequence; *bar* — *bar* gene (phosphinothricin acetate transferase gene), Nos pro — nopaline synthase promoter, Nos ter — nopaline synthase terminator, 35S prom — promoter of cauliflower mosaic virus gene (CaMV), Ocs ter — octopine synthase terminator, *fbpB* (Δ TMD) — gene, coding tuberculosis antigen Ag85B; *sgfp* — superfolder green fluorescent protein gene, Ω — regulatory sequence enhancer

lation of inflorescences in the flowering phase, as well as the collection of mature seeds.

The vectors pCB125 of *A. tumefaciens* and pCB131 of *A. rhizogenes* A4 (Fig. 1 and 2) allowed us to check and evaluate the function of heterologous DNA sequences in amaranth plants. The *bar* gene is responsible for herbicide resistance. Nos terminator is termination sequence of the nopaline synthase gene, the function of this sequence is to signal the termination of the gene expression.

Transformation of plants. The Helios and Karmin cultivars of *A. × caudatus* were transformed according to the «floral-dip» method [12]. The peculiarities of the transformation process and the detailed description of all the stages of transformation were described in our previous article [9]. The mature seeds (T_1) were harvested and stored according to Curtis [13, 14].

Herbicide selection. To evaluate the transfer of T-DNA sequence of *Agrobacterium rhizogenes* A4 gene vector pCB131 and *A. tumefaciens* GV3101 pCB125 gene vector in amaranth tissues, we carried out herbicide selection (the Basta[®] herbicide, Bayer CropScience AG, Germany). For this purpose, the lowest lethal concentration of Basta herbicide on amaranth seedlings was identified in our previous experiments (160 mg/l of herbicide) [8].

To screen for the presence of *bar* gene, 2,000 seeds obtained from «floral-dipped» plants and 200 seeds obtained from control plants (grown without *Agrobacterium* treatment) were sown separately. Seeds were grown in plastic pots with soil in greenhouse conditions (22–26 °C, 14-hour light period, illumination 3,000–4,500 lx). The seedlings obtained from «floral-dipped» and control plant seeds were treated at the age of two weeks with

the herbicide in a concentration of 160 mg/l (screening for the resistant plants possessing a *bar* gene). After performing these stages of the experiment, a statistical analysis of the *bar*-positive plants was carried out.

DNA purification. Genomic DNA was isolated by the CTAB method [15]. For the PCR analysis, we used a reaction mixture of the following composition: 10.25 µl Milli-Q H₂O, 2 µl 10x Reaction buffer B (Solis BioDyne), 2 µl 1 mM cresol 60 % sucrose (Solis BioDyne), 1.6 µl 25 mM MgCl₂ (Solis BioDyne), 0.5 µl of forward and 0.5 µl of reverse (10 µl) primers, 2 µl 2 mM deoxyribonucleotide triphosphate (dNTP) (Thermo Fisher Scientific), 0.15 µl 5 U/µl FirePol DNA Polymerase (Solis BioDyne), 1.5 µl DNA (20–30 ng/ml DNA). The volume of the reaction mixture was 20 µl. The amplification reaction was carried out in thermal cyclers — Arctic Thermal Cycler (Thermo Fisher Scientific) and Mastercycler gradient (Eppendorf).

PCR analysis (polymerase chain reaction). To identify the *bar* gene, primers 5'-ACA TCG AGA CAA GCA CGG TC-3' and 5'-GCC AGA AAC CCA CGT CAT GC-3' (Metabion, Germany) were used [16]. Amplification conditions: initial denaturation at 94 °C for 4 min, denaturation at 94 °C for 30 s, annealing at 65 °C for 30 s, extension at 72 °C for 1 min for the first cycle followed by 34 cycles each. Final polymerization was at 72 °C for 5 min. The size of the expected amplicon was 405 base pairs (bp).

To identify the *NOS terminator*, primers 5'-GAA TCC TGT TGC CGG TCT TG-3' and 5'-GCG GGA CTC TAA TCA TAA AAA CC-3' were used [17]. Amplification conditions: initial denaturation at 94 °C for 4 min, denaturation at 94 °C for 30 s, annealing at 57 °C for 1 min, extension at 72 °C for 30 s, for the first cycle followed by 35 cycles each. Final polymerization was at 72 °C for 5 min. The size of the expected amplicon was 125 base pairs (bp).

To identify the β-tubulin gene, primers 5'-ATG TGG GAT GCC AAG AAC ATG ATG TG-3' and 5'-TCC ACT CCA CAA AGT AGG AAG AGT TCT-3' were used [18, 19]. Amplification conditions: initial denaturation at 94 °C for 3 min, denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, extension at 72 °C for 20 s, for the first cycle followed by 34 cycles each. Final polymerization was at 72 °C for 5 min. The size of the expected amplicon was 157 base pairs (bp).

To identify the *virC* gene, primers 5'-ATC ATT TGT AGC GAC T-3' and 5'-AGC TCA AAC CTG CTT C-3' were used [20]. Amplification conditions: initial denaturation at 94 °C for 3 min, denaturation at 94 °C for 30 s, annealing at 55 °C for 47 s, extension at 72 °C for 30 s, for the first cycle followed by 40 cycles each. Final polymerization was at 72 °C for 5 min. The size of the expected amplicon was 720 base pairs (bp).

GeneRuler™ DNA Ladder Mix (Thermo Fisher Scientific) molecular weight markers were used to determine the amplification products.

DNA electrophoresis. Separation of amplified products was conducted by horizontal electrophoresis. The electrophoresis was performed in agarose gel containing LB-buffer with 1.2 % agarose and 5 µg/ml ethidium bromide (for identification of *bar*, *vir C* genes) and agarose gel containing LB-buffer with 0.8 % agarose with 5 µg/ml ethidium bromide (for identification

of *nos* terminator and β -tubulin gene). For sample analysis, 100 ng of total plant DNA, previously dissolved in TE buffer pH 7.6, was used. Electrophoresis was performed under 5 V/cm for 45 min. A UV light source and a digital Canon camera were used to obtain the gel image. The resulting image was processed using a GIMP graphical editor.

Results and discussion



Fig. 3. The effect of Basta herbicide on the seedlings of non-transformed *Amaranthus caudatus* cv. Helios: A — 0 mg/l (control); B — plants sprayed with herbicide (160 mg/l) after 7 days

Usually, advances in plant genetic engineering concern the optimization of agrotransformation methods and factors influencing the transfer of a selective marker and/or reporter gene via *Agrobacterium* to the plant cell. This is followed by the alien gene's integration in the recipient plant genome and subsequently its expression and stability in the transgenic plants. Considering the useful properties of *Amaranthus* plants, the present work was conducted with the aim of identifying the functioning of the pCB131 gene vectors of *A. rhizogenes* and the pCB125 gene vector of *A. tumefaciens* in amaranth tissues and obtaining transgenic amaranth plants after genetic transformation using the GV3101 strain of *A. tumefaciens* (pCB125) and A4 strain of *A. rhizogenes* (pCB131).

Herbicide selection. Due to possible escape rates (obtaining false positive results) of non-transformed plants, it was necessary to accept a compromise regarding the survival percentages of the presumably transformed plants and the non-transformed plants. That is why the first step of the experiment was to determine the lowest lethal concentration of herbicide on non-transformed amaranth seedlings. The lowest lethal concentration of herbicide was 160 mg/l (Fig. 3).

This concentration of herbicide (160 mg/l) was lethal for 100 % of non-transformed *Amaranthus* plants, but a certain quantity of presumably transformed plants remained alive which may indicate they were resistant and it can be assumed that they had a built-in *bar* gene [21]. For the first time, we have obtained transgenic seeds for the cultivars of *A. caudatus*.

After spraying with herbicide, resistant plants were obtained for two cultivars: Helios and Karmin (Fig. 4). The percentage of *A. × caudatus* cv. Helios plants (with vector pCB125) tolerant to the effect of the herbicide

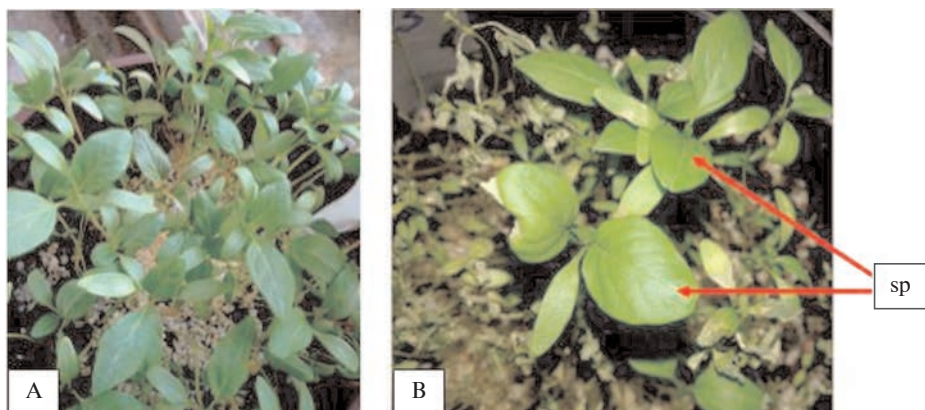


Fig. 4. The effect of herbicide on presumably transformed seedlings of *Amaranthus caudatus*, cv. Helios: A — plants before spraying with herbicide; B — plants at 7th day after spraying with herbicide (160 mg/l); sp — seedlings, which survived

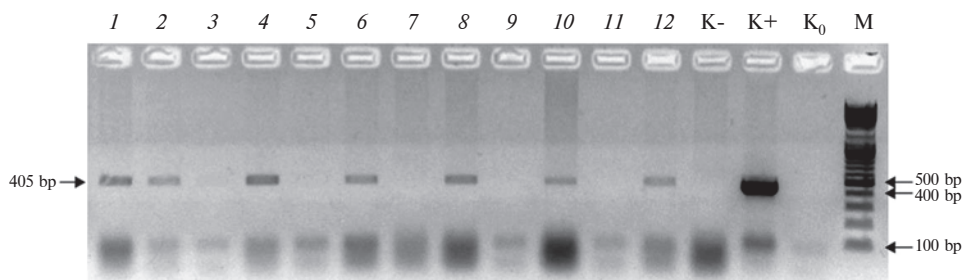


Fig. 5. PCR analysis of amaranth plants using *bar3F* and *bar4R* primers for the *bar* gene: lanes 1–12 — total DNA of experimental samples (1, 11–12 — cv. Karmin, 2–10 — cv. Helios; 1 — sample with vector pCB131, 2 — sample with vector pICH5290, 3–12 — samples with vector pCB125); K- — negative control, DNA of non-transformed plants, K+ — positive control, DNA of transgenic *N. tabacum*, K₀ — no-DNA, M — Fisher Thermo Scientific GeneRuler™ DNA Ladder Mix; 405 bp — the expected size of the amplicon, specifying *bar* gene in the leaf genomic DNA of expected transgenic lines; samples 1–2, 4, 6, 8, 10–12 — *bar* positive plants

(160 mg/l) was 4.05 % (81 plants of 2,000). The percentage of cv. Karmin plants (with vector pCB125) tolerant to the effect of the herbicide (160 mg/l) was 2.4 % (48 plants of 2,000). The percentage of tolerant *A. × caudatus* cv. Karmin plants (with vector pCB131) was 0.6 % (12 plants of 2,000).

Sometimes the main drawback of the transformation process is the unpredictability of the pattern of transgenes integration in the host genome and their expression. That is why, as the final step of the experiment, it was necessary to confirm the results with PCR analysis, as it is a more reliable method than herbicide selection alone.

PCR analysis. During the analysis of 130 samples of the tested *A. × caudatus* Helios and Karmin cultivars, the presence of DNA fragment with 405 bp was detected. This confirms the presence of the *bar* gene in the transformed plant (Fig. 5). The percentage of *bar*-positive plants for cv. Helios (gene vector pCB125) was 0.6 % of the total initial quantity of

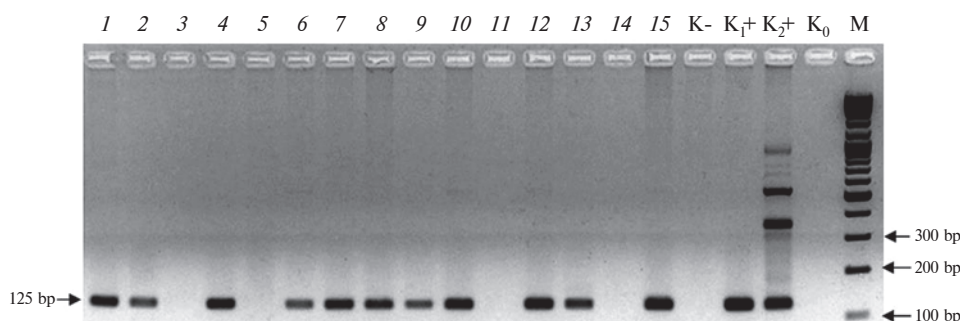


Fig. 6. PCR analysis of amaranth plants using primers U-NOS, D-NO for the *nos* terminator: lanes 1–15 – total DNA of experimental samples (1, 11–15 – cv. Karmin, 2–10 – cv. Helios; 1 – sample with vector pCB131, 2–15 – samples with vector pCB125); K- – negative control, DNA of non-transformed plants; K₁+ – positive control, plant DNA of transgenic *N. tabacum*; K₂+ – DNA of *A. tumefaciens* subtype C58, K₀ – no-DNA; M – Fisher Thermo Scientific GeneRuler™ DNA Ladder Mix; 125 bp – the expected size of the amplicon, specifying *nos terminator* in the leaf genomic DNA of expected transgenic lines; samples 1–2, 4–6, 8–10, 15, 17–18, 21 – NOS terminator positive plants

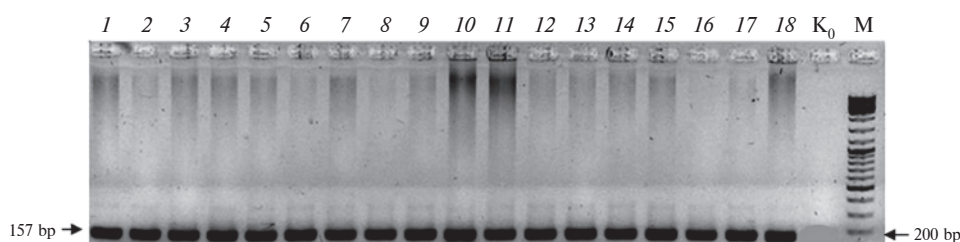


Fig. 7. PCR analysis of amaranth plants using primers tubF and tubR for the reference gene of β -tubulin: lanes 1–18 – total DNA of experimental samples (1, 11–18 – cv. Karmin, 2–10 – cv. Helios; 1, 2 – sample with vector pCB131, 3–18 – samples with vector pCB125); K₀ – no-DNA; M – Fisher Thermo Scientific GeneRuler™ DNA Ladder Mix; 157 bp – the expected size of the amplicon, specifying the β -tubulin gene in the leaf genomic DNA of expected transgenic lines; samples 1–18 – β -tubulin positive plants

plants (12 plants of 2,000) and for cv. Karmin (gene vector pCB131) was 0.1 % of the total initial quantity of plants (2 plants of 2,000).

Also, the presence of a DNA fragment with 125 bp was detected. This confirms the presence of the *nos* terminator sequence in the transformed plant (Fig. 6). The percentage of *nos* terminator-positive plants for cv. Helios was 0.15 % of the total initial quantity of plants (3 plants of 2,000).

The presence of DNA fragment 157 bp long was detected. This confirms the presence of the β -tubulin gene in the transformed plant (Fig. 7). The percentage of β -tubulin-positive plants for cultivars of *A. × caudatus* was 100 %. β -tubulin is reference gene. Reference genes are used in PCR reactions as indicators of quality and quantity of extracted DNA. The reference gene is detected by PCR, even if it is present in one copy. A positive PCR reaction on the reference gene indicates that the quantity and quality of the isolated DNA is sufficient for further PCR reactions on the presence of other genes.

No presence of DNA fragment with 720 bp was detected. This confirms that plants did not contain the *vir C* gene and means that there was no live *Agrobacterium* in the plant tissues (Fig. 8).

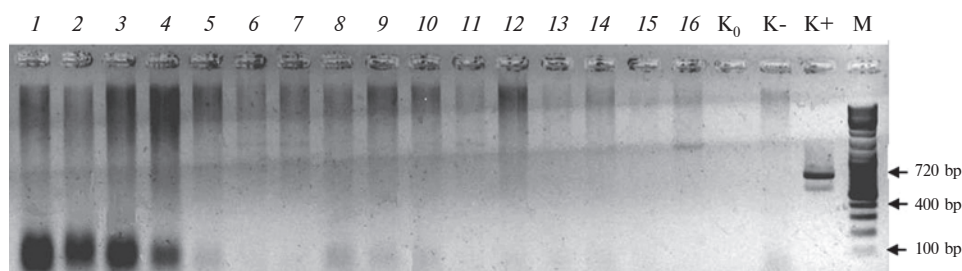


Fig. 8. PCR analysis of amaranth plants using primers Vir CF, Vir CR for the reference gene of *vir C*: lanes 1–16 — total DNA of experimental samples (1, 11–16 — cv. Karmin, 2–10 — cv. Helios; 1, 2 — sample with vector pCB131, 3–16 — samples with vector pCB125); K₀ — no-DNA; K⁻ — DNA of non-transformed plants; K⁺ — total DNA of *A. tumefaciens* subtype C58; M — Thermo Fisher Scientific GeneRuler™ DNA Ladder Mix; 720 bp — the expected size of the amplicon, specifying *vir C* gene in the leaf genomic DNA of the expected transgenic lines; samples 1–16 — *vir C*-negative plants

Initial experiments concerned with the transformation of amaranth species using *Agrobacterium* strains were unsuccessful [22]. At present, there exists a proof that it is possible to obtain transgenic amaranth plants with *A. rhizogenes* and *A. tumefaciens*. However, there are relatively few works dealing with the transformation of amaranth. Positive results were obtained in transforming *A. tricolor* L. [3] and *A. spinosus* L. [4] using wild strains of *Agrobacterium rhizogenesis* A 4. The authors obtained transgenic roots.

Positive results were obtained in the transformation of amaranth species with strains of *Agrobacterium tumefaciens*. There were obtained transgenic plants of *A. hypochondriacus* L., cv. Azteca using an *Agrobacterium* vector with marker genes [5]. Transgenic *A. tricolor* L. was obtained by two different groups of scientists [6, 23], a vector with marker genes was used in [6].

In addition, there are two studies dedicated to the transformation through amaranth inflorescence. Munusamy et al. [24] used the vector with selective genes, and Taipova [7] conducted experiments with *A. retroflexus*, *A. viridis*, *A. cruentus*. Both works asserted that positive and promising results were achieved and that transgenic seeds were obtained. However, their results do not seem convincing. Munusamy et al. [24] did not indicate the species of the amaranth they worked with, while Taipova [7] did not specify the types of bacteria used. The results of the biochemical and genetic analyses, on the basis of which it would be possible to state with accuracy that they obtained transgenic seeds, are not shown.

In our experiments, the frequency of amaranth transformation by the «floral-dip» method was quite similar compared to that obtained for *Arabidopsis thaliana*. Frequency of transformation for *Arabidopsis* was in the range of 0.5 % to 3 % [14].

The «floral-dip» method is used as an alternative to *Agrobacterium*-mediated transformation in those cases where, for whatever reason, the latter cannot be performed in the laboratory. There may be various possible explanations. One is the presence of biochemical compounds in plant tissues that have strong antibacterial properties and inhibit the growth of agrobacteria at the initial stages of transformation. Second is the small amount of regeneration or its complete absence in plants (in our case,

amaranth is characterized by the presence of high levels of auxins in tissues, which complicates the formation of both additional lateral buds and regenerants). Third is the possible high degree of contamination of the original plant material with fungal agents.

The floral-dip method of plant transformation is quite simple and less expensive than the *Agrobacterium*-mediated transformation of plants under sterile conditions. This is because it does not require the presence of a laminar box, large amounts of sterile laboratory glassware, or a culture conditions of thermal room for growing plants *in vitro*.

Nevertheless, when applying the floral-dip method, difficulties may arise at the stage of screening potentially transformed plants. It is not always possible to select only transformed plants using a herbicide. The effective dose of herbicide for screening potentially transformed plants should be first accurately determined on non-transformed plants. It should also be considered that resistance to the action of herbicide may appear in non-transformed plants as a result of spontaneous mutations. This can also give a false positive result. It is therefore necessary to use several different screening methods when selecting transformed plants after transformation by the «floral-dip» method.

It is essential to check for the presence of transferred genes in plants using molecular-genetic methods (i.e. PCR analysis and electrophoresis). After transformation by the «floral-dip» method, the plants are not treated with special sterilizing agents, so it is likely that living bacteria can survive in the seeds of the T_0 and in the tissues of the T_1 generations of plants. Due to the possible presence of agrobacterial contamination in T_1 plant tissues, it is necessary additionally to check for the presence of the *vir C* or *vir D* bacteria genes by PCR analysis and electrophoresis, in order to avoid false positive results. Also, the plants should be checked for the presence of target components that should begin to be synthesized by the plant after the effective insertion of the transferred genes. Additionally, if laboratory operating conditions permit, a check should be carried out for the presence of transferred genes in plant tissues using Southern-blot analysis. Scientists should weigh all the advantages and disadvantages before choosing the most suitable transformation method.

So, in our experiments the transfer of T-DNA sequence of pCB125 vector from *Agrobacterium tumefaciens* and pCB131 vector from *A. rhizogenes* was evaluated in the first generation amaranth plants. Seeds of the Helios and Karmin amaranth cultivars were obtained after treatment with *A. tumefaciens* and *A. rhizogenes* by the «floral-dip» method.

After spraying with the herbicide, tolerant plants were obtained for both cultivars: Helios and Karmin. The percentage of *A. × caudatus* cv. Helios (vector pCB125) plants tolerant to the herbicide effect (160 mg/l) was 4.05 % (81 plants of 2,000), cv. Karmin (vector pCB125) — 2.4 % (48 plants of 2,000). The percentage of tolerant *A. × caudatus* cv. Karmin (vector pCB131) plants was 0.6 % (12 plants of 2,000).

The presence of foreign genes in amaranth tissue was proved with PCR analyses. The positive results were obtained for both cultivars. The percentage of *bar*-positive plants was 0.6 % (cv. Helios, gene vector pCB125) and 0.1 % (cv. Karmin, gene vector pCB131) of the total initial quantity of plants. The percentage of *nos* terminator-positive plants for

cv. Helios was 0.15 % out of the total initial quantity of plants. The presence of a DNA fragment with 157 bp was identified.

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АНАЛІЗИ ПЛР РОСЛИН ПЕРШОГО ПОКОЛІННЯ *AMARANTHUS CAUDATUS* L. ПІСЛЯ ГЕНЕТИЧНОЇ ТРАНСФОРМАЦІЇ МЕТОДОМ «FLORAL-DIP»

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Амарант — C_4 -рослина, яка використовується в різних галузях промисловості і є перспективним об'єктом для генетичної трансформації. Після «floral-dip» трансформації сортів Helios і Karmin *A. caudatus* L. з використанням штаму *Agrobacterium rhizogenes* A4 і штаму *Agrobacterium tumefaciens* GV3101 були отримані трансформовані рослини першого покоління. Вектори pCB125 і pCB131 містять ген *bar*, що надає стійкості до Basta (комерційний гербіцид). Насіння сортів амаранту Karmin і Helios було отримано після обробки суспензією, що містить *A. tumefaciens* і *A. rhizogenes* методом «floral-dip» («занурення квіткових суцвіть»). Перенесення генного вектора pCB131 штамом *Agrobacterium rhizogenes* A4 і генного вектора pCB125 штамом *Agrobacterium tumefaciens* GV3101 оцінювали шляхом відбору стійких рослин амаранту після обробки гербіцидом. Після обприскування гербіцидом, були отримані стійкі рослини двох сортів: Helios і Karmin. Відсоткова частка рослин *A. × caudatus* сорту Helios (з вектором pCB125), толерантних до дії гербіциду (концентрація 160 мг/л), становила 4,05 %, сорту Karmin (з вектором pCB125) — 2,4 %. Відсоток стійких рослин *A. × caudatus*

сорту Karmin (з вектором pCB131) становив 0,6 %. Було проаналізовано присутність генів вектора pCB125 (штам *A. tumefaciens* GV3101) і генів вектора pCB131 (штам *Agrobacterium rhizogenes* A4) в тканинах амаранту. Інтеграція і транскрипція гена *bar* і *nos*-термінатора в рослинах була підтверджена результатами аналізу ПЛР. Відсоток *bar*-позитивних від загальної початкової кількості рослин становив 0,6 % для сорту Helios, генетичний вектор pCB125 (12 рослин із 2000) і 0,1 % для сорту Karmin, генетичний вектор pCB131 (2 рослин із 2000). Відсоток *nos*-термінатор-позитивних рослин для сорту Helios становив 0,15 % від загальної початкової кількості рослин (3 рослини із 2000).

Ключові слова: *Amaranthus*, *Agrobacterium*, трансформація, генетично модифіковані рослини.