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Research Article

Achievements in Genetic Engineering of *Amaranthus* L. Representatives

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Abstract: Despite the fact that in the modern world more than a thousand edible plants are used for food, only 3 staple cereal crops are grown worldwide: wheat, rice, and maize. Growing a limited number of crops often causes many problems: ranging from the loss of biodiversity, due to the constant cultivation of the same monocultures in the same areas, to the deterioration of soil quality. A way out of this situation is the selection of new untraditional and neglected plants that could grow in a wide range of temperatures, produce high yields and at the same time have a balanced amino acid composition. Pseudocereals of the genus *Amaranthus* L. meet these criteria. Amaranth grain and plant raw materials are used in many industries: food, medicine, cosmetics.
Modern technologies do not stand still. Along with traditional methods of plant breeding, the rapid pace of development involves genetic engineering of plants, which allows the process of creating improved plants to be speeded up several times.
The purpose of this study is to analyze and systematize the achievements in the field of regeneration and genetic transformation of representatives of the *Amaranthus* genus. The results can be used for a practical application: the genetic transformation of species of the genus *Amaranthus* and other close genera of plants.

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1. INTRODUCTION

Amaranth is a high-yielding plant. From 1 plant it is possible to obtain more than 5,000 seeds. Moreover, amaranth has a uniquely balanced amino acid composition that ensures easy digestion. Amaranth is a rich source of protein and essential amino acids, deficits of which cannot be compensated by traditional agricultural crops.

Furthermore, amaranths are used in medicine. Amarantin substance ($C_{29}H_{31}N_2O_{19}$) was derived from some species of amaranth (*A. caudatus* L., *A. tricolor* L., *A. cruenthus* L.) (Yaacob *et al*., 2012). Amarantin relates to alkaloids-betalains. It has useful antioxidant properties in the human organism (Burd, 2006).

Due to the fact that amaranths are indifferent to the type of soil and are drought-resistant, they are grown as a grain crop in countries with a temperate climate (Western Europe), as well

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as in hot-climate countries, where many traditional crops grow poorly: Mexico, the USA, African countries, India.

Given that amaranth is one of the main food crops in India and Africa, has a unique rich amino acid composition with a high nutritional value, and can serve as a source of biologically active substances for further use in medicine, amaranth plants have undergone improvements for many decades using hybridization, selection and mutagenesis methods.

In recent years, the chemical composition of plants and some agronomic properties have begun to improve using biotechnological methods, namely genetic engineering. Genetic engineering methods make it possible to improve not only the useful properties of a plant, but also to provide additional useful characteristics during plant transformation.

Since it is known that the percentage of *Agrobacterium* - mediated transformation of plants is often low, usually even before this transformation possible ways of obtaining a large number of transformed plants from a single parent plant are consequently worked out. One of the optimal methods of rapidly increasing the number of plants is considered to be direct regeneration of plants *in vitro* conditions.

Therefore, we first consider the main achievements related to obtaining regenerants of amaranths *in vitro*.

2. ACHIEVEMENTS IN REGENERATION OF *AMARANTHUS L.* SPECIES

To date, there have been many studies on the regeneration and callus formation of amaranth. Basically, the researchers who obtained calluses, had as primary objective their use as a source of secondary metabolites and other valuable substances. In this connection, the largest number of studies devoted to amaranths have had a biochemical orientation.

Amin and colleagues verified the possibility of obtaining the *Amaranthus gangeticus* L. callus. The leaves, stems and roots were used as initial explants. The scientists observed the formation of calluses in 99.7% ± 0.2% of explants which were derived from stem calluses on MS medium supplemented with 2.0 mg/l NAA(α-Naphthalene acetic acid) + 1.0 mg/l BA (6-benzylaminopurine) (Amin *et al*., 2015).

The group headed by Bennici studied the morphogenesis and growth of calluses. As an object of investigation, they chose lines of several species: *A. caudatus* L., (PI490458, AMES15114, AMES5461), *A. cruentus* L. (434, 622, AMES2248, AMES2247, PI511731, PI777913), *A. hybridus* L. (1047), *A. hypochondriacus* L. (1221, 718, 674, 722, 412, PI540446). The stem segments derived from 15-day sprouts were used for explants (Bennici *et al*., 1997).

Callus tissue was obtained from the explants of the lines *A. caudatus* L. (Bennici *et al*., 1997), *A. cruentus* L. (Bennici *et al*., 1997) and *A. hypochondriacus* L. (Bennici *et al*., 1997) on MS medium with the addition of 2.3 μM 2.4-D (2,4-dichlorophenoxyacetic acid) + 2.3 μM KIN (kinetin); NAA from 0.5 μM to 5.4 μM + BA from 0.4 μM to 13.3 μM.

The callus formation was observed in 100% of explants, with the exception of two lines of *A. caudatus* L. and three lines of *A. cruentus* L. and *A. hypochondriacus* L.. Different concentrations of NAA + BA did not induce callus formation on the *A. caudatus* explants line AMES5461, while 5.4 μM NAA + 13.3 μM BA caused callus formation only in 43% of PI490458 *A. caudatus* L. explants. *A. cruentus* L. lines formed calluses in percentage ratios of less than 100%: AMES2247, 71% on MS medium, with addition of 5.4 μM NAA + 4.4 μM BA; PI511731, 60% on MS medium with addition of 2,4-D + KIN and 67% on MS medium with addition of 5.4 μM NAA + 13.3 μM BA; PI477913 – 75% on 2,4-D + KIN and 79% on MS medium with addition of 5.4 μM NAA + 4.4 μM BA.

Plant regenerants were obtained for *A. hybridus* L. (line 1047) and for *A. hypochondriacus* L. (line 674). The rate of regeneration was low – 8.5% (*A. hybridus* L.) and 14.3% (*A. hypochondriacus* L.). Regenerants were also obtained for *A. hybridus* L., *A. hypochondriacus* L., *A. cruentus* L. on MS medium with addition of 2.7 μM NAA + 2.5 μM 2iP (N^6-(2-isopentenyl)adenine), 2.7 μM NAA + 2.3 μM KIN. The regenerants of *A. cruentus* L. line 434 and 1034 were obtained on MS medium with addition of 2.7 μM NAA + 4.4 μM BA. The general conclusion of the authors was as follows: the absolute majority of species and lines of amaranths are able to form calluses on most media tested by the authors (almost 100% of callus formation). There was no clear connection between regeneration of shoots and the use of growth regulators. This is due to the strong influence of the genotype of plants on organogenesis. Amaranths have high levels of cytokinins (auxins), which inhibit regeneration processes. The authors believe that the best stimulator of amaranth regeneration was BA.

Mousumi Biswas and colleagues conducted experiments aimed at obtaining calluses for further isolation of betacianins from them (Biswas *et al*., 2013). The biggest volumes of callus synthesizing betacianins were obtained from explants of stem origin on MS medium supplemented by NAA (0.25 mg/l) + BA (2 mg/l). In addition, researchers found red-purple amaranthine pigment in the callus lines, 2 new yellow pigments and 18 other biologically active phenylpropanoids. A new betaxanthin has been identified and a methyl derivative of arginine betaxanthin was also identified. Pigments were purified by size exclusion chromatography (Biswas *et al*., 2013).

Flores and colleagues studied the formation of callus and regeneration for the *A. hypochondriacus* L., *A. cruentus* L. and *A. tricholor* L. species. They observed a rapid growth of calluses and abnormal roots on *A. hypochondriacus* L. and *A. cruentus* L. leaf disks on MS medium in the presence of 0.1–1.0 mg/l of 2.4-D. At higher levels (1.0–10.0 mg/l) of 2,4-D, embryo-like structures formed from the surfaces and veins of the leaf discs. Shoots were formed from hypocotyl derivative callus on the medium B5 + 0.1 mg/l NAA and 0.1–1.0 mg/l ZEA (zeatin). Lower ratios of ZEA/NAA stimulated the formation of roots from hypocotyl segments (Flores *et al*., 1982).

Gajdošová, with a team of researchers, selected the ideal conditions for the regeneration and cultivation of *Amaranthus cruentus* L. 'Ficha' and *Amaranthus hybridus* (Gajdosova *et al*., 2007; Gajdosova *et al*., 2013) 'K-433'. As explants, they used epicotyls with the first pair of leaves, hypocotyls, roots and segments of the leaves of 10-day seedlings. For both species studied, the most effective media for direct regeneration from epicotyls were MS_{30}, supplemented with 5 mg/l BA + 0.01 mg/l NAA, MS_{30} supplemented with 1 mg/l TDZ (thidiazuron), MS_{30} supplemented with 3mg/l TDZ + 0.01 mg/l NAA. The most effective medium for induction of callus was MS_{30} with 6 mg/l NAA + 0.1 mg/l BA (for *Amaranthus cruentus* L. 'Ficha') and MS_{30} + 2 mg/l 2.4 D + 0.5 mg/l BAP (for *Amaranthus hybridus* L. "K-433"). The authors made the following conclusions: in order to obtain regenerants, it is necessary to use mediums with a high cytokinin content: auxins; amaranths are characterized by a high callus forming ability, almost 100% on all tested mediums; regenerants were obtained only from epicotyl segments; the ability to regenerate strongly depends on the genotype, age of plants and used types of explants; the overall regeneration frequency was low (Gajdosova *et al*., 2007; Gajdosova *et al*., 2013).

Flores and colleagues investigated the regeneration ability and the callus formation of the following species: *A. hypohondriacus* L., *A. cruentus* L., A. *tricolor* L.. Parts of the hypocotyls were used as explants. The regeneration was indirect (first, callus tissue was obtained). The scientists concluded that the optimal medium for regeneration is B5 supplemented with 0.1mg/l NAA + 0.1–1.0 mg/l ZEA. The callus tissue was obtained from leaf discs of *A. hypohondriacus* L. and *A. cruentus* L. Intensive growth of the callus was observed on MS_{30} medium with 0.1–1

mg/l 2,4-D. However, after addition to the MS_{30} medium of 0.2 mg/l BA + 2 mg/l NAA and 10% coconut water, they observed shoot induction from callus tissue (Flores & Teutonico, 1986).

The team of researchers headed by Bennici intended to obtain regenerants for the following species: *A. hypohondriacus* L., *A. cruentus* L., *A. hybridus* L., *A. caudatus* L. As explants, hypocotyls were used. Regeneration was obtained for 2 species as a result: *A. hypochondriacus* L. (MS_{30} + 3 mg/l BA + 1 mg/l NAA), *A. caudatus* L. (MS_{30} + 3 mg/l KIN + 0.3 mg/l IAA (indole-3-acetic acid). The percentage of regeneration was low (26%). At the same time as the main objective of obtaining regenerants, researchers obtained a callus tisssue. Rapid and intensive callus formation from hypocotyl explants was observed for *A. cruentus* L. (6 mg/l NAA + 0.1 mg/l BA) and *A. hybridus* L. (6 mg/l 2.4-D + 0.1 mg/l KIN (Bennici *et al*., 1992). Arya and colleagues chose *A. paniculatus* L. as an object of research. Parts of the inflorescence were used as explants. When transferring the explants on the MS_{30} medium with 8–15 mg/l KIN or MS_{30} + 5–10 mg/l BA, secondary inflorescences were formed from stems and leaves of the primary inflorescence buds (Arya *et al*., 1993). Bui van Le and colleagues received regenerants of *A. edulis* L. from thin cell layers. For experiments, they used thin slices (0.2–0.4 mm) of cotyledons, hypocotyls, roots, tissues from the apical and sub-apical areas. Explants were obtained from 7-day seedlings (Bui van Le *et al*., 1998). Regenerants were obtained solely from tissues taken from the apical and sub-apical zone. Only callus tissue was obtained from all other types of explants.

Initially, embryonic buds were formed from the tissues of the apical and sub-apical zone on a medium of MS_5 + 2 μM TDZ + 10 μM of CPPU (forchlorfenuron). These embryonic buds were then transferred on MS_5 + 10 μM BAP for elongation of stems (Bui van Le *et al*., 1998). Tisserat and Galletta obtained only callus tissue for *A. gangenticus*, *A. hypochondriacus*, *A. caudatus* L., *A. viridis* L., *A. retroflexus* L. (Tisserat & Galletta, 1988). Callus tissue was obtained by Yaacob and colleagues. Callus was obtained for further extraction of biologically active substances using leaves, stems, roots on MS_{30} + 1.5 mg/l IAA + 0.5 mg/l of ZEA or MS_{30} + 1 mg/l IAA medium (Yaacob *et al*., 2012).

A team of researchers headed by Bagga, studied the regeneration ability and callus formation of *A. paniculatus* L. The hypocotyls were used as the explants. Regeneration of 1-2 shoots from one end of the hypocotyls explants was obtained on medium B_5 + 1ppm KIN + 1 ppm NAA; on medium B_5 + 0.5 mg/l KIN + 0.1 mg/l NAA numerous buds formed (10–14 pieces), from which stems developed later. Intensive callus growth was observed on medium B_5 + 1 mg/l GA_3 (gibberellic acid) + 1 mg/l KIN + 1mg/l 2,4-D (Bagga *et al*., 1987).

Jofre-Garfias and co-authors obtained embryos from the cotyledons of *A. hypochondriacus* L. cv. Azteca on medium MS_3 + 10% coconut milk and MS_3 + 10μM 2.4-D (Jofre-Garfias *et al*., 1997). Pal and colleagues obtained *A tricolor* regenerants from hypocotyls and epicotyls of 7-day seedlings on MS_{30} + 13.2 μM BA +1.8 μM NAA (Pal *et al*., 2013 a). In another study, Pal argued that he and his colleagues received regenerants of *A. spinosus* from the culture of "hairy" roots. Regenerants were obtained on MS_{30} medium without growth regulators (spontaneous regeneration) and on MS_{30} medium + 2 mg/l ZEA (Pal *et al*., 2013 a).

Swain and his colleagues obtained *A. tricolor* regenerants from the culture of "hairy" roots. Regenerants were obtained (on MS_{30} medium without growth regulators (spontaneous regeneration) and on MS_{30} medium + 2 mg/l ZEA (Swain *et al*., 2009; Swain *et al*. 2010).

For clarity, the achievements in the field of callus formation and regeneration is presented in tabular form (Table 1).

Table 1. Achievements in amaranth regeneration.

Species of amaranth, cultivar, hybrid, line	Most effective medium for regeneration	Type of explants, age	Authors, year of publication Title
A. cruentus L. 'Ficha', *A. hybridus* 'K-433'.	MS_{30} + 5 mg/l BA + 0.01 mg/l NAA	epicotyls with 1st pair of leaves	(Gajdošová *et al*., 2013)
A. cruentus L. 'Ficha', *A. hybridus* 'K-433'.	MS_{30} + 1 mg/l TDZ, MS_{30} + 3mg/l TDZ + 0.01mg/l NAA	epicotyls of 10-day seedlings	(Gajdošová *et al*., 2007)
A. hypohondriacus L., *A. cruentus* L., *A. tricolor* L.	B_5 + 0.1mg/l + 0.1-1.0 mg/l ZEA	hypocotyls	(Flores *et al*., 1982)
A. hypohondriacus L., *A. cruentus* L.	MS_{30} + 2mg/l NAA + 0.2 mg/l BA + 10% coconut water	hypocotyls (non-direct regeneration), leaf discs	(Flores & Teutonico, 1986)
A. caudatus L., *(PI490458, AMES15114, AMES5461),* *A. cruentus* L., *434, 622, AMES2248, AMES2247, PI511731, PI477913)* *A. hybridus* L. *1047,* *A. hypochondriacus* L.)*, 1221, 718, 674, 722, 412, PI540446)*	MS_{30} + 2.7µM NAA+ 2.5µM 2iP, 2.7µM NAA + 2.3µM KIN). 2.7 µM NAA + 4.4 µM BA	stems	(Bennici *et al*., 1997)
A. caudatus L., *A. hypochondriacus* L.	MS_{30} + 0.3 mg/l IAA + 3mg/l KIN; MS30 + 1mg/l IAA + 3mg/l BA; MS_{30} + 6mg/l 2,4-D + 0.1 mg/l KIN; MS_{30} + 6mg/l NAA + 0.1 mg/l BA	hypocotyls (non-direct regeneration)	(Bennici *et al*., 1992)
A. paniculatus L.	MS_{30} + 8-15 mg/l KIN or 5-10 mg/l BA; MS_{30} + 0.5 – 10mg/l 2.4-D+ 0.5 – 10 mg/l NAA	inflorescence	(Arya *et al*., 1993)

Table 1. Continued.

A. edulis L.	MS30 + 2 µMTDZ, MS30 + 10µM CPPU	thin cell layers, obtained from the apical and sub-apical meristems of 7-day seedlings	(Bui van Le et al., 1998)
A. paniculatus L.	B5 KIN (0.5 ppm) and NAA 0.1 ppm), B5 + 1 mg/l GA3 + 1 mg/l KIN + 1 mg/l 2,4-D.	hypocotyls	(Bagga *et al*., 1987)
A. hypochondriacus, cv 'Azteca' L.	MS_{30} + 13.2 µM BA+1.08µM NAA	epicotyls and hypocotyls 7 day seedlings	(Jofre-Garfias *et al*., 1997)
A. spinosus L.	MS_{30}, MS_{30} +2mg/l ZEA	"hairy" roots	(Pal *et al*., 2013 b)
A. tricolor L.	MS_{30} + 13.2 µM BA +1.8 µM NAA	epicotyls and hypocotyls 7-day seedlings	(Pal *et al*., 2013 a)
A. tricolor L.	MS_{30}, MS30 +2mg/l ZEA	"hairy" roots	(Swain *et al*., 2009; Swain *et al*. 2010)
A. gangenticus L.	MS_{30} + 2 mg/l NAA + 1 mg/l BA	Leaves, stems, roots	(Amin *et al*., 1993)
A. cruentus L.	MS_{30} + 1.5 mg/l IAA + 0.5 mg/l ZEA; MS_{30} + 1 mg/l IAA	Leaves, stems, roots	(Yaacob *et al*., 2012)

3. ACHIEVEMENTS IN THE TRANSFORMATION OF AMARANTHUS SPECIES AND FUTURE PROSPECTS

The next step after obtaining regenerated plants is genetic transformation. The number of studies devoted to genetic transformation of *Amaranthus* is rather small.

So far, it is reported that genetically transformed parts or whole plants of amaranth have been obtained by two different methods: *Agrobacterium*-mediated transformation and transformation using the "floral-dip" method.

The *Agrobacterium* – mediated transformation method was developed on the basis of a natural process. Wild soil bacterium *Agrobacterium rhizogenes* or *tumefaciens* is able to infect plants, causing the appearance of "hairy" roots (*A. rhizogenes*) or tumors – crown galls (*A. tumefaciens*). At the same time as the infection process, the transfer and integration of two groups of genes into the plant genome occurs. Genetically modified *Agrobacterium* transfers the genes of interest or selective genes needed by humans into the plant's genome.

The first experiments on the transformation of amaranths were unsuccessful (De Cleene & De Ley, 1976). At present, it has been proved that transgenic amaranth plants can be obtained through *Agrobacterium*-mediated transformation. But still there are very few studies devoted to amaranth transformation.

Transgenic roots were obtained for *Amaranthus tricolor* L. (Swain *et al*., 2010) and *A. spinosus* L. (Pal & Swain, 2013). Transgenic plants were obtained for *A. hypochondriacus* L. and *A. tricolor* L. (Pal & Swain, 2013; Swain *et al*., 2009; Swain *et al*., 2010), *A. retroflexus* L. (Taipova & Kuluev, 2015), *A. viridis* L. (Taipova & Kuluev, 2015), *A. cruentus* L. (Taipova & Kuluev, 2015).

There is no information on the transformation of *A. caudatus*, varieties of which are also used in agriculture.

Transgenic roots were obtained for *A. tricolor* L. plants by Swain and colleagues (Swain *et al*., 2010) and for *A. spinosus* L. by Pal and colleagues (Pal & Swain, 2013). The transformation of amaranths was carried out using a wild strain of *Agrobacterium rhizogenesis* A4. Research group Taipova, Kulaev and others obtained transgenic roots for *A. cruenthus* L. from epicotil segments (Taipova *et al*., 2019 a; Taipova *et al*., 2019 b).

Positive results were also obtained in the transformation of amaranth species using strains of *Agrobacterium tumefaciens*. Jofre-Garfias and co-authors transformed the Azteca variety of *A. hypochondriacus* L. They used the vector from *Agrobacterium tumefaciens* with marker genes (Jofre – Garfias *et al*., 1997). Transgenic *A. tricholor* L. was obtained by two different groups of scientists – Swain and colleagues and Pal with co-authors (Swain *et al*., 2009; Pal *et al*., 2013). A team of researchers headed by Pal used a vector with marker genes.

Taipova and Kulaev obtained regenerated transformed plants from epicotil explants after *Agrobacterium*-mediated transformation (Taipova *et al*., 2019 b; Taipova & Kuluev, 2018).

Castellanos-Arévalo with colleagues obtained transgenic *A. hypochondriacus* L. and *A. hybridus* L. from "hairy" roots culture after transformation by *A. rhizogenes* strains ATCC 15834, A4 and HRI. They obtained transgenic plants with *rolB*, *bar*, *gfp*, *uid*A genes (Castellanos-Arévalo *et al*., 2020).

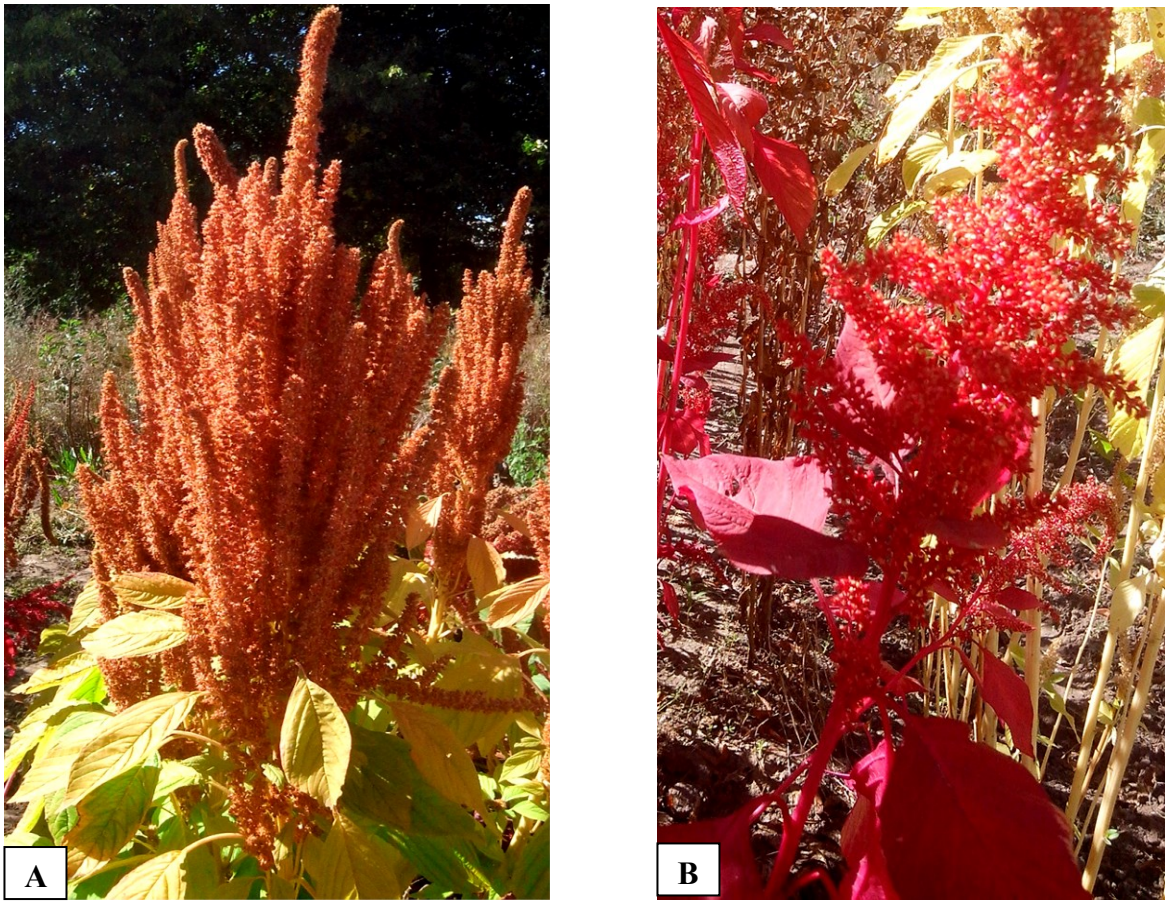
There are also 3 studies devoted to amaranth transformation through inflorescences by the "floral-dip" method – Umaiyal Munusamy and co-authors. They used a vector with selective genes (Munusamy *et al*., 2013).

Another group of researchers – Taipova and Kuluyev – carried out experiments on the transformation of *A. retroflexus* L. (Kuluev *et al*., 2017; Taipova & Kuluev, 2015; Taipova *et al*., 2019 a; Taipova *et al*., 2019 b), *A. viridis* (Kuluev *et al*., 2017; Taipova & Kuluev, 2015;

Taipova *et al*., 2019 a; Taipova *et al*., 2019 b), *A. cruentus* L. (Kuluev *et al*., 2017; Taipova & Kuluev, 2015; Taipova *et al*., 2019 a; Taipova *et al*., 2019 b). They used inflorescences of immature plants for transformation by the "floral-dip" method.

Yaroshko, Kuchuk and co-authors obtained transgenic plants of *A. caudatus* L. local cultivars Karmin and Helios with *bar* gene, after transformation by the "floral-dip" method (Yaroshko *et al*., 2018; Yaroshko & Kuchuk, 2018) (Figure 1).

Figure 1. *Amaranthus caudatus* L. cultivars Helios (A) and Karmin (B).



"Floral-dip" method protocols are described in detail in the articles of several authors (Curtis, 2005; Martins *et al*., 2015). This method was first successfully applied to *Arabidopsis thaliana* transformation (Clough & Bent, 1998; Bent, 2006; Harrison *et al*., 2006; Zhang *et al*., 2006). In addition, successfully transformed by this method were *Brassica rapa via* (Hu *et al*., 2019), *Setaria* (Saha & Blumwald, 2016; Sood & Prasad, 2017; Van Eck, 2018; Van Eck & Swartwood, 2015), rice (Ratanasut *et al*., 2017), *Schrenkiella parvula* (Wang *et al*., 2019), sugarcane (Mayavan *et al*., 2015), tomato (Sharada *et al*., 2017), *Eustoma grandiflorum* (Fang *et al*., 2018). The researchers from the Umaiyal Munusamy group, as well as Taipova and Kuluyev, assured that they had obtained viable transgenic seeds.

Yaroshko and Kuchuk obtained transgenic plants of *A. caudatus* L. and hybrids *A. caudatus* L.x *A. paniculatus* L. after floral-dip transformation (Yaroshko & Kuchuk, 2018). The researchers Murugan and Sathishkumar obtained only transgenic callus for *A. trisis* (Murugan & Sathishkumar, 2016), after transformation of parts of leaves with the *Agrobacterium tumefacies* strain EHA 105 harboring pCAMBIA 1301 (Murugan & Sathishkumar, 2016). The achievements in the field of amaranth transformation are presented in tabular form below (Table 2).

Table 2. Achievements in amaranth transformation.

Species of amaranth, cultivar	Parts of plants used for transformation	Strain of *Agrobacterium* used for transformation	Result	Authors, year of publication
A. hypochondriacus L. *"Azteca"*	germs and cotyledons	*A.tumefaciens* pgv2260 (pEsc4 with genes of *npt* II (neomycin phosphotransferase gene) - kanamycin resistance and *uid*A (gene of *β*-glucuronidase)	transgenic plants	(Jofre – Garfias *et al*., 1997)
A. tricolor L.	internodes and leaf blades	*A. rhizogenes* A4	transgenic plants	(Swain *et al*., 2009)
A. tricolor L.	internodes and leaf blades	*A. rhizogenes* A4, LBA9402	"hairy" roots, transgenic plants***	(Swain *et al*., 2010)
A. spinosus L.	internodes and leaf blades	*A. rhizogenes* LBA9402	"hairy" roots, transgenic plants***	(Pal & Swain, 2013)
A. tricolor L.	epicotyls	*A. tumefaciens EHA 105, LBA 4404 (p35SGUSINT* with genes of *npt II* - kanamycin resistance and *uid*A for each strain)	transgenic plants	(Pal *et al*., 2013)
Amaranthus L.*	inflorescence of adult plants	*A. tumefaciens AGL1 (p5b5, p5d9, p5f7* with gene of *hph* (gene codes hygromycin-B-phosphotransferase protein)	transgenic plants	(Munusamy *et al*., 2013)
A. trisis Willd. (trisis is the synonym of *Amaranthus dubius Mart. ex Thell.*	segments of leaf explants	*A. tumefacies strain EHA 105 harbouring pCAMBIA 1301*	transgenic callus	(Murugan & Sathishkumar, 2016)

Table 2. Continued.

A. retroflexus L.	inflorescence of adult plants	*A. tumefaciens* strain AGL0, which contained gene construction in the vector pCAMBIA 1301 (with ARGOS-like gene from *A. thaliana* (ARL)	transgenic plants	(Kuluev *et al*., 2017)
A. cruentus L.	epicotyls	*A. tumefaciens* strain AGL0, which contained gene construction in the vector pCAMBIA 1301 (with ARGOS-like gene from *A. thaliana* (ARL)	transgenic plants	(Taipova *et al*., 2019)
A. caudatus L.cv. Karmin, cv. Helios	inflorescence of adult plants	*A. tumefaciens* strain GV3101 (with *uid*A and *bar* (phosphinothricin N-acetyltransferase) genes)	transgenic plants	(Yaroshko *et al*., 2018)
A. caudatus L.	hypocotyls	*A. rhizogenes* A4	"hairy" roots	(Yaroshko & Kuchuk, 2018)

Note: * – name of amaranth species not stated;
** – name of *Agrobacterium* strain not stated;
*** –authors did not provide enough results in the publication that would confirm exactly the fact of obtaining transgenic plants.

Thus, at the moment, transgenic amaranth plants have been already obtained with selective genes, marker genes and genes of interest. Research into the transformation of amaranth continues. In the near future, transgenic amaranths may appear that have an improved biochemical composition and new useful properties.

4. CONCLUSION

Amaranth is unique plant. Its nutritional value and optimal amino acid composition have already been evaluated in many countries around the world. In Western Europe, the plant has already gained popularity and it is possible find products with amaranth on the shelves of supermarkets. In Ukraine, we also have a small range of products that include amaranth.

At the moment, plant regenerants have been obtained for 9 species of amaranth (*A.cruentus* L., *A. hybridus* L., *A.hypohondriacus* L., *A. caudatus* L., *A. paniculatus* L., *A. edulis* L., *A. spinosus* L., *A. tricolor* L., *A. gargenticus* L.), transformed plants for 6 species (*A.hypohondriacus* L., *A. tricolor* L., *A. spinosus* L., *A. retroflexus* L., *A. viridis* L., *A. cruentus* L.), transformed organs and tissues for 4 species (*A. spinosus* L., *A. trisis* L., *A. caudatus* L., *A. tricolor* L.).

As can be understood from our previous experimental work and the work of other authors, there are difficulties in achieving regeneration for many species of amaranths. If regenerants are obtained, the percentage of regeneration does not exceed 30 percent, which is clearly not enough for further obtaining transformed plants after agrobacterial transformation.

Therefore, other transformation techniques are being developed, for which it is not necessary to obtain regenerated plants. The alternative transformation method is called “floral-dip’. According to published studies, transformed plants have been obtained using this method.

At present, mainly transgenic amaranth plants have been obtained, which were transformed by agrobacteria that carried vectors containing selective genes. Only one group of authors obtained transgenic plants with not only selective genes, but also genes of interest.

In the near future, a greater number of amaranth species will be obtained, which will present additional useful features, such as, for example, protein synthesis, which can be used in medicine. The authors hope, that in the near future, amaranth will achieve the position of a recognized niche of the food and medicine industries.

Declaration of Conflicting Interests and Ethics

The author declares no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the author.

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