PLANT TISSUE CULTURE IN BIOTECHNOLOGY: RECENT ADVANCES IN TRANSFORMATION THROUGH SOMATIC EMBRYOGENESIS

V. A. SIDOROV

Monsanto Co., 700 Chesterfield Parkway W., St. Louis, MO 63017, USA

E-mail: vladimir.sidorov@monsanto.com

Plant genetic transformation has become an important biotechnology tool for the improvement of many crops. A solid foundation for the fast development and implementation of biotechnology in agriculture was provided by achievements in plant tissue culture. On the 30th anniversary of plant transformation, I report the advancements, recent challenges and shifts in methodology of transformation. The main focus of this paper will be on conventional and novel approaches for genetic improvements of soybean, cotton and corn. I will also highlight results on the transformation of these crops that have considerably been improved by modern biotechnology.

Key words: plant tissue culture, transformation, genetic improvement of crops.

The development of cell, tissue and organ culture methods was rapidly accelerated in the second half of the previous century after establishing robust cell culture techniques and media formulation for *in vitro* growth of plant material [1−8]. A stunning number of articles have been published on *in vitro* induction and maintenance of non-differentiated cells and the regeneration the plants from them whether through organogenesis or somatic embryogenesis. Fine protocols were established for the culture of enzymatically isolated single cells and protoplasts [9], which were able to regenerate into plants [10−12]. *In vitro* culture methods became an essential part of many micropropagation protocols. Culture of plant cells and organs in bioreactors were used for the production of different secondary metabolites and pharmaceuticals. *In vitro* techniques were used for production of mutants, haploids, virus-free material, and also for maintenance and preservation of rare genotypes and specific cell cultures [13−15].

The first positive results on *Agrobacterium*-mediated transformation in plants were reported in 1983 [16−18]. A simple method for transferring genes into plants through the inoculation of leaf discs with *Agrobacterium tumefaciens* followed by *in vitro* culture and regeneration of whole plants was reported by Horsch et al. [19]. Different methods for DNA delivery into plant cell, including electroporation [20], PEG treatment [21, 22], microinjection [23], sonication [24], biolistics or particle bombardment [25], silicon carbide Whiskers™ treatment [26, 27], were used for specific transformation purposes and different types of cells and genotypes. However, *Agrobacterium*-mediated transformation became a preferred method. *Agrobacterium tumefaciens* is a natural vector system for transgenes delivery into a wide range of plants species, providing an efficient and «clean» insertion of DNA into the plant genome and deserved to be called the «tzar of genetic engineering» [28]. In the past 30 years the discovery and application of new transformation technologies essentially sped up the improvement of major cultivated crops. The first, really commercially grown plants hit the market in the mid — 1990s.

Practically all transformation systems were based on *in vitro* culture methods. It was impossible to transform whole plant organism at once. All techniques were based on transforming single cells of callus, leaves, pollen, roots or other organs and than regeneration of plants through somatic embryogenesis or organogenesis. Routine and highly efficient transformation methods for many important crop and particular genotypes were implemented in many biotechnology companies.

However, laborious and time consuming *in vitro* methods of transformation limited the production of transgenic events. The biotechnology industry requires simple, high throughput, genotype independent transformation systems, which also could be marker- and selection-free. Whole plants, seeds, mature embryos, flowers, meristems, stolons and other plant organs became a target for transformation with a purpose to produce transgenic plants without using *in vitro* methods. The application of meristematic cultures has become a valuable tool for transformation of some recalcitrant species since it is less genotype dependant and due to possible short culture period had less potential problems with somaclonal variation [29].

In this report, as a tribute to cell biologists and their huge impact in development of modern biotechnology, the overview of conventional techniques used for transformation of important crop plants and some advancement in this area will be presented. Comprehensive reviews on molecular aspects of transformation and on milestones in plant tissue culture can be found elsewhere [30, 31]. Herein the information on transformation will be updated with emphasize on transformation through embryogenesis in some legumes, woody plants and cereals which are commonly considered as recalcitrant.

Soybean transformation

Roundup Ready® soybean developed by Monsanto was one of the first transgenic crop commercialized in 1996. For the development of this new product a bacterial gene for a glyphosate-tolerant variant of EPSP syntase (CP4) [32] was transferred, by particle bombardment, into embryonic axes of excised soybean embryos, which were regenerated into plants by organogenesis [33]. Further improvements in soybean transformation were with the development of *Agrobacterium*-mediated transformation of cotyledon explants, which also underwent organogenesis [34]. Considerable advancement in soybean transformation was observed after development of new high throughput technology based on *Agrobacterium*-mediated transformation of excised mature embryos [35, 36]. In the meantime a lot of research was concentrated on the development of embryogenic culture transformation and/or regeneration of plants through somatic embryogenesis [37, 38]. First efficient induction of embryogenic culture from immature cotyledons was described by Lippmann and

Lippmann [39]. The most reproducible particle bombardment transformation system was based on soybean embryogenic culture protocols which came from the labs of Finer and Parrott [40–42]. Soybean embryogenic cultures were also transformed by using *Agrobacterium* [43, 44] but this method turned out to be less efficient and not always reproducible.

Although embryogenic cultures of soybean are not the best target for transformation some biotechnology laboratories and companies are still using it for commercial production of transgenic plants. Since it was demonstrated that somatic embryos could be comparable to seeds in terms of quantity and especially quality of oil and protein [45–47], transformation of embryogenic cultures and production of transgenic soybean somatic embryos has been used in assays for rapid analysis of seed traits [48]. To some extend the wide application of embryogenic culture in transformation was limited due to genotype dependence. The cultivar Jack has given the best *in vitro* response, however, other genotypes can also be used for induction of embryogenic culture but with lower efficiency.

For the initiation of somatic embryos from immature cotyledons usually MSD40 medium containing 40 mg/l 2,4-D [49] is used. MSD20, which is the same medium as MSD40 but with 20 mg/l 2,4-D, is used for maintenance of embryogenic culture. For liquid culture the FN Lite medium [50] with 10 mg/l 2,4-D plus 0.5 mg/l Picl (or 0.1 mg/l Kin) is recommended. Embryogenic cultures on MSD20 are more developed while on FN Lite the culture is more globular and much greener. Established embryogenic cultures are usually transformed by biolistic methods. Detailed condition and media for induction of culture and transformation with biolistics can be found in different publications [41, 51, 52]. For selection of transgenic embryo-cultures hygromycin was mainly used.

At Monsanto our research confirmed the feasibility of using embryogenic cultures for transformation and developed a transformation system with *NPTII* as selectable marker. Embryogenic cultures were established from immature cotyledons of Jack and other genotypes. The scheme for isolation of immature cotyledons from pods and the induction of somatic embryos is shown on Fig. 1, *A*. Initiation of somatic embryos from immature cotyledons and the morphology of callus on MS40 and FN Lite medium can be seen on Fig. 1, *B, C, D*.
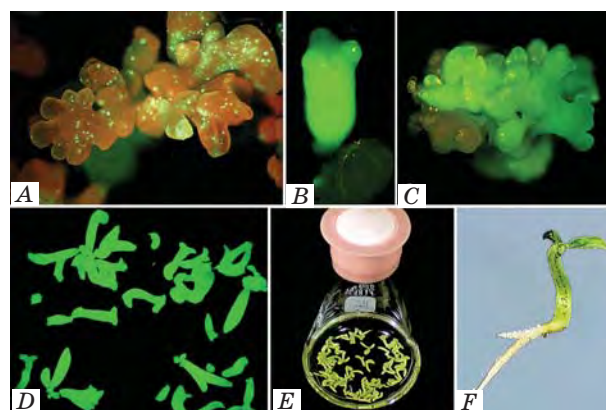
Efficient delivery of foreign DNA (*NPTII*/GFP construct) was established by particle bombardment. As a target for bombardment we
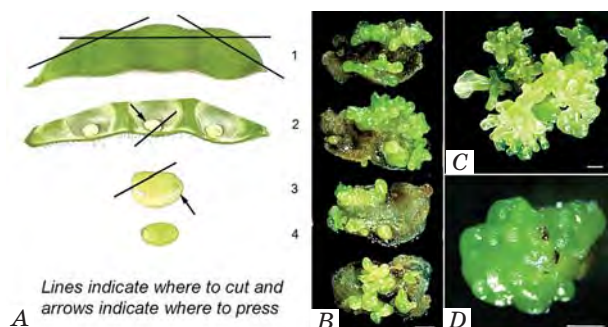
Fig. 1. Scheme for isolation of immature cotyledons from a soybean pod (*A*), induction of somatic embryos on MS40 medium (*B*) and embryogenic callus on MS20 and FN Lite media (*C, D*). Bar: 1 mm

Fig. 2. *A* — Transient GFP expression in soybean embryogenic callus (1 day after bombardment with NPTII/GFP construct). *B, C, D* — GFP expression in single somatic embryo, callus and mature somatic embryos obtained from embryogenic callus after 9 days of culture on SHAM medium. *E, F* — Maturation of somatic embryos in suspension culture and their germination.

used a young embryogenic callus grown in a dim light on MS20 medium. In spite of efficient DNA delivery into cells (high transient GFP expression was observed after 24 h of culture, Fig. 2, *A*) the recovery of stable transformants was very low. Several antibiotics and different selection pressure were tested for selection. It was found that kanamycin even at concentration of 200 mg/l did not bleach and inhibit the growth of green embryogenic cultures. Suitable for selection was paromomycin with an optimal concentration 50 mg/l. First stable transgenic events were usually identified after 3 weeks of selection (Fig. 2, *B*). Selected callus was propagated in the presence of paromomycin and than transferred to a new medium for embryogenesis (Fig. 2, *C, D*). Very fast embryogenesis and embryo maturation was established on SHAM medium (modification of FNL0S3 [52]). The embryos after 9 days of culture on this medium are shown (in a blue and day light) on Fig. 2, *D, E*. After maturation the developed embryos were germinated on SHAM or MS media without plant growth regulators (PGR).

In general, the low transformation frequency (TF) of soybean embryogenic cultures, observed in our work and in other reports, is probably due to the origin of the transformation target which is highly developed multicellular somatic embryos, and their sensitivity to selection agents. Even in spite of repetitive formation of somatic embryos observed in *in vitro* cultures, the chances for recovery of transgenic events are low. Due to development of novel and extremely robust alternative transformation techniques it is difficult to see a wide application of soybean embryogenic cultures in commercial production of genetically modified soybean plants although it is still very valuable for fundamental research and for development of different transformation assays.

Cotton transformation

Cotton is another economically important agricultural crop which is transformed by *Agrobacterium* or particle bombardment and mainly regenerated via somatic embryogenesis. Insect resistant, Bollgard® cotton, which was first transformed in 1987 [53, 54], was commercially released in 1996. Cotton is woody dicotyledonous plant, and it is truly recalcitrant to *in vitro* regeneration with very strict genotype dependence. Since the first reports on transformation, successful regeneration via embryogenesis is mainly limited to cotton varieties with a Coker pedigree. Although regeneration of cotton via organogenesis has been reported [55] there are only a few available publications on transformation and regeneration through organogenesis [56, 57]. *Agrobacterium*-mediated transformation of Coker genotype with regeneration by means of somatic embryogenesis was the most efficient method for generating transgenic cotton plants [58, 59]. This method, however, is labor-intensive and time-consuming. *Agrobacterium*-mediated transformation system *via* embryogenesis usually requires a period of up to twelve months for production of transgenic cotton plants and in comparison to other crops is rather inefficient. Regeneration and transformation methods were also established for other cotton genotypes [60–63] and, in spite of low efficiencies, were utilized for specific needs.

Other methods, like particle bombardment [64, 65] or Whiskers™-mediated transformation [66], have been exploited for the transfor-

mation of embryogenic cotton suspension cultures. There are also a few reports on pollen and pollen-tube-pathway transformations [67, 68]. In several protocols transformation of the meristem in the shoot apex with particle bombardment or *Agrobacterium* was used [69–71]. A similar approach was applied by Balasubramani et al. [56] who conducted *Agrobacterium* transformation of the embryonic axis of germinated seeds. In these intact plant tissue cases the transformation is rapid and genotype-independent. Since there is no callus stage and practically no cell dedifferentiation (it is based on multiple shoot formation) the chances of somaclonal variation are low. At the same time very light selection pressure used for meristem transformation can often be associated with chimerism of the produced shoots and plants [59]. No doubts that such methods with further improvements will be used as the foundation and essence for new industrial technologies.

Here it will be summarized the improvements made at Monsanto on the conventional transformation of Coker 130 genotype through embryogenesis which were presented earlier [72]. This protocol is similar to others and includes several steps: 1) *Agrobacterium tumefaciens* inoculation of hypocotyls cuttings and co-culture; 2) Induction of «creamy and soft» friable, undifferentiated callus; 3) Initiation and selection of embryogenic callus (EC); 4) Embryo maturation and germination; 5) Transplanting into soil. We developed and implemented a liquid-based culture system that drastically increases the efficiency of plant production. For protocol development *Agrobacterium tumefaciens*, a modified C58 strain of the bacterium with *NPTII*/GFP and *NPTII*/GUS constructs and kanamycin selection (70 mg/l) were used. Some production steps of transgenic cotton are illustrated on Fig. 3.

Callus induction was performed in plates with liquid medium. With a new culture system we could speed up the production of EC and eliminate multiple sub-culturing. Higher concentration of gelling agent in the regeneration medium especially covered with nylon «mesh» (100% nylon organza fabric) provided faster conversion of embryogenic callus and maturation of embryos (Fig. 3, *F, G, H*). Overall, with an optimized liquid transformation system the time frame for plant production could be cut in half.

Further improvements were connected with the development of *Agrobacterium*-mediated transformation system based on using EC as an initial explant. The initial EC material was easy to maintain on medium without growth regulators and could be transformed with *Agrobacterium* only if EC was desiccated during co-culture with the bacteria. Earlier it was demonstrated that desiccation during co-culture enhances the T-DNA delivery into plant cells and could be critical for transformation of callus [74]. Using GFP and GUS as reporter marker it was shown that transgenic plants could be produced in about 3 months after transformation (Fig. 4).
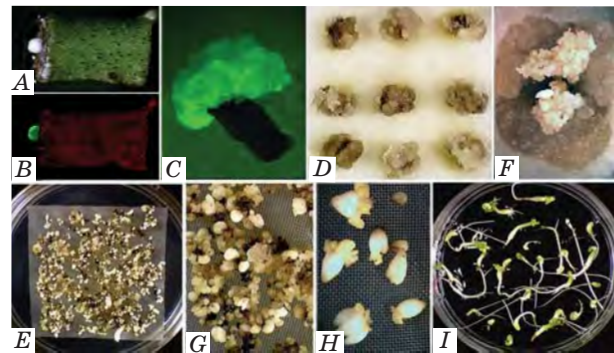
Fig. 3. **Different stages of cotton hypocotyls transformation:**
A-E — Transgenic callus formation in liquid WPSEL medium which contains Lloyd an McCown salts according to Phytotechnology Labs, 2 ml/l Gamborgs B5 vitamins (500x, Phytotechnology Labs, 0.1 mg/l 2,4-D, 0.5 mg/l kinetin, 30 g/l glucose, pH 5.8. *A, B* — 7 d old callus under day and blue light; *C* — GFP expression in 1 month old callus; *D* — 1 month old callus, cultured in a liquid medium; *E* — EC formation after 3 month of selection; *F, G* — Formation of globular embryos on TRP medium (MS salts and 2 ml/l Gamborg B5 vitamins according to Phytotechnology Labs, 0.1 g/l casein hydrolysate, 30 g/l glucose, 2.5 g/l Phytagel®, pH 5.8); *H* — Embryo maturation on TRP medium with 7 g/l agarose; *I* — Embryo germination on ShSu medium (Stewart and Hsu salts and vitamins [73], 5 g/l sucrose, 2.5 g/l Phytagel, pH 6.8).
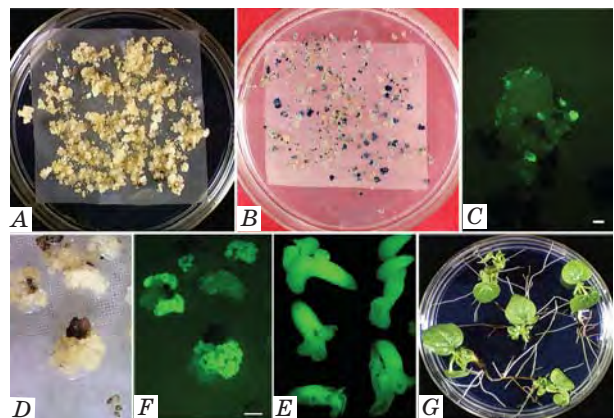
Fig. 4. **Agrobacterium-mediated transformation of embryogenic callus:**
A — Friable EC used for transformation; *B* — Transient GUS expression in embryogenic callus; *C* — GFP expression after 14 days of selection; *D, E* — Callus culture under blue and day light after 1.5 month; *F* — GFP expression in somatic embryos during maturation after 2.5 months; *G* — Plantlet formation from somatic embryos after their maturation. Bar: 1 mm

Corn transformation

Corn is one of the most economically important crops which was considerably improved by modern biotechnology. Although the first positive results on the transformation of corn and regeneration of transgenic plants were reported in 1990 [75, 76] only in 1997 did Monsanto Company bring to the marketplace the GMO plant, insect-protected corn in the form of YieldGard$^®$ Corn Borer. Similar products were developed by other companies. In 1996 Ciba-Geigy and Mycogen introduced E-176 corn and in 1997 Novartis introduced Bt-11corn. Particle bombardment of corn embryogenic cultures was used for producing the first product. Later not only single traits but stacked traits were added to corn products. To create biotech products two transformation techniques for transgenes delivery have mainly been employed: *Agrobacterium*-mediated transformation [77−80] and particle bombardment transformation [81, 82]. Although corn, like other cereals, is not a natural host for *Agrobacterium* sp., *Agrobacterium*-based transformation is the preferred method and has several advantages over biolistics. New *Agrobacterium*-transformation methods are very efficient and in comparison to bombardment provide a higher number of events with single intact transgenes. Now new products like Genuity$^™$ SmartStax$^™$ (Monsanto), Herculex$^®$ Xtra Roundup$^®$ Corn 2 (Dow Agrosciences and Pioneer Hi-Bred), Agrisure$^®$ GT/CB/LL (Syngenta), Optimum$^®$ AcreMax$^®$ Insect Protection (Pioneer Hi-Bred, DuPont) and many other have combinations of stacked traits against different insects and tolerance to a number of herbicides for broad spectrum weed control. In 2011 twelve countries have planted biotech crops with two or more traits.

Type I culture in transformation. From the very first publication on corn *in vitro* regeneration [83] it was accepted that somatic embryogenesis is the principal way of plant regeneration in corn and other cereals. The induction of so-called «Type I» callus was established from immature embryos (IE) of inbred line A188. The same compact, organized «embryogenic» type of callus was induced from IEs of number genotypes [84−88]. Right now in most transformation protocols the plants are regenerated through Type I culture. The transformation of IEs and regeneration through somatic embryogenesis or organogenesis is the most popular choice for transgenic plant production. The wide application of *Agrobacterium*-mediated transformation of monocotyledonous species and particularly corn became feasible after development of an efficient transformation method for rice described by Hiei et al. [89]. The disarmed *Agrobacterium*, which was induced by acetosyringone and carried a «super binary» vector with selectable marker genes, was used to establish a corn transformation protocol with freshly isolated IEs [78, 80, 90, 91]. After considerable improvement *Agrobacterium*-mediated transformation of freshly isolated or precultured IEs became a routine practice for efficient production of transgenic corn [80, 92]. However, some corn genotypes possess a high competence for embryogenesis and regeneration but they are not susceptible to *Agrobacterium*. In Fig. 5 a high level of GUS and GFP transient expression in IEs of proprietary genotype, L1 is shown (Fig. 5, *B*, *C*), and poor transient GUS expression in IEs of another tested proprietary genotype L2 (Fig. 5, *A*).

In order to obtain a high TF with «difficult» genotype it could be necessary to do an essential protocol modification and media optimization. Very often it can be easier to screen the breeders potential candidate-genotypes for «culturability» (competent for *in vitro* culture and regeneration) and «transformability» (competent for transformation) using several standard protocols. Sometimes it could be a good idea to test for transformation of other explants. Thus, one genotype which was difficult to transform using freshly isolated IEs (Fig. 5, *A*) turned to be highly transformable when young callus of cultured IEs were used as initial explants for transformation (Fig. 5, *D*).

High TF can be achieved with freshly isolated IEs or propagated *in vitro* callus obtained from IEs or seedlings [80, 93]. Because of potential somaclonal variation, extended maintenance of callus *in vitro* is not recommended. Production of seedling-derived callus using mature seeds has a high impact on trans-
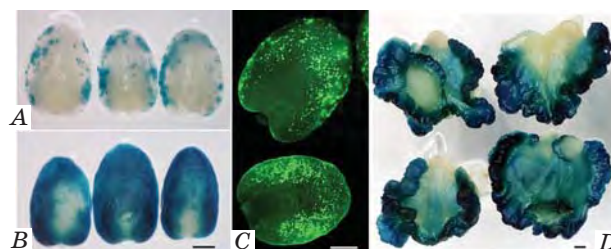

Fig. 5. **Transient GUS and GFP expression in freshly isolated (*A*, *B*, *C* different genotypes) and transient GUS expression in callus cells after culturing of IEs for 8 days. GUS staining for 3 hours. Bar = 0.5 mm**

formation technology since it is considered as «greenhouse-independent» (it does not require growing plants for IEs). Transformation technology based on using seedling-derived callus can be efficiently utilized in dihaploid programs and potentially speed up the breeding process. Haploid Type I callus can be produced from seedlings of haploid seeds conventionally obtained after crossing a corn variety with a haploid inducer line. Haploid seeds are easy to identify due to the presence in the inducer line of visible pumule and cap markers. Routinely about 75% of haploid callus lines from seedlings remain haploid. Isolated haploid callus can be transformed and after doubling homozygote dihaploid transformed plants can be regenerated [94, 95].

Different selection scheme can be used for preferential growth of transformed cells and regeneration of transgenic plants. For commercial production of transgenic plant genes conferring resistance to antibiotics, herbicides and mannose have been used [96]. Selectable marker genes can be removed since they are not required for expression of the gene of interest. There are several methods available for marker removal from transgenic plants: co-transformation with 2 unlinked T-DNAs («2T transformation») followed by segregation of the marker gene in progeny [97-99], homologous recombination between direct repeats [100] and site-specific recombination, including the most popular Cre/*lox* system [101, 102]. Extremely appealing is marker-free, selection-free transformation technology [103, 104].

Only with very high TF it is possible to do the transformation without selection and conduct identification of transgenics by molecular screening of all produced shoots or plants. Since we developed a very efficient IEs transformation protocol for L1 line with TF in some experiments higher than 60% we checked the feasibility of using no selection for production transgenic plants.

Using ABI *Agrobacterium* containing a construct with the *uidA* (GUS) reporter gene we tried to track the formation of stable transgenic events during different stages of culture and evaluate the efficiency of transformation without selection. After 10 days of culture post- transformation with no selection it was found that almost all IEs formed a callus with several GUS positive regions. After several weeks of culture the callus derived from each IE was divided into 10−20 pieces and cultured further (Fig. 6). It was found that all callus pieces from a single IE were GUS negative, a few were chimeric and one was non-chimeric, GUS positive. A similar picture was observed in several other cases when the batch of callus pieces derived from single IE were stained. Among plants regenerated without selection we also identified several GUS positive (Fig. 6, *D*). Although in our experiments the TF was very low this method after improvements of screening at the callus stage can be useful. Such a marker-free/selection-free protocol allows doing the transformation of corn with 1T constructs without any additional markers and in comparison to transformation with 2T constructs it reduces the breeding timeframe and simplifies the downstream breeding process.

Type II culture in transformation. A new type of embryogenic callus named as «Type II» was described by Green [105, 106] and Armstrong, Green [107]. Type II culture derived from immature embryos is a very fine, friable callus which can directly form somatic embryos. Efficient induction and sustainable
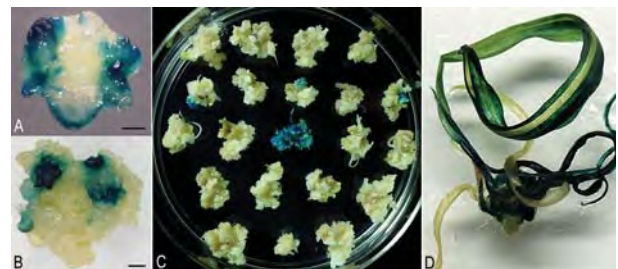
Fig. 6. **GUS expression in callus derived from IEs on different stages of culture and regeneration (without selection) after transformation with GUS construct:**
A, B — Transient GUS expression in IEs callus after 6 days and 14 days of culture; *C* — Callus pieces on regeneration medium; one transformant and few himeric clumps were identified among callus pieces originated from one IE; *D* — GUS positive plantlets from no-selection experiment. Bar: 1 mm

propagation of embryognic callus was established with A188 inbred genotype on N6 [7] medium supplemented with 6 mM proline [107]. The induction of this type of callus is highly genotype dependent. It was demonstrated that many hybrids which have A188 as one parent also demonstrate the embryogenic response found in A188. The well known genotype with Type II response is «Hi-II» derived from progeny of A188×B73 cross [108]. Quantitative trait loci (QTLs) that control regenerable callus formation and plant regeneration in maize have been identified for the Type II callus response [109, 110]. Embryogenic Type II callus was usually initiated from

IEs but the induction of such culture from immature tassels of HiII genotype has also been reported [111]. The Hi-II line is not an inbred but Lowe et al. [112], after crossing Hi-II with the FBLL genotype followed by multiple backcrosses transferred the Type II embryogenic response developing the FBLL-MAB inbred line. The availability of suitable inbred lines for transformation is particularly desirable since modern breeding of corn is based on the utilization of inbred parents for production of hybrid seeds possessing «hybrid vigor» or heterosis. It is typical that breeding/biotechnology companies have their own proprietary germplasm that are bred for traits like yield or disease resistance not for *in vitro* «culturability» and «transformability». Some of these elite genotypes require, for *in vitro* growth, completely new combinations of PGR in media for them to be transformed with high efficiency. It was demonstrated that Type II callus is not limited to A188 genotypes or close relatives and can be obtained from different genotypes [113, 114]. Type II callus can be directly used for transformation [76] and can be a good source for protoplast isolation and production of transgenic plants [115]. Still, genotype specificity is much higher for Type II callus than for Type I callus. Due to the high genotype dependence the regeneration through somatic embryogenesis from Type II callus has been used less and less in the commercial transformation of corn.

Meristem culture in transformation. Corn and other cereals can also be regenerated via organogenesis. First multiple shoot formation from apical meristem of immature embryos was reported in 1992 [116]. Induction of organogenic cultures from corn seedling meristem was described by Zhong et al. [117, 118]. The same type of culture was initiated from meristematic tissue of the nodal area of seedlings, leaf bases of young leaves and other explants containing highly meristematic cells. The culture medium for multiple shoot induction usually contains high concentration of cytokinins (0.5−10 mg/l 6BA) and no or low concentration of auxins (0−0.5 mg/l 2,4-D). The same or similar types of media were used for multiple shoot induction in oat [119], sorghum [120], millet [121], wheat [122], barley [123] and other monocots. Meristem culture depending on the stage of development may have multiple buds resembling shoot apex with apical meristem and primordial leaf, enlarged buds, highly meristematic zones with multiple buds or more developed multiple shoots (Fig. 7). Meristem shoot culture can be maintained for

Fig. 7. Different development stages of corn shoot meristem culture:
A — Enlarged bud; *B* — Adventitious buds formation from highly meristematic tissue; *C* — Established meristem shoot culture. Bar: 2 mm

extended period of time and can easily produce plants on the medium without PGR [124].

Shoot apical meristems and derived organogenic cultures were recommended for use as sustainable explants for genetic transformation of cereal crops [119]. This organogenic type of culture, referred to as apical meristem culture, shoot meristematic culture, multiple shoot culture, and multiple bud culture, has been transformed by particle bombardment [125, 126]. Induction of this type of tissue appears is less dependent on specific genotype in comparison to Type II and Type I cultures [119]. For wide implementation of organogenic culture in production work it still requires the development of efficient delivery of foreign DNA via *Agrobacterium*.

General remarks on corn morphogenesis. The morphology, characteristics of Type I, Type II, and meristem culture with the possible conversion pathway of one type culture to another are presented on Fig. 8. Depending on the genotype the conversion of one type of culture to another can be very fast but often requires several subcultures. Conversion of organogenic meristem culture of LH198 x HiII and L1 line into Type I callus is presented below (Fig. 9, *A, B*). Conversion of FBLL-MAB Type II callus into shoot meristem culture is shown on Fig. 9, *C, D, E*.

In scientific lexicon Type I culture is commonly described as «embryogenic» which is not completely correct. It is known that term somatic embryogenesis describes a developmental process of somatic cells which results in a morphological structures similar to zygotic embryos and typically had a distinct developmental stages. In comparison to Type II callus which is truly embryogenic, Type I callus is not. It is more organogenic since at light condition it is easy converts into leafy structures and forms shoots (Fig. 10). Detailed histochemical and ultrastructural study show that Type II callus does contains embryogenic units (which give rise to somatic embryos) while Type I callus has mainly a meristematic cells and extensive vascular network [127, 128].
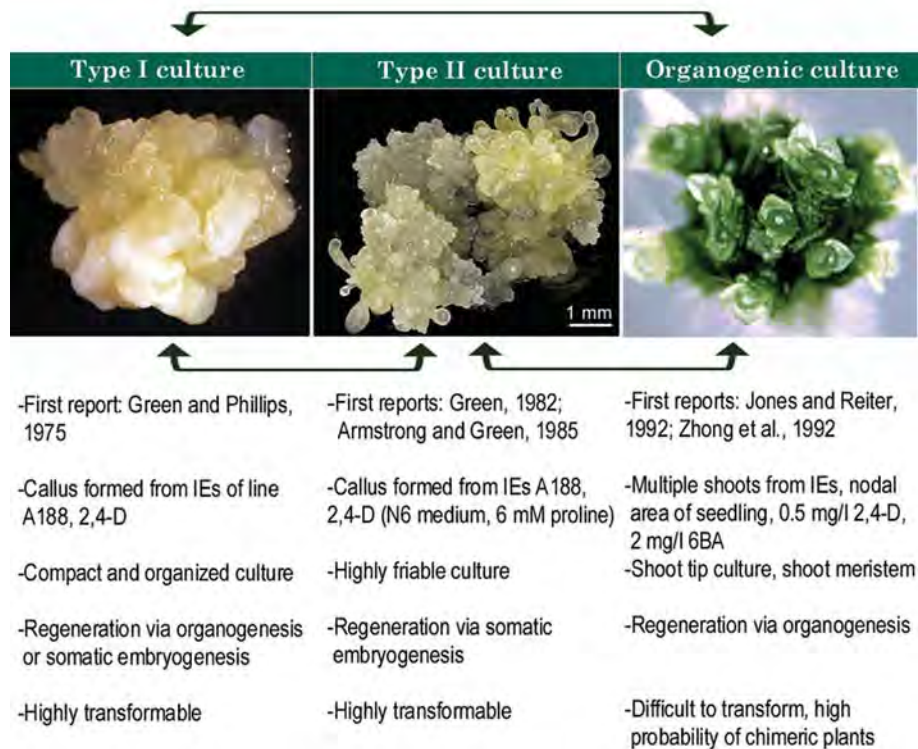
Type I culture	Type II culture	Organogenic culture

-First report: Green and Phillips, 1975

-Callus formed from IEs of line A188, 2,4-D

-Compact and organized culture

-Regeneration via organogenesis or somatic embryogenesis

-Highly transformable

-First reports: Green, 1982; Armstrong and Green, 1985

-Callus formed from IEs A188, 2,4-D (N6 medium, 6 mM proline)

-Highly friable culture

-Regeneration via somatic embryogenesis

-Highly transformable

-First reports: Jones and Reiter, 1992; Zhong et al., 1992

-Multiple shoots from IEs, nodal area of seedling, 0.5 mg/l 2,4-D, 2 mg/l 6BA

-Shoot tip culture, shoot meristem

-Regeneration via organogenesis

-Difficult to transform, high probability of chimeric plants

Fig. 8. **Regenerable types of in vitro corn culture, their main characteristics, and conversion pathway of one type culture to another**
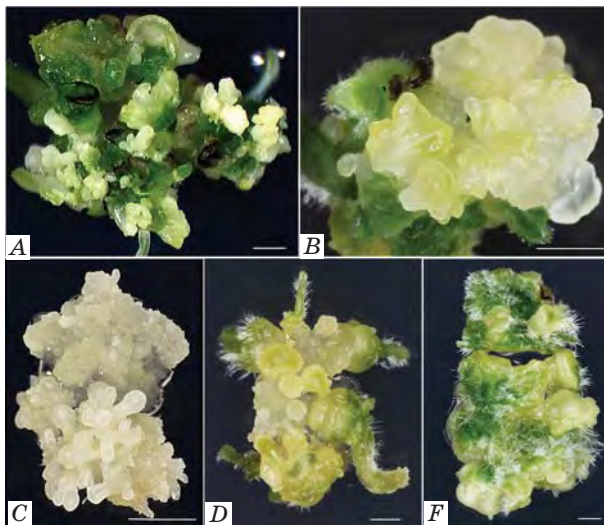

Fig. 9. **Conversion of organogenic meristem culture of LH198 x HiII (*A*) and L1 line (*B*) into Type I callus. Bar: 2 mm. *C, D, E* — Conversion of FBLL-MAB Type II callus into meristem culture with multiple buds; 2 and 4 weeks culture on MSV34 medium [80]. Bar: 0.9 mm**

Fig. 10. *A* — **Type I callus formation from IE LH198 x HiII (in dark). *B* — The same IE cultured another 10 days on the same medium (on light). *C* — Piece of callus on higher magnification which converts into leafy structures. Bar: 1 mm**

Greening of this type of callus, formation of leafy structures, followed by multiple buds and shoot formation are especially pronounced in production experiments where for a fast regeneration the callus is exposed for a short time to 6BA (MSBA medium 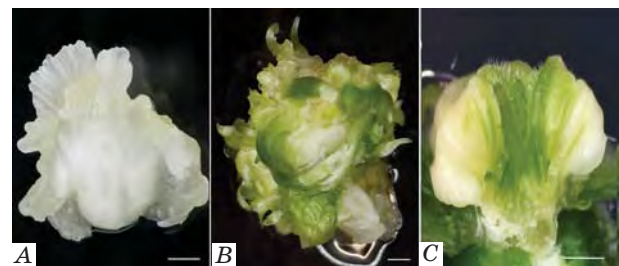according to [80]). As it is shown on Fig. 11, Type I callus after a short culture in light on 6BA medium formed green leafy structures with multiple buds at the base of them. On PGR-free medium these buds formed shoots which could be later rooted. Formation of multiple buds (apical meristem) from Type I callus in corn is demonstrated on Fig. 11, *A, B*. Because of this regeneration pathway there is no stage of somatic embryogenesis and it is properly to call this way of morphogenesis as organogenesis. At the same time the regeneration of Type I callus through embryogenesis is also possible. In several publications compact Type I callus was regarded as «fused deformed and normal
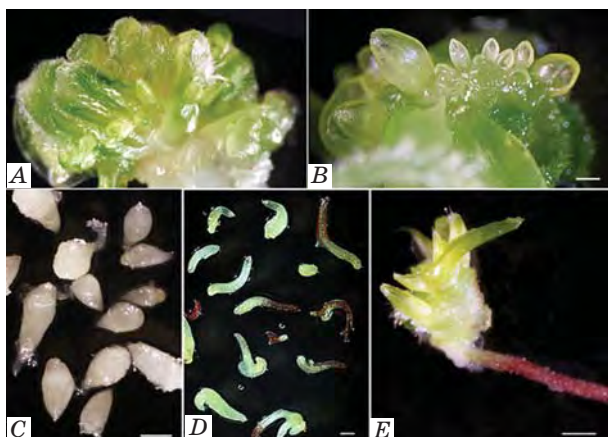
***Fig. 11. A, B* — Multiple buds and shoots forma-
tion in corn after 1 week culture on 6BA contain-
ing medium, followed by PGR-free medium.
Bar: 0.5 mm;
C, D, E — Germination of corn somatic embryos
of L1 line on PGR-free medium;
C — Bar: 1 mm; *D, E* — Bar: 2 mm.**

somatic embryos with an incomplete shoot-
root axis» [129, 130]. Biochemical analyses of
Type I callus also have shown the presence of
specific markers of embryogenesis in this type
of callus [131, 132]. It was confirmed that

Type I callus on PGR-free medium in darkness
can produce very fine callus clumps which in
suspension culture could initiate single soma-
tic embryos (Fig. 11, *C, D, E*). Because of pre-
sence of two types of cells which regenerate
through organogenesis or embryogenesis pro-
bably it would be more correct simply to call
Type I callus as regenerable callus and only
Type II callus refer as embryogenic.

In conclusion, it is expected that the pre-
sented results on corn, especially in compari-
son to the data on other species regenerated
through embryogenesis, will clarify some
peculiarities of corn culture and transforma-
tion. Hopefully this will be useful for resear-
chers working on further improvements of
transformation technologies.

REFERENCES

1. *Murashige T., Skoog F.* A revised medium for
rapid growth and bioassays with tobacco tissues
cultures // Physiol. Plant. — 1962. —
V. 15. — P. 473–479.
2. *Eriksson T.* Studies on the growth require-
ments and growth measurements of cell cultures
of *Haplopappus gracilis* // Ibid. — 1965. —
V. 18. — P. 976–993.
3. *Gamborg O. L., Miller R. A., Ojima K.* Nutrient
requirement of suspensions cultures of soybean
root cells // Exp. Cell Res. — 1968. —
V. 50. — P. 148–151.
4. *Nitsch J. P., Nitsch C.* Haploid plants from pol-
len grains // Science. — 1969. — V. 163,
N 3862. — P. 85–89.
5. *Schenk R. U., Hildebrandt A. C.* Medium and
techniques for induction and growth of mono-
cotyledonous and dicotyledonous plant cell
cultures // Can. J. Bot. — 1972. — V. 50. —
P. 199–204.
6. *Kao K. N., Michayluk M. R.* Nutritional requi-
rements for growth of *Vicia hajastana* cells
and protoplasts at a very low population densi-
ty in liquid media // Planta. — 1975. —
V. 126. — P. 105–110.
7. *Chu C. C., Wang C. C., Sun C. S. et al.*
Establishment of an efficient medium for ant-
her culture of rice through comparative expe-

riments on the nitrogen sources // Scientia
Sinica. — 1975. — V. 18. — P. 659–668.
8. *Lloyd G., McCown B.* Commercially feasible
micropropagation of mountain laurel, *Kalmia
latifolia*, by use of shoot-tip culture // Proc.
Int. Plant Prop. Soc. — 1980. — V. 30. —
P. 421–427.
9. *Cocking E. C.* A method for the isolation of
plant protoplasts and vacuoles // Nature. —
1960. — V. 187. — P. 962–963.
10. *Nagata T., Takebe L.* Cell wall regeneration
and cell division in isolated tobacco mesop-
hyll protoplasts // Planta. — 1970. —
V. 2. — P. 301–308.
11. *Frearson E. M., Power J. B., Cocking E. C.* The
isolation, culture, and regeneration of
Petunia protoplasts // Develop. Biol. —
1973. — V. 33. — P. 130–137.
12. *Nagy J. I., Maliga P.* Callus induction and
plant regeneration from mesophyll proto-
plasts of *Nicotiana sylvestris* // Z.
Pflanzenphysiol. — 1976. — V. 78. —
P. 453–455.
13. *Gamborg O. L., Phillips G. C.* Plant Cell,
Tissue and Organ Culture. — Springer-
Verlag, 1995. — 348 p.
14. *Hall R. D.* Plant Cell Culture Protocols. —
Humana Press. Totowa, New Jersey, 1999. —
421 p.

15. *Singh M. P., Kumar S.* Plant Tissue Culture. — New Delhi: APH Publishing, 2009. — 286 p.

16. *Fraley R. T., Rogers S. B., Horsch R. B.* Use of a chimeric gene to confer antibiotic resistance to plant cells // Advances in Gene Technology: Molecular Genetics of Plants and Animals. Miami Winter Symposia. — 1983. — V. 20. — P. 211–221.

17. *Framond A. J., Bevan M. W., Barton K. A. et al.* Mini-Ti plasmid and a chimeric gene construct: new approaches to plant gene vector construction // Ibid. — 1983. — V. 20. — P. 159–170.

18. *Schell J., Van Montagu M., Holsters M.* Ti plasmids as experimental gene vectors for plants. // Ibid. — 1983. — V. 20. — P. 191–209.

19. *Horsch R. B., Fry J. E., Hoffmann N. L. et al.* A simple and general method for transferring genes into plants // Science. — 1985. — V. 227. — P. 1229–1231.

20. *Fromm M. E., Taylor L. P., Walbot V.* Stable transformation of maize after gene transfer by electroporation // Nature. — 1986. — V. 319, N 6056. — P. 791–793.

21. *Lorz H., Baker B., Schell J.* Gene transfer to cereal cells mediated by protoplast transformation // Mol. Gen. Genet. — 1985. — V. 199. — P. 178–182.

22. *Potrykus I., Saul M. W., Petruska J. et al.* Direct gene transfer to cells of a graminaceous monocot // Ibid. — 1985. — V. 199. — P. 183–188.

23. *Reich T. J., Iyer V. N., Miki B. I.* Efficient transformation of alfalfa protoplasts by the intranuclear microinjection of Ti plasmid // Biotechnology. — 1986. — V. 4. — P. 1001–1004.

24. *Joesbro M., Brunstedt J.* Direct gene transfer to plant protoplasts by mild sonication // Plant Cell Rep. — 1990. — V. 9. — P. 207–210.

25. *Klein T. M., Wolf E. D., Wu R., Sanford J. C.* High velocity microprojectiles for delivering nucleic acids into living cells // Nature. — 1987. — V. 327. — P. 70–73.

26. *Kaeppler H. F., Gu W., Somers D. A. et al.* Silicon carbide fiber-mediated DNA delivery into plant cells // Plant Cell Rep. — 1990. — V. 8. — P. 415–418.

27. *Frame B. R., Drayton P. R., Bagnall S. V. et al.* Production of fertile transgenic maize plants by silicon carbide whisker-mediated transformation // Plant J. — 1994. — V. 6. — P. 941–948.

28. *Tzfira T., Citovsky V.* *Agrobacterium*-mediated genetic transformation of plants: biology and biotechnology // Curr. Opin. Biotech. — 2006. — V. 17. — P. 147–154.

29. *Duncan D. R.* Organogenesis and embryogenesis in plant genetic transformation / Plant Transformation Technology Revolution in Last Three Decades. — V. 1. Yinghui Dan and David W. Ow (eds.) // Science. — 2011. — P. 46–54.

30. *Tzfira T., Citovsky V.* (eds). *Agrobacterium*: From Biology to Biotechnology. Springer, 2008. — 737 p.

31. *Vasil I. K.* A history of plant biotechnology: from the Cell Theory of Schleiden and Schwann to biotech crops // Plant Cell Rep. — 2008. — V. 27. — P. 1423–1440.

32. *Barry G., Kishore G., Padgette S. et al.* Inhibitors of amino acid biosynthesis: Strategies for imparting glyphosate tolerance to crop plants / B. K. Singh et al. (eds). Biosynthesis and Molecular Regulation of Amino Acids in Plants. Amer. Soc. Plant Physiol., Rockville, MD. — 1992. — P. 139–145.

33. *McCabe D. E., Swain W. F., Martinell B. J., Christou P.* Stable transformation of soybean (*Glycine max*) by particle acceleration // Bio/Technol. — 1988. — V. 6. — P. 923–926.

34. *Hinchee M. A., Ward D. C., Newell R. E. et al.* Production of transgenic soybean plants using *Agrobacterium*-mediated DNA transfer // Ibid. — 1988. — V. 6. — P. 915–922.

35. *Martinell B. J., Julson L. S., Emler C. A. et al.* Soybean transformation method. US Patent 7,002,058 B2. — 2006.

36. *Martinell B. J., Julson L. S., Emler C. A. et al.* Soybean transformation method. US Patent 08030076. — 2011.

37. *Parrott W. A., Clemente T. E.* Transgenic soybean / Soybean: Improvements, Production, and Uses, 3rd ed. Agronomy Monograph no 16. — 2004. — P. 265–301.

38. *Yamada T., Takagi K., Ishimoto M.* Recent advances in soybean transformation and their application to molecular breeding and genomic analysis // Breed Sci. — 2012. — V. 61, N 5. — P. 480–494.

39. *Lippmann B., Lippmann G.* Induction of somatic embryos in cotyledonary tissue of soybean, *Glycine max* L. Merr. // Plant Cell Rep. — 1984. — V. 3. — P. 215–218.

40. *Finer J. J., Nagasawa A.* Development of an embryogenic suspension culture of soybean (*Glycine max* Merrill.) // Plant Cell Tissue Organ Cult. — 1988. — V. 15. — P. 125–136.

41. *Finer J. J., McMullen M. D.* Transformation of soybean via particle bombardment of embryogenic suspension culture tissue // In Vitro Cell. Dev. Biol. — 1991. — V. 27. — P. 175–182.

42. *Parrott W. A., Williams E. G., Hildebrand D. F., Collins G. B.* Effect of genotype on somatic embryogenesis from immature cotyledons of soybean // Plant Cell Tissue Organ Cult. — 1989. — V. 16. — P. 15–21.

43. *Parrott W. A., Hoffman L. M., Hildebrand D. F. et al.* Recovery of primary transformants of soybean // Plant Cell Rep. — 1989. — V. 7. — P. 615–617.

44. *Trick H. N., Finer J. J.* Sonication-assisted *Agrobacterium*-mediated transformation of soybean [*Glycine max* (L.) Merrill] embryogenic suspension culture tissue // Ibid. — 1998. — V. 17. — P. 482–488.

45. *Cahoon E. B, Marillia E-F., Stecca K. L. et al.* Production of fatty acid components of meadow-foam oil in somatic soybean embryos // Plant Physiol. — 2000. — V. 124. — P. 243–251.

46. *Herman E. M., Helm R. M., Jung R., Kinney A. J.* Genetic modification removes an immunodominant allergen from soybean // Ibid. — 2003. — V. 132. — P. 36–43.

47. *Schmidt M. A., Tucker D. M., Cahoon E. B., Parrott W. A.* Towards normalization of soybean somatic embryo maturation // Plant Cell Rep. — 2005. — V. 24. — P. 383–391.

48. *Lightner J. E., Okuley J. J., Hitz W. et al.* Genes for microsomal delta-12 fatty acid desaturases and hydroxylases from plants. US Patent 6,372,965. — 2002.

49. *Bailey M. A., Boerma H. R., Parrott W. A.* Genotype effects on proliferative embryogenesis and plant regeneration of soybean // In Vitro Cell Dev. Biol. — 1993. — V. 29. — P. 102–108.

50. *Samoylov V. M., Tucker D. M., Parrott W. A.* Soybean [*Glycine max* (L.) Merrill] embryogenic cultures: the role of sucrose and total nitrogen content on proliferation // Ibid. — 1998. — V. 34. — P. 8–13.

51. *Trick H. N., Dinkins R. D., Santarem E. R. et al.* Protocols. Recent advances in soybean transformation // Plant Tissue Culture Biotechnol. — 1997. — V. 3, N1. — P. 9–26.

52. *Samoylov V. M., Tucker D. M., Thibaund-Nissen F., Parrott W. A.* A liquid-medium-based protocol for rapid regeneration from embryogenic soybean cultures. // Plant Cell Rep. — 1998. — V. 18. — P. 49–54.

53. *Umbeck P., Johnson G., Barton K., Swain W.* Genetically transformed cotton (*Gossypium hirsutum* L.) plants // Biotechnology. — 1987. — V. 5. — P. 263–266.

54. *Firoozabady E., DeBoer D. L., Merlo D. J. et al.* Transformation of cotton (*Gossypium hirsutum* L.) by *Agrobacterium tumefaciens* and regeneration of transgenic plants // Plant. Mol. Biol. — 1987. — V. 10. — P. 105–116.

55. *Khan T., Singh A. K., Pant R. C.* Regeneration via somatic embryogenesis and organogenesis in different cultivars of cotton (*Gossipium* spp.) // In Vitro Cell. Dev. Biol. Plant. — 2006. — V. 42. — P. 498–501.

56. *Balasubramani G., Amudha J., Mayee C. D.* Genetic transformation of cotton (*G. hirsutum* L.) by *Agrobacterium tumefaciens* and regeneration by direct shoot organogenesis // Adv. Plant Sci. — 2005, — V. 18. — P. 497–504.

57. *Yuceer S. U., Koc N. K. Agrobacterium*-mediated transformation and regeneration of cotton plants // Russ. J. Plant Physiol. — 2006. — V. 53. — P. 413–417.

58. *Wilkins T. A., Mishra R., Trolinder N. L. Agrobacterium*-mediated transformation and regeneration of cotton // Food, Agricul. Environ. — 2004. — V. 2. — P. 179–187.

59. *Duncan D. R.* Cotton transformation / Cotton, Biotechnology in Agriculture and Forestry. U. B. Zehr (ed.). — Springer-Verlag, Berlin Heidelberg, 2010. — V. 65. — P. 65–77.

60. *Sakhanokho H. F., Zipf A., Rajasekaran K. et al.* Induction of highly embryogenic calli and plant regeneration in upland (*Gossypium hirsutum* L.) and pima (*Gossypium barbadense* L.) cottons // Crop Sci. — 2001. — V. 41. — P. 1235–1240.

61. *Mishra R., Wang H. Y., Yadav N. R., Wilkins T. A.* Development of a highly regenerable elite Acala cotton (*Gossypium hirsutum* cv. Maxa) — a step towards genotype-independent regeneration. // Plant Cell Tissue Organ Cult. — 2003. — V. 73. — P. 21–35.

62. *Wu J. H., Zhang X. L., Nie Y. C. et al.* Factors affecting somatic embryogenesis and plant regeneration from a range of recalcitrant genotypes of Chinese cottons (*Gossypium hirsutum* L.) // In Vitro Cell. Dev. Biol. Plant. — 2004. — V. 40. — P. 371–375.

63. *Sun Y., Zhang X., Huang C. et al.* Somatic embryogenesis and plant regeneration from different wild diploid cotton (*Gossypium*) species // Plant Cell Rep. — 2006. — V. 25. — P. 289–296.

64. *Finer J. J., McMullen M. D.* Transformation of cotton (*Gossypium hirsutum* L.) via particle bombardment // Ibid. — 1990. — V. 8. — P. 886–889.

65. *Rajasekaran K., Hudspeth R. L., Cary J. W. et al.* High-frequency stable transformation of cotton (*Gossypium hirsutum* L.) by particle bombardment of embryogenic cell suspension cultures // Ibid. — 2000. — V. 19. — P. 539–545.

66. *Beringer J., Palta A. M., Baker L. W. et al.* Transgenic cotton via Whiskers™-mediated transformation // Recent Res. Devel. Crop Sci. — 2004. — V. 1. — P. 335–347.

67. *Li X., Wang X. D., Zhao X., Dutt Y.* Improvement of cotton fiber quality by transforming the *acsA* and *acsB* genes into *Gossypium hirsutum* L. by means of vacuum infiltration // Plant Cell Rep. — 2004. — V. 22. — P. 691–697.

68. *Huang G. C., Dong Y. M., Sun J. S.* Introduction of exogenous DNA into cotton via the pollen-tube pathway with GFP as a reporter // Chin. Sci. Bull. — 1999. — V. 44. — P. 698–701.

69. *McCabe D. E., Martinell B. J.* Transformation of elite cotton cultivars via particle bombardment of meristem // Biotechnology. — 1993. — V. 11. — P. 596–598.

70. *Gould J. H., Magallanes-Cedeno M.* Adaptation of cotton shoot apex culture to *Agrobacterium*-mediated transformation // Plant. Mol. Biol. Rep. — 1998. — V. 16. — P. 1–10.

71. *Zapata C., Park S. H., El-Zik K. M., Smith R. H.* Transformation of a Texas cotton cultivar by using *Agrobacterium* and the shoot apex // Theor. Appl. Genet. — 1999. — V. 98. — P. 252–256.

72. *Sidorov V., Subbarao S., Layton J. et al.* Transformation system improvements in cotton. 12th IAPB Congress, June 6–11, 2010, St. Louis, Missouri. Poster Abstracts. — 2010. — P–166.

73. *Stewart J. Mc.D., Hsu C. L.* In ovulo embryo culture and seedling development of cotton (*Gossypium hirsutum* L.) // Planta. — 1977. — V. 137. — P. 113 –117.

74. *Cheng M., Hu., Layton J. et al.* Desiccation of plant tissue post-*Agrobacterium* infection enhavces T-DNA delivery and increases stable transformation efficiency in wheat // In Vitro Cell. Dev. Biol. — Plant. — 2003. — V. 39. — P. 595–604.

75. *Fromm M. E., Morrish F., Armstrong C. et al.* Inheritance and expression of chimeric genes in the progeny of transgenic maize plants // Biotechnology. — 1990. — V. 8. — P. 833–839.

76. *Gordon-Kamm W. J., Spencer T. M., Mangano M. L. et al.* Transformation of maize cells and regeneration of fertile transgenic plants // Plant Cell. — 1990. — V. 2. — P. 603–618.

77. *Grimsley N., Hohn T., Davis J. W., Hohn B.* *Agrobacterium*-mediated delivery of infectious maize streak virus into maize plants // Nature. — 1987. — V. 325. — P. 177–179.

78. *Ishida Y., Saito H., Ohta S. et. al.* High efficiency transformation of maize (*Zea mays* L.) mediated by *Agrobacterium tumefaciens* // Nature Biotechnol. — 1996. — V. 14. — P. 745–750.

79. *Miller M., Tagliani L., Wang N. et. al.* High efficiency transgene segregation in co-transformed maize plants using an *Agrobacterium tumefaciens* 2 T-DNA binary system // Transgenic Res. — 2002. — V. 11. — P. 381–393.

80. *Sidorov V., Duncan D.* *Agrobacterium*-mediated maize transformation: Immature embryos *versus* callus. — Methods in Molecular Biology: Transgenic Maize. — V. 526. M. Paul Scott (ed.). — Humana Press, 2009. — P. 47–58.

81. *Brettschneider R., Becker D., Lorz H.* Efficient transformation of scutellar tissue of immature maize embryos // Theor. Appl. Genet. — 1997. — V. 69. — P.737–748.

82. *Shou H., Frame B., Whitham S., Wang K.* Assessment of transgenic maize events produced by particle bombardment or *Agrobacterium*-mediated transformation // Mol. Breed. — 2004. — V. 13. — P. 201–208.

83. *Green C. E., Phillips R. L.* Plant regeneration from tissue culture of maize // Crop Sci. — 1975. — V.7. — P. 417–421.

84. *Lu C., Vasil I. K., Ozias-Akins P.* Somatic embryogenesis in *Zea mays* L. // Theor. Appl. Genet. — 1982. — V. 62. — P. 109–112.

85. *Lu C., Vasil V., Vasil I. K.* Improved efficiency of somatic embryogenesis and plant regeneration in tissue culture of maize (*Zea mays* L.) // Ibid. — 1983. — V. 66. — P. 285–289.

86. *Duncan D. R., Williams M. E., Zehr B. E., Widholm J. M.* The production of callus capable of plant regeneration from immature embryos of numerous *Zea mays* L. genotypes // Planta. — 1985. — V. 165. — P. 322–332.

87. *Close K. R., Ludeman L. A.* The effect of auxin-like plant growth regulators and osmotic regulation on induction of somatic embryogenesis from elite maize inbreds // Plant Sci. — 1987. V. 52. — P. 81–87.

88. *Carvalho C. H. S., Bohorova N., Bordallo P. N. et al.* Type II callus production and plant regeneration in tropical maize genotype // Plant Cell. Rep. — 1997. — V. 17. — P. 73–76.

89. *Hiei Y., Ohta S., Komari T., Kumashiro T.* Efficient transformation of rice (*Oryza sativa* L.) mediated by *Agrobacterium* and sequence analysis of the boundaries of the T-DNA // Plant. J. — 1994. — V. 6. — P. 271–282.

90. *Zhao Z. Y., Gu W., Cai T., Pierce D. A.* Methods for *Agrobacterium*-mediated transformation // US Patent 5,981,840. — 2001.

91. *Negrotto D., Jolley M., Beer S. et. al.* The use of phosphomannose-isomerase as a selectable marker to recover transgenic maize plants (*Zea mays* L.) via *Agrobacterium* transformation // Plant Cell Rep. — 2000. — V. 19. — P. 798–803.

92. *Zhao Z. Y., Ranch J.* Transformation of maize via *Agrobacterium tumefaciens* using a binary co-integrate vector system. — Methods in Molecular Biology: Plant Cell Culture Protocols. — V. 318. V. M. Loyola-Vargas, F. Vazquez-Flota (eds.). — Humana Press, 2006. — P. 315–323.

93. *Sidorov V., Gilbertson L., Addae P., Duncan D.* *Agrobacterium*-mediated transformation of seedling-derived maize callus // Plant Cell Rep. — 2006. — V. 25. — P. 320–328.

94. *Sidorov V., Brar G., Foley T., Duncan D.* Transformation of haploid corn and the production of dihaploid transgenic plants. Plant & Animal Genome XIV. January 14–18, 2006. San Diego, Ca. Abstracts. — P. 272.

95. *Armstrong C. L., Duncan D. R., Sidorov V. A.* Method for *Agrobacterium* transformation for dihaploid corn plants // US Patent 07,572,635. — 2009.

96. *Jones T. J.* Maize tissue culture and transformation: the first 20 years. — Molecular

genetic Approaches to Maize Improvement. Biotechnology in Agriculture and Forestry, V. 63. — Springer-Verlag, 2009. — P. 7–22.

97. *Depicker A., Herman L., Jacobs A. et al.* Frequencies of simultaneous transformation with different T-DNAs and their relevance to the *Agrobacterium*/plant cell interaction // Mol. Gen. Genet. — 1985. — V. 201. — P. 477–484.

98. *De Frammond A., Back E., Chilton W. et al.* Two unlinked T-DNAs can transform the same tobacco plant cell and segregate in the F1 generation // Ibid. — 1986. — V. 202. — P. 125–131.

99. *McCormac A. C., Fowler M. R., Chen D. F., Elliott M. C.* Efficient co-transformation of *Nicotiana tabacum* by two independent T-DNAs, the effect of T-DNA size and implications for genetic separation // Trans. Res. — 2001. — V. 10. — P. 143–155.

100. *Zubko E., Scutt C., Meyer P.* Intrachromosomal recombination between attP regions as a tool to remove selectable marker genes from tobacco transgenes // Nature Biotechnol. — 2000. — V. 18. — P. 442–445.

101. *Dale O. C., Ow D. W.* Gene transfer with subsequent removal of the selection gene from the host genome // Proc. Natl. Acad. Sci. USA. — V. 88. — P. 10558–10562.

102. *Zhang W., Subbarao S., Addae P. et al.* Cre/*lox*-mediated marker gene excision in transgenic maize (*Zea mays* L.) plants // Theor. Appl. Genet. — 2003. — V. 107. — P. 1157–1168.

103. *De Vetten N., Wolters A. M.; Raemakers K. et al.* A transformation method for obtaining marker-free plants of a cross-pollinating and vegetatively propagated crop. // Nature Biotechnol. — 2003. — V. 21. — P. 439–442.

104. *Jia H., Liao M., Verbelen J. P., Vissenberg K.* Direct creation of marker-free tobacco plants from agroinfiltrated leaf discs // Plant Cell Rep. — 2007. — V. 26. — P. 1961–1965.

105. *Green C. E.* Somatic embryogenesis and plant regeneration from the friable callus of *Zea mays* / Plant Tissue Culture. A. Fujiwara (ed.). — Maruzen, Tokyo, 1982. — P. 107–108.

106. *Green C. E.* New developments in plant tissue culture and regeneration / Basic Biology of New Developments in Biotechnology / A. Hollaender, A. I. Laskin, P. Rogers (eds.). — Plenum, New York, 1983. — P. 195–209.

107. *Armstrong C. L., Green C. E.* Establishment and maintenance of friable, embryogenic maize callus and the involvement of L-proline // Planta. — 1985. — V. 1985. — P. 207–214.

108. *Armstrong C. L., Green C. E., Phillips R. L.* Development and availability of germplasm with high type II culture formation response // Maize Genet. Coop. Newsl. — 1991. — V. 65. — P. 92–93.

109. *Armstrong C. L., Romero-Severson J., Hodges T.* Improved tissue culture response of an elite maize inbred through backcross breeding, and identification of chromosomal regions important for regeneration by R.F.L.P. analysis // Theor. Appl. Genet. — 1992. — V. 84. — P. 755–762.

110. *Cowen N. M., Johnson C. D., Armstrong K. et al.* Mapping genes controlling in vitro androgenesis in maize using RFLP analysis // Ibid. — 1992. — V. 84. — P. 720–724.

111. *Songstad D. D., Petersen W. L., Armstrong C. L.* Establishment of friable embryogenic (Type II) callus from immature tassels of *Zea mays* (*Poaceae*) // Amer. J. Bot. — 1992. — V. 79, — No. 7. — P. 761–764.

112. *Lowe B. A., May M. M., Rout J. et al.* Molecular assisted breeding for transformability in maize // Mol. Breed. — 2006. — V. 18. — P. 229–239.

113. *Morocz S., Donn G., Nemeth J., Dudits D.* An improved system to obtain fertile regenerants via maize protoplasts isolated from a highly embryogenic suspension culture // Theor. Appl. Genet. — 1990. — V. 80. — P. 721–726.

114. *Huang X-Q., Wie Z-L.* Successful *Agrobacterium*-mediated genetic transformation of maize elite inbred lines // Plant Cell Tissue Organ Cult. — 2005. — V. 83. — P. 187–200.

115. *Golovkin M. V., Abraham S., Morocz S. et al.* Production of transgenic maize plants by direct DNA uptake into embryogenic protoplasts // Plant Sci. — 1993. — V. 90. — P. 41–52.

116. *Jones T. J., Reiter K. L.* The establishment of proliferative shoot meristem cultures from immature embryos of maize // Maize Genetics Conference Abstracts 34 (73), Asilomar. — 1992.

117. *Zhong H., Srinivasan C., Sticklen M. B.* Morphogenesis of corn (*Zea mays* L.) in vitro I. Formation of multiple shoot clumps and somatic embryos from shoot tips // Planta. — 1992. –V. 187. — P. 490–497.

118. *Zhong H., Srinivasan C., Sticklen M. B.* Morphogenesis of corn (*Zea mays* L.) in vitro II. Transdifferentiation of shoots, tassels, and ear primordia from corn shoot tips // Planta. — 1992. — V. 187. — P. 483–489.

119. *Zhang S., Zhong H., Sticklen M. B.* Production of multiple shoots from shoot apical meristems of oat (*Avena sativa* L.) // J. Plant Physiol. — 1996. — V. 148. — P. 667–671.

120. *Zhong H., Wang W., Sticklen M. B.* In vitro morphogenesis of *Sorghum bicolor* (L.) Moench: efficient plant regeneration from shoot apices // Ibid. — 1998. — V. 153. — P. 719–726.

121. *Devi P., Zhong H., Sticklen, M. B.* In vitro morphogenesis of pearl millet (*Pennisetum glaucum.* L.): efficient production of multiple shoots and inflorescences from shoot apices. // Plant Cell Rep. — 2000. — V. 56. — P. 546–550.

122. *Ahmad A., Zhong H., Wang W., Sticklen M. B.* Shoot apical meristem: in vitro regeneration and morphogenesis in wheat (*Triticum aestivum* L.) // In Vitro Cell. Dev. Biol. Plant. — 2002. — V. 38. — P. 163–167.

123. *Bregitzer P., Tonks D.* Inheritance and expression of transgenes in barley // Crop Sci. — 2003. — V. 43. — P. 4–12.

124. *Sticklen M. B., Oraby H. F.* Shoot apical meristem: a sustainable explant for genetic transformation of cereal crops // In Vitro Cell. Dev. Biol. Plant. — 2005. — V. 41. — P. 187–200.

125. *Zhong H., Zhang S., Sun B. et. al.* The competence of maize shoot meristems for integrative transformation and expression of transgenes // Plant Physiol. –1996. — V. 110. — P. 1097–1107.

126. *Zhang S., Williams-Carrier R., Lemaux P.* Transformation of recalcitrant maize elite inbreds using in vitro shoot meristematic cultures induced from germinated seedlings // Plant Cell Rep. — 2002. — V. 21. — P. 263–270.

127. *Fransz P. F., Schel J. H. N.* Cytodifferentiation during the development of friable embryogenic callus of maize (*Zea mays*) // Can. J. Bot. — 1991. — V. 69. — P. 26–33.

128. *Fransz P. F., Schel J. H. N.* An ultrastructural study on the early development of *Zea mays* somatic embryos // Ibid. — 1991. — V. 69. — P. 858–865.

129. *Ozias-Akins P., Vasil I. K.* Plant regeneration from cultured immature embryos and inflorescences of *Triticum aestivum* L. (wheat): Evidence for somatic embryogenesis // Protoplasma. — 1982. — V. 110. — P. 243–250.

130. *Fransz P. F., Schel J. H. N.* Ultrastructural studies on callus development and somatic embryogenesiss in *Zea mays* L. // Biotechnology in Agriculture and Forestry, V. 25. Maize (ed. Y. P. S Bajaj). — Springer-Varlag, Berlin Heidelberg, 1994. — P. 50–65.

131. *Everett N. P., Wach M. Y., Ashwork D. J.* Biochemical markers of embryogenesis in tissue cultures of the maize inbred B73 // Plant Sci. — 1985. — V. 41. — P. 133–140.

132. *Duncan D. R., Kriz A. L., Paiva R. Widholm J. M.* Globulin-1 gene expression in regenerable *Zea mays* (maize) callus // Plant Cell Rep. — 2003. — V. 21. — P. 684–689.

КУЛЬТУРА ТКАНИН РОСЛИН У БІОТЕХНОЛОГІЇ: ОСТАННІ ДОСЯГНЕННЯ В ГАЛУЗІ ТРАНСФОРМАЦІЇ ЗА ДОПОМОГОЮ СОМАТИЧНОГО ЕМБРІОГЕНЕЗУ

В. А. Сидоров

Корп. Монсанто, США

E-mail: vladimir.sidorov@monsanto.com

Генетична трансформація рослин стала важливим інструментом біотехнології для вдосконалення багатьох сільськогосподарських культур. Міцну основу для швидкого розроблення та впровадження біотехнологій у сільському господарстві було закладено досягненнями методу культури тканин рослин. До 30-річчя трансформації рослин у статті описано успіхи, проблеми та останні зміни в методології трансформації. Основну увагу приділено традиційним і новим підходам для генетичного вдосконалення сої, бавовни і кукурудзи. Наведено також результати трансформації цих культур, які були суттєво вдосконалені завдяки здобуткам сучасної біотехнології.

Ключові слова: культура тканин рослин, трансформація, генетичне вдосконалення сільськогосподарських культур.

КУЛЬТУРА ТКАНЕЙ РАСТЕНИЙ В БИОТЕХНОЛОГИИ: ПОСЛЕДНИЕ ДОСТИЖЕНИЯ В ОБЛАСТИ ТРАНСФОРМАЦИИ ПОСРЕДСТВОМ СОМАТИЧЕСКОГО ЭМБРИОГЕНЕЗА

В. А. Сидоров

Корп. Монсанто, США

E-mail: vladimir.sidorov@monsanto.com

Генетическая трансформация растений стала важным инструментом биотехнологии для усовершенствования многих сельскохозяйственных культур. Прочная основа для быстрой разработки и внедрения биотехнологий в сельском хозяйстве была заложена достижениями метода культуры тканей растений. К 30-летию трансформации растений в статье описаны успехи, проблемы и последние изменения в методологии трансформации. Основное внимание уделено традиционным и новым подходам для генетического совершенствования сои, хлопка и кукурузы. Представлены также результаты трансформации этих культур, которые были значительно усовершенствованы благодаря достижениям современной биотехнологии.

Ключевые слова: культура тканей растений, трансформация, генетическое усовершенствование сельскохозяйственных культур.