Cultivos Tropicales, Vol. 46, No. 1, January-March 2025, e-ISSN: 1819-4087, p-ISSN: 0258-5936, https://ediciones.inca.edu.cu

Cu-ID: https://cu-id.com/2050/v46n1e12

Bibliographic review

The tissue culture and genetic transformation in *Gossypium* spp

El cultivo de tejidos y la transformación genética en *Gossypium* spp

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ABSTRACT: The cotton plant is cultivated mainly by the fiber, the oil that is extracted of the seed that can be used like eatable oil and the use of the cotton cake like forage. This plant is resistant to conditions of drought and salinity of the soil. However, it possesses some characters that limit their productivity. It is for it that requires of programs of plant breeding, but the programs for traditional methods are limited by several factors in this cultivation, for what the biotechnical techniques constitute alternatives to achieve these objectives. In the work was carried out a brief revision of the national and international scientific literature about the origin, the distribution and the cultivation importance, as well as the antecedents of the regeneration of plants and the methods of breeding, by means of the genetic transformation, in the cotton cultivation. It seeks to put on to the reader's disposition a summary of results as preamble for the development of future investigations in the regeneration of plants and breeding genetics of *Gossypium* spp. for biotechnical methods.

Key words: cotton, plant breeding, biotechnology, *in vitro*, *Agrobacterium*.

RESUMEN: La planta de algodón se cultiva principalmente por la fibra, el aceite que se extrae de la semilla que puede utilizarse como aceite comestible y el aprovechamiento de la torta de algodón como forraje. Esta planta es resistente a condiciones de sequía y salinidad del suelo. Sin embargo, posee algunos caracteres que limitan su productividad. Es por ello que requiere de programas de mejoramiento genético, pero los programas por métodos tradicionales están limitados por varios factores en este cultivo, por lo que las técnicas biotecnológicas constituyen alternativas para lograr los objetivos de la mejora. En el trabajo se realizó una breve revisión de la literatura científica nacional e internacional sobre el origen, la distribución e importancia del cultivo, así como los antecedentes de la regeneración de plantas y los métodos de mejoramiento, mediante la transformación genética, en el cultivo del algodón. Se pone a disposición del lector un compendio de resultados como preámbulo para el desarrollo de futuras investigaciones en la regeneración de plantas y mejora genética de *Gossypium* spp. por métodos biotecnológicos.

Palabras clave: algodón, mejora genética, biotecnología, *in vitro*, *Agrobacterium*.

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Received: 07/08/2024

Accepted: 09/12/2024

Conflict of interest: Authors declare that they have no conflict of interest.

Authors' contributions: Conceptualization- María Esther González Vega, Silvio de Jesús Martínez Medina, Luke Leroy Theodore Nedd. **Information Recording and Processing-** Silvio de Jesús Martínez Medina Luke Leroy Theodore Nedd. **Organization of Writing by Topics-** María Esther González Vega, Silvio de Jesús Martínez Medina, Luke Leroy Theodore Nedd. **Writing the Document-** Luke Leroy Theodore Nedd. **Review and Revision of the Initial Draft-** Silvio de Jesús Martínez Medina, María Esther González Vega, Luke Leroy Theodore Nedd. **Review and Revision of Second Draft-** María Esther González Vega, Luke Leroy Theodore Nedd. **Review and Approval of the last draft-** María Esther González Vega, Silvio de Jesús Martínez Medina, Luke Leroy Theodore Nedd.

INTRODUCTION

Cotton (*Gossypium* spp.) is one of the most widely grown oilseeds worldwide for oil extraction, with a total of 32.26 million tons of oil produced in 2020 (1). The cotton plant is grown mainly for its fiber, the oil extracted from the seed that can be used as edible oil, and the use of cotton cake as fodder. The seed hull can be used as raw fodder and bedding for livestock, as fertilizer or fuel (2). This plant is resistant to drought conditions and soil salinity; however, it has some unfavorable characteristics such as the long vegetative period, the fiber is thick and of short length, in addition to susceptibility to soil pathogens such as *Rhizoctonia solani*, *Pythium* spp., *Fusarium* and *Thielaviopsis basicola*, which cause seedling drop (3).

In view of this problem, it is necessary to develop genetic breeding programs for this crop. Although it should be noted that in cotton, the use of traditional methods of genetic breeding is limited by several factors; among them, the complexity of the character to be breeded, the influence of the environment and the long periods of selection, aspects that make them significantly more expensive (4). The use of biotechnological methods constitutes an alternative for the development of these crop breeding programs, although in order to establish biotechnological breeding methods, it is essential to have an efficient and reproducible plant regeneration methodology (5).

This paper provides a brief introduction to the origin, distribution and importance of cotton cultivation, the background for *in vitro* plant regeneration and the methods of genetic breeding in this species, with emphasis on biotechnological techniques. It is intended to make available to the scientific community a compilation of results as an introduction for the development of future research in the propagation and genetic breeding of *Gossypium* spp. through the application of biotechnological methods.

DEVELOPMENT

Origin, taxonomic classification and distribution

The cotton genus (*Gossypium* spp.) includes approximately 50 species distributed in arid and semi-arid regions of the tropics and subtropics. It includes four species that have been independently domesticated for their fiber, two in Africa, and one in Asia and another in America (6).

Its taxonomic classification is as follows (7): Kingdom: Plantae, Division: Tracheophyta, Class: Magnoliopsida, Order: Malvales, Family: Malvaceae, Genus: *Gossypium*.

This parallel domestication process involved four species, two from America: *G. hirsutum* and *G. barbadense*, and two from Africa and Asia: *G. alborean* and *G. herbaceum* (7). There are different criteria on the origin of cotton. The discussion on the centers of origin, according to recent studies, states that it has not been determined. However, it has been described that the primary centers of biological diversity for this genus are Central and South America with 18 species, among which 11 are found in west-central and southeastern Mexico and two in Peru and the Galapagos Islands (one species from Hawaii). In Northeast Africa and Arabia 14 species are identified, while in Australia 17 species (8).

Importance of cultivation

Cotton is grown in more than 75 countries on five continents, generating income and rural employment, which is a vital source of income for the economy of rural households and a contribution to the food security of family farming. The cotton value chain generates agricultural products, fibers, textiles and industrial and handcrafted garments, as well as the use of co-products as business opportunities for food, health, cosmetics, among others, which boosts national and regional economies (9).

At the end of the 2020/2021 season, world cotton demand totaled 25.5 million tons, an increase of 12.4 %. In the 2021/2022 season, world cotton production reached 25 million tons, an increase of 3 % (10).

In vitro plant regeneration methods

Modern biotechnology involves the establishment of tissue culture and gene transfer systems, which guarantees the obtaining of specific desired characteristics, which contributes to the breeding of the crop in question and implies the ability to regenerate a large number of plants (11). The main ways of *in vitro* plant regeneration in cotton are: organogenesis (12) and somatic embryogenesis (13).

Organogenesis

Organogenesis is the formation of organs (leaves, stem, and roots) from buds or primordia developed on the surface of callus or explants; generally, it starts with the formation of leaf and stem, and continues with the formation of roots (14). Organogenesis involves the formation of monopolar structures that establish a vascular connection with the tissue from which they derive; their origin is multicellular. Normally, stems and roots are formed independently and are characterized by the lack of union between vascular elements of both structures (15).

Organogenesis involves the regeneration of shoots directly from meristematic cells or from tissues close to them. Shoots can arise from explant tissues with or without the callus phase. Moreover, it is less genotype-dependent compared to somatic embryogenesis, as each plant has axillary meristems capable of regeneration. However, the response is different among cultivars, which depends on the vigor, level of growth of explants *in vitro* and sensitivity to the components of the culture medium (16).

In this sense, and during plant regeneration via organogenesis in three cotton cultivars of *Gossypium hirsutum*, and using apices as initial explant, the highest range of shoot elongation was observed with 11.1 µM 6-benzylaminopurine (6-BAP) and 0.1 % (w/v) activated carbon, as well as an increase in growth in the presence of Kinetin (kin). In addition, high rooting efficiency was obtained with 0.98 µM Indolbutyric acid (IBA) and activated charcoal (17).

Somatic embryogenesis

Somatic embryogenesis is a biotechnological technique that makes it possible to obtain embryos from plant somatic cells without the union of gametes. This technique and plant tissue culture, in general, are based on the principle of cell totipotency (18), which consists in the fact that all plant cells have the capacity to regenerate complete plants.

There are two types of somatic embryogenesis: direct and indirect. The direct route involves the formation of embryos directly from a segment of the explant without prior callus formation. The indirect pathway occurs through an intermediate stage of callus formation (19) and is observed more frequently than direct somatic embryogenesis (20). In both cases, for monocotyledonous plants, somatic embryos pass through stages similar to those observed in a zygotic embryo: globular, heart-shaped, torpedo, cotyledon and mature embryo (21).

In 1979, the first studies on somatic embryogenesis in *Gossypium koltzchianum* were reported, but without obtaining complete plants (22). Subsequently, and since the first half of the 1980s, some methods of plant regeneration by this route have been developed in *Gossypium* spp. in this sense, for the species *G. hirsutum* L. cv. Coker, plant regeneration by somatic embryogenesis was described for the first time in 1983 (23). Since then, significant progress has been made in the use of this regeneration pathway in cotton tissue culture (24). Similar to other cultures, the phases of induction and formation, proliferation, maturation, germination and conversion of somatic embryos are very well defined (25).

The induction of the process consists of the pattern termination of the expression of genes present in the explant tissue; and it is replaced by an expression program of the gene or genes in the cells of the cultured tissue, which can give rise to somatic embryos (26). These cells depend on different factors to achieve a high frequency of callus formation, among them, the genotype, the type of donor plant, the age or developmental stage of the explant, the *in vitro* environment that includes the composition of the culture medium and the physical conditions (light, temperature, relative humidity) (27). There are also references on the influence of the type of explant in the formation of callus, whether leaves, petioles, roots, seeds, cotyledons, meristems, zygotic embryos, among others (28).

Factors influencing the development of somatic embryogenesis in cotton

Genotype

The regeneration capacity of plants shows wide differences among families, genera, species, and even among genotypes of the same species. Generally, dicotyledonous plants regenerate more easily than monocotyledonous plants. The regeneration capacity in the family Malvaceae, and particularly in the genus *Gossypium*, is considered very low (29). Plant regeneration and genetic transformation of cotton by genetic engineering techniques is closely associated with genotype, and most protocols have been adjusted for model varieties. However, many of the elite varieties of this crop are recalcitrant and do not respond favorably to genetic manipulation (17). Also, differences have been observed in the regeneration and *in vitro* propagation ability of cotton plants from various cultivars of *G. hirsutum* species (30). These aspects corroborate the need to study each genotype and to adjust or optimize plant regeneration protocols.

Explant

There is evidence that all tissues have the capacity to form callus *in vitro*, although not all are embryogenic. Embryonic tissues and very young tissues are the ones that have an active embryogenic response. An important aspect to consider for the establishment of an efficient plant regeneration protocol is the type of initial explant (31). In cotton cultivation, the use of different types of explants for the development of somatic embryogenesis has been described. Among the most commonly used are those from sexual reproduction such as ovaries, ovules, zygotic embryos, anthers, as well as roots, leaves, segments of young seedlings (cotyledons, hypocotyls) (32), and stem segments from *in vitro* germinated seed plants (3).

In addition, the use of young leaf segments of *Gossypium barbadense* L. cultivar 'MSI', from seedlings grown under *in vitro* conditions, as initial explant for callus formation was recently reported (33). However, this type of explant has been successfully used for the development of plant regeneration protocols in species such as *Secale cereale* L. (34), *Handroanthus heptaphyllus* (35) and *Lavandula angustifolia* L. (36).

Oxidation of phenolic compounds

One of the factors that frequently affects the isolation of explants in *in vitro* culture is the oxidation of phenolic compounds released by damaged cells during the process of dissection of the explant source organs. Some practices to counteract this effect include the use of antioxidant substances such as ascorbic acid, citric acid, cysteine, activated charcoal, among others, or mixtures of some of these compounds (37).

Ascorbic acid is one of the most widely used antioxidants in plant tissue culture, it acts as a redox buffer in plants and has an important role in their metabolism. This acid is a cofactor of enzymes and is involved in several physiological processes in plants, including cell division, cell wall metabolism and cell expansion, apical meristem formation, root development, photosynthesis, regulation of flowering and leaf senescence (38).

In the national and international scientific literature, there are few references to the use of antioxidants to control the oxidation of phenolic compounds in cotton cultivation. The authors of a study carried out on the cotton cultivar 'MSI' observed that, during the formation of callus with embryogenic structures, these compounds were formed; this was controlled with the addition to the culture medium of 60 mg L^{-1} of ascorbic acid. The addition of this antioxidant

to the medium, in the presence of 2,4-dichlorophenoxyacetic acid (2,4-D), contributed to the formation of a greater number of total callus and embryogenic callus (33).

Culture medium

The culture medium composed of Murashige-Skoog (MS) salts (39) is the most commonly used medium for somatic embryogenesis in dicotyledonous species (40). In general, and particularly in cotton, several modifications have been made to this culture medium for plant regeneration by this morphogenetic route. In this sense, the MS medium has been modified with the addition of Gamborg B5 vitamins. Evidence of the above are the favorable results during callus formation, using MS culture medium, in different cultivars of *Gossypium* spp. (41). Also, callus formation has been successfully achieved in this genus when using MSB culture medium (MS salts plus Gamborg B5 vitamins) (40-43).

Growth Regulators

Plant growth regulators are compounds that have a regulatory rather than a nutritional role in the growth and development of plant tissues. In the callus formation process, the growth regulator used plays a fundamental role (44). 2,4-D is one of the most commonly used in tissue culture for these purposes (45). The regeneration potential is also influenced by the presence of the regulatory compounds in the culture medium. For this reason, various types and concentrations of growth regulators are used in protocols for plant regeneration.

With respect to cotton, there are different criteria on the concentration of 2,4-D to be used in the callus formation process. This auxin has been very useful for the induction of callus with embryogenic structures, from leaves and cotyledons, in *G. arboreum* cultivars 'BD-I' and 'BD-6', as well as in *G. hirsutum* cultivars 'SH-131' and 'LH-900' (46). In these cultivars, 2,4-D was also found to be effective in inducing callus from explants of different provenance (47).

The combination of auxins and cytokinins, in cotton cultivation, stimulates the formation of callus with embryogenic structures, thus, for example, it has been pointed out that the incorporation of 2,4-D and kinetin in the culture medium induces the development and proliferation of somatic embryos. Some authors report that the formation of callus, with embryogenic structures, was achieved when using concentrations of 9.04 µM of 2,4-D combined with 0.46 µM of kin. Similarly, callus formation has been reported in cultivars of this genus with the addition of only 4.52 µM 2,4-D and 2.32 µM kin to the medium (41,42,47). Induction of somatic embryos has been obtained by transferring embryogenic callus to growth regulator-free culture medium and subsequent subculture on MS medium enriched with 1.9 g L^{-1} KNO_3 (42).

There are references on obtaining friable and green callus in *Gossypium klotzschianum*, from hypocotyls cultivated in medium with 0.9 µM 2,4-D and 2.32 µM kin (48). Likewise, embryo development and proliferation have been achieved with 0.045 µM 2,4-D; 0.93 µM kin and 2.46 µM AIB. On the other hand, embryo differentiation has also been achieved in liquid medium with 0.226 µM 2,4-D and 0.93 µM kin, observing the different stages of the somatic embryo: globular, heart, torpedo and mature cotyledonary embryo (48).

In this sense, a protocol was developed for somatic embryogenesis and plant regeneration of five recalcitrant cultivars of cotton (*G. hirsutum*), allowing to expand the range of genotypes manipulated *in vitro* for genetic breeding, and the greatest formation of somatic embryos was achieved by combining AIB (0.49 µM), kin (0.46 µM) and 2,4-D (0.45 µM) (49).

In other protocols developed for the regeneration of cotton plants of *G. hirsutum* and *G. barbadense* species, in the presence of 10.74 µM 2,4-D and 4.64 µM kin, callus with pre-embryogenic structures were formed (50). The authors observed the formation of friable callus, with small cells and very dense cytoplasm, when transferred to maturation culture medium. Also, high percentages of callus formation have been achieved in *G. hirsutum* with 5.37 µM naphthalene acetic acid (NAA) in combination with 0.46 µM Kin or 0.44 µM 6-BAP, callus formed with the addition of kinetin were characterized by compactness and the presence of a large number of roots (51).

These plant growth regulators have been very useful for callus induction in cotton from cotyledons of *G. arboreum* cultivars 'BD-I' and 'BD-6', as well as *G. hirsutums* cultivars 'SH-131' and 'LH-900', achieving callus formation with concentrations of 9.04 µM 2,4-D combined with 0.464 µM kin (46).

Other authors highlight callus formation when using two combinations of growth regulators: 0.90 µM of 2,4-D + 0.89 µM of 6-BAP and 4.92 µM of 2,4-D + 0.89 µM of 6-BAP, under dark and light conditions, obtaining the highest callus proliferation in the presence of light and with a higher concentration of 2,4-D, in the presence of 6-BAP (52). On the other hand, the formation of a granular, partially friable and light brown callus was obtained in the presence of 2-isopentyladenine (2iP) and 2,4-D; it was observed that, as the concentrations of these regulators increased, the callus presented a dark brown color and necrotic portions (53).

Some authors have reported 0.45 µM 2,4-D as the most effective concentration for embryogenic callus formation in cotton (4). In *G. barbadense* “native cotton”, the highest induction of embryogenic callus (82.5 %) was obtained with 0.45 µM 2,4-D and 100 mL L^{-1} of coconut water (3). Other authors found with 11.31 and 13.58 µM of 2,4-D and the addition of 60 mg L^{-1} of ascorbic acid the highest percentages of total callus formation and with embryogenic appearance, with 88.05 and 83.50 %, respectively (35). These callus were characterized by being compact, bright yellow, very dense, compatible with isodiametric cells. The results show that 2,4-D is necessary for callus formation, at least for the explants mentioned above, because in the culture medium without this growth regulator, callogenesis cannot be induced (33).

Growing condition

Light is one of the environmental factors necessary in the processes of photosynthesis and photomorphogenesis,

which are facilitated by pigments present in the tissues that absorb radiation of certain wavelengths (3). Photosynthesis carried out in most plant tissues grown *in vitro* is relatively low, so the cultures depend on an external source of sucrose. In these circumstances, light is important for its effect on photomorphogenesis, because it induces the rapid change in gene expression that leads to the normal pattern of development (20).

In *Gossypium* spp. embryogenic callus formation and induction have been achieved under both continuous dark and photoperiod conditions, the latter being the most common. For example, in some investigations on this crop, callus formation and proliferation was initiated with 16 h light/8 h dark photoperiod (3,55,56), while in others it has been achieved in continuous darkness (57).

Undefined organic supplements

Organic substances of undefined chemical nature are used in the callus formation of different plant species. Among the organic substances are coconut water, hydrolyzed casein and yeast extract. Coconut water is the liquid endosperm of the coconut fruit, and its components include amino acids, organic acids, nucleic acids, purines, sugars, polyols, vitamins, minerals and growth regulators, the concentrations of which can vary (20).

Some authors have evaluated the use of coconut water in callus induction, in combination or absence of other supplements. For example, callus was obtained from ovules of *Gossypium hirsutum* and *G. barbadense*, using MS medium enriched with 100 - 120 mL of coconut water, 1 g L^{-1} of hydrolyzed casein, 1 g L^{-1} of yeast extract and different concentrations of growth phyto-regulators (58).

The highest proliferation of friable callus (82.5 %) was found in *Gossypium barbadense* L. “native cotton” brown color, from explants obtained from seed plant segments germinated *in vitro* and grown in MS medium enriched with 0.1 mg L^{-1} of 2,4-D and 100 mL L^{-1} of coconut water and incubated under a photoperiod of 16 h light/ 8 h dark (3).

In vitro regeneration of cotton plants is difficult because, among other things, the morphogenetic response is genotype dependent. Somatic embryogenesis is the most commonly used method, because the regenerated plants are of unicellular origin and there is no vascular connection between the somatic embryo and the maternal tissue (17).

Genetic transformation methods

Different methods have been developed to introduce foreign genes into plants. A common characteristic is that the transforming deoxyribonucleic acid (transforming DNA) has to overcome different barriers; first, it has to enter the plant cell, crossing the cell wall and the plasma membrane; subsequently, it has to reach the nucleus and integrate into the resident chromosomes. For most species, gene transfer is carried out using explants that are competent for regeneration, thus facilitating the procurement of complete fertile plants. This involves the use of tissue culture technology. Although gene transfer technology has become routine in several species, in others the limiting step is not transformation per se, but the absence of efficient regeneration protocols (59). Transformation methods can be divided into two main categories: direct and indirect transformations, which are detailed in the following sections (60).

Indirect transformation

In these methods, plants are transformed using *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* to introduce the plasmid construct carrying the target gene into the target cell (60).

Direct transformation

Direct transformation methods do not use bacterial cells. The most commonly used direct methods include microprojectile bombardment or protoplast transformation. Problems with regeneration of transiently low transgene expression plants arise as a result of protoplast transformation, mainly in monocotyledonous plants. Among the transformation techniques used in cotton are: silicon carbide fiber-mediated transformation, microinjection, infiltration, embryo electrophoresis, and transformation via the pollen tube pathway, cell and tissue electroporation, and liposome-mediated transformation (60).

Commonly used processing techniques in cotton

Among the most commonly used transformation techniques in cotton cultivation are Agrobacterium-mediated transformation and biobalistic transformation.

Agrobacterium-mediated transformation

In 1907, some authors demonstrated that the soil bacterium *Agrobacterium tumefaciens*, a member of the Rhizobiaceae family, produced crown gall tumors (61). Furthermore, they noted that the formation of these tumors occurred as a result of Bactrian infection, usually at damaged sites, in dicotyledonous and some monocotyledonous plants (62). This discovery did not have major repercussions until Armin Braun demonstrated that tumor cells were transformed and that the uncontrolled proliferation of these cells was not dependent on the continued presence of *Agrobacterium*, implying the presence of a principle of induction of transformation (63).

This *Agrobacterium* system has several advantages over other transformation systems and is considered the first choice for transforming plants. Among the advantages are that the DNA segments, which are integrated into plant cells, are in a single copy in a significant percentage of transformation events (64); numerous vectors containing the T-DNA borders and a variety of reporter and selection genes are now available, allowing researchers to choose the most appropriate combination for inserting heterologous genes and selecting transformed cells in their study model; large DNA fragments including yeast artificial chromosomes can be transferred (65), and direct transformation in plants without the need for tissue culture is possible in *Arabidopsis thaliana* and *Medicago trunculata* species (66).

Agrobacterium-mediated transformation of cotton was first reported a decade ago with hypocotyl and cotyledon as explants (67). Several useful genes have been introduced into cotton by *Agrobacterium*-mediated transformation, including genes with insect and herbicide resistance (68). Explants such as hypocotyl, cotyledon, and callus generated from hypocotyl and cotyledon, as well as immature embryos, have been used for transformation by *Agrobacterium* (69-71). However, transformation rates were generally low, ranging from 20 to 30 % when the hypocotyl was used as explant (72,73).

The validity of octopine as a marker of transformation is questionable as octopine has been found in several plant species certainly not transformed by infection with *A. tumefaciens* (74). A more recent report indicated that the transformation efficiency of cotyledon was approximately 20 to 30 % (75). The transformation efficiency was even lower when the particle bombardment procedure was used (76). A difference in the type of explants used for transformation could have had a significant effect on transformation and regeneration efficiency.

Cotton transformation is highly genotype dependent (68). Besides a few cultivars that are regenerable and transformable, such as *Gossypium hirsutum* cv. Coker 312 and *G. hirsutum* Jin 7, most of the other important elite commercial cultivars, such as *G. hirsutum* cv. D&P 5415, are not regenerable and transformable by these procedures.

Agrobacterium-mediated transformation followed by somatic embryogenesis is the best method for cotton transformation. However, the regeneration aspect of the transformation process remains more difficult and options are limited in the case of cotton, one of the most difficult crops for transformation. The fact that *Agrobacterium* is highly attracted to phenolic compounds, this transformation method is not preferable in monocots due to phenolic production, whereas it can be used for dicots (77,78).

The efficiency of transformation via *Agrobacterium tumefaciens* is closely related to the strain used for infection. In 1989, a markedly increased transformation of cotton shank tips was described by simultaneous addition of acetosyringone and nopaline at the time of infection (79). Unfortunately, this system has several drawbacks. One is that the host range is more limited than in *A. tumefaciens* and another is that expression of T-DNA genes in plants confers aberrant phenotypes on the plants. To solve this type of problem, the B-role gene has been introduced into a binary vector of *A. tumefaciens* (80).

In this regard, an additional problem arises from the fact that all *Agrobacterium*-mediated cotton transformation methods require regeneration of an embryogenic callus from the explant containing the transformed cells as an intermediate operation in the regeneration of transgenic cotton plants. The absence of a highly efficient plant regeneration procedure has been considered the major obstacle to the application of *Agrobacterium*-mediated transformation to cotton (71).

Transformation by Biobalistics

The bioballistics method was developed as a necessity to transform plant species originally recalcitrant to transformation by the *Agrobacterium* system, including economically important cereals. This method consists of the introduction of projectiles, usually of tungsten or gold coated with DNA and propelled into the target cells by acceleration. The particle velocity can be generated by the explosion of a conventional gun or a discharge by high-pressure gases, such as helium or carbon dioxide (81,82). Molecular analysis of bioballistics-transformed plants reveals a complex pattern of transgene integration; however, it has been shown that multiple copies are arranged in a single locus and segregate in a Mendelian pattern (83). As with *Agrobacterium*, a large number of diverse plant species have been transformed by the bioballistics method (84). Some advantages of the bioballistics method are the following: 1) A wide variety of explant types can be used to bombard and obtain fertile plants; 2) There is no need to use specialized transformation vectors; and 3) It is the only reliable method for chloroplast transformation.

Bioballistics allows the integration of multiple copies of transgenes in the genome of transformed plants, and there are references of up to 100 copies of a transgene (85). Explants (such as hypocotyl, cotyledon, callus generated from hypocotyl and cotyledon, as well as immature embryos) have been used in cotton culture for transformation by particle bombardment (8,70,71). In addition, meristematic tissue from cleaved embryonic axes has been used for the transformation of cotton by particle bombardment (86).

In cotton, procedures have been used for the regeneration of genetically modified plants after transformation by bombardment of cotyledons (71). There are references of successful use of DNA bombardment on cotton embryonic apices (86).

In general, genetic transformation techniques of cotton by somatic embryogenesis involve processes between 10 and 14 months, while transformation of meristematic apices and microinjection of ovules involve processes between 6 and 10 months. Transformation efficiencies in cotton may vary, depending on the technique and operational capacity, but it is always around 0.1 % (87).

Genetic breeding of cotton through genetic transformation

Genetic engineering emerged in 1973 and the first transgenic plant was developed in 1983. All the knowledge emanated through the history of the green revolution, based on biotechnology, soon led to the modern biotechnology revolution prevailing in the development of plants of agricultural crops of interest, generating new varieties carrying certain genes; even those that do not even belong to plants, but to organisms from another kingdom. In 1996, the first commercial transgenic cotton line was developed: Bollgardi® cotton, to which the Cry1ac gene was transferred from the bacterium *Bacillus thuringiensis*; this gene gives the cotton plant resistance to attack by insects of the lepidopteran order, since it expresses a protein that is toxic to these insects, causing their death (88).

In 1997, cotton resistant to glyphosate herbicide appeared, since the cp4-epsps gene was transferred to the cells of the cotton plant, coming from the *Agrobacterium* spp. bacterium, strain cp4, which expresses a key enzyme in the synthesis of aromatic amino acids that plants, bacteria and some fungi possess. This enzyme destroys the active ingredient of glyphosate (N-phosphonomethyl-glycine); however, the EPSPS enzyme of the isolated bacterium is resistant to glyphosate, so the plant is not damaged.

In 2000, a transgenic cotton line resistant to the herbicide glufosinate ammonium was obtained, to which the pat gene, isolated from the *Streptomyces viridochromogenes* bacterium, was transferred; this gene expresses an enzyme that transforms the active ingredient of the herbicide into a non-toxic substance. Subsequently, in 2002, transgenic cotton seeds were developed and commercialized with dual technology, resistant to lepidopteran insects, due to the cry1Ac gene, and tolerant to the herbicide glyphosate, thanks to the cp4-epsps gene (89).

The lack of knowledge about the safety and behavior of transgenic plants soon led to the collapse of the biotechnology transferred to cotton, so that insects of the lepidopteran order resistant to the toxic protein expressed by the Cry1ac gene appeared; therefore, in 2003, Bollgardi® cotton emerged, which is a variant of Bollgardi®. In addition to expressing the Cry1ac gene, to which some insects had already developed resistance, the Cry1ab gene, also isolated from the bacterium *Bacillus thuringiensis* (Bt), was transferred to it, in order to confront the insects with a new toxin, so that there should be no resistance. Along with this new technology came a campaign for the implementation of refuges, in order to prevent the emergence of tolerant insects, as had happened in the past intolerant insects, as happened with Bollgardi® cotton (90).

In 2006, a dual technology transgenic cotton was commercialized, resistant to lepidopteran insects based on Bollgardi® technology and also tolerant to glyphosate herbicide, known as Bt-rr cotton, where Bt (*Bacillus thuringiensis*) refers to the genes that make it resistant to insects (Cry1ac and Cry2ab), and rr refers to its tolerance to glyphosate, specifically to the Roundup Ready® brand (90).

Transgenic cotton has continued to undergo transformations, as there are transgenic varieties tolerant to herbicides other than glyphosate and with other properties. Currently, new knowledge and new technologies are being generated, so that in the coming years modifications will be made in various ways, some perhaps more precise, which will provide new solutions to the crop's problems.

The development of non-sexual methods for gene transfer, such as genetic transformation, makes it possible to overcome the limitations of traditional genetic breeding, opening up new perspectives in plant breeding (91). Genetically modified cotton is the third most widely grown transgenic crop in the world. It currently occupies 70 % of the world's cotton area, with insect-resistant Bt varieties being the most widely cultivated. Among the countries that grow the most Bt cotton are China, India, Pakistan, South Africa and Burkina Faso, among others. In these countries, more than 15 million small farmers enjoy its economic, social and environmental benefits (92).

According to the International Cotton Advisory Committee - ICAC, world production amounted to 25.5 million in the 2021-2022 harvest, with a 12.4 % increase in demand (93). Transgenic cotton occupied third place (14 %) of the crops produced by this method in the world, behind soybean (48 %) and corn (32 %). GM cotton currently in production and trade has been modified to be herbicide tolerant, insect resistant or a combination of both (94).

By using genes of interest, genetically modified cotton has made crops more economically and environmentally sustainable (17). *Bacillus thuringiensis* is a very common bacterium found in the soil and can produce a protein, Cry d-endotoxin, which is toxic to larvae of certain insects, for example, moths such as cotton bollworms that attack this crop, and its action is specific to control these insects (95). Of the transgenics currently available for commercial production, two offer tolerance to herbicides and one is resistant to cotton bollworms, known as Bt cotton, because it expresses toxins from the bacterium *Bacillus thuringiensis* (96).

There are cotton cultivars with insect resistance capacity, which is used to control the bollworm and pink bollworm; it also exerts some control over other bollworms such as the so-called soldier bollworm and false bollworm; it is also resistant to the herbicides glyphosate and glufosinate, being useful for the farmer to control weeds without affecting the plants of this crop (95).

CONCLUSIONS

- In cotton cultivation, cultivars are shown to be genotype-dependent given their differentiated response to *in vitro* culture conditions and genetic transformation.
- Although transgenic plants have been produced worldwide in cotton, the existing transformation and regeneration methodologies present low efficiency and frequent obtaining of chimeric plants and are specific for cultivars that express a better embryogenic response.
- Among the regeneration methods, somatic embryogenesis is more used than organogenesis, because the regenerated plants have a unicellular origin and there is no vascular connection between the somatic embryo and the maternal tissue, in addition, they offer higher multiplication coefficients.
- In cotton there are several techniques for genetic transformation, but the most used in cultivation are *Agrobacterium*-mediated transformation and transformation by bioballistics; their effectiveness depends on the type of tissue used, age, genotype and susceptibility to infection with the bacterium.
- Transformation techniques using *A. tumefaciens* are characterized by being simple, low cost, result in few copies of the transgenes, and have reduced expression problems. However, although cotton is a dicotyledonous plant and host of *Agrobacterium*, it presents difficulties for transformation by means of this vector, and genetic transformation of somatic embryos is limited.

ACKNOWLEDGE

To the colleagues of the Laboratory of Phytogenetic Resources of the Agricultural Research Center attached to the Faculty of Agricultural Sciences of the Central University "Marta Abreu de Las Villas", for all the support in the search of scientific literature for the elaboration of this document. Ivonny Hernández Chaviano, director of the Provincial Seed Testing Laboratory of the Ministry of Agriculture in Villa Clara province, for her unconditional support during the literature search.

BIBLIOGRAPHY

1. National Cooperative Dairy Federation of India Ltd. (NCDFI), Cottonseed Oil Cake. 2020; 15-21p.
2. SAGARPA. Análisis de la cadena de valor en la producción de algodón en México , Secretaría de Agricultura, Desarrollo Rural, Pesca y Alimentación, Organización de las Naciones Unidas para la Alimentación y la Agricultura, Ciudad de México, 2014, 71 p.
3. Teruya MS. Evaluación de fitorreguladores del crecimiento en la inducción de callo embriogénico en *Gossypium barbadense* L. 1753 "algodón nativo" color pardo. Tesis para optar el Título Profesional de Licenciada en Biología. Facultad de Ciencias Biológicas, Escuela Profesional De Biología, Universidad Ricardo PALMA, Lima, Perú. 201; 69 p.
4. Martínez SJ, Rafael Gómez- Kosky R, Saucedo O. El sorgo: su cultivo y mejora en Cuba. Editorial Académica Española. 2014; 100 p. ISBN: 978-3-8473-6942-4.
5. Martínez SJ. Regeneración de plantas de sorgo granífero [*Sorghum bicolor* (L.) Moench] cultivar 'CIAP 132R-05' vía embriogénesis somática. Tesis presentada en opción al grado científico de Doctor en Ciencias Agrícolas. Universidad Central "Marta Abreu" de Las Villas. 2018; 101 p.
6. Conabio. (2005). Sistema de Información de Organismos Vivos Modificados. Recuperado el 10 de Abril de 2011, de Bioseguridad.
7. Roskov Y, Abucay L, Orrell T, Nicolson D, Flann C, Bailly N, Kirk P, Bourgoin T, DeWalt R.E., Decock W, De Wever A, eds. Species 2000 & ITIS Catalogue of Life, 2016; Annual Checklist.
8. Pérez M. Documento base de la especie *Gossypium hirsutum* L. para el análisis de riesgo ambiental. Distrito Federal, México: Instituto Nacional de Ecología. 2012.
9. FAO. Día Mundial del Algodón 2021 – Conmemoración Latinoamérica y África. 2021. Consultado.6 de junio del 2022 en: https://www.fao.org/in-action/program-brazil-fao/news/ver/fr/c/1441913/
10. FAO. Producción mundial de algodón. 2021. Consultado.6 de junio del 2022 en: https://www.icac.org/Publications/Details?publicationId=81
11. Naz S, Ali A, Siddique F.A. and Iqbal J. Multiple shoot formation from different explants of chick pea (*Cicer arietinum* L.), Pak. J. Bot. 2007; 39(6): 2067-2073 p.
12. Zapata C, Srivatanakul M, Park S.H., Lee B.M., Salas M.G., and Smith R.H. Improvements in shoot apex regeneration of two fiber crops: cotton and kenaf, Plant Cell Tissue Org. Cult. 1999; (12):43-50.
13. Firoozabady E, DeBoer D.L. Plant regeneration via somatic embryogenesis in many cultivars of cotton (*Gossypium hirstum* L.). *In vitro* Cell. Dev. Biol. Plant. 1993; 9:166-173.
14. Thorpe, T.A. Morphogenesis and regeneration in tissue culture. In: Genetic Engineering. Application to agriculture. Beltsville Symposia in Agricultural Research (L.D. Owens, ed.).1983; 285-303 p. Rowman and Allanheld, Publishers
15. Segura J. Morfogénesis *in vitro*. En: Fisiología y Bioquímica Vegetal (J. Azcón-Bieto, M. Talón, eds) .1993; 381-392 p. Interamericana-McGraw Hill, Madrid.
16. Olhoft P.M., Somers D.A. Soybean. En: EC Pua, M.R. Davey (Eds.) Biotechnology in Agriculture and Forestry. 2007; 61. Transgenic Crops VI. Springer-Verlag, Berlin Heidelberg.
17. Gonzalez A.J. Evaluación *in vitro* de materiales de algodón *Gossypium hirsutum* L. en relación a la capacidad de regeneración y respuesta a estrés abiótico. Análisis de variedades comerciales de INTA, líneas avanzadas. Tesis para obtener el grado de Magister en Genética Vegetal, presentada en la Facultad de Ciencias Agrarias, Universidad Nacional de Rosario, 2015; 193 p.
18. Radice S. Morfogénesis. En: Levitus G, Echenique V, Rubinstein C, Hopp E, Mroginski L, eds. Biotecnología y Mejoramiento Vegetal II. Buenos Aires: INTA; 2010; 26–33 p.
19. Bedoya C, Ríos A. Inducción de la embriogénesis somática en *Crinum x powellii "album"* (Amaryllidaceae) [Tesis de pregrado]. Pereira: Universidad Tecnológica de Pereira; 2010.
20. George EF, Hall MA, De Klerk GJ. Plant propagation by tissue culture. 3rd ed. Vol. 1. Dordrecht: Springer; 2008.
21. Kamle M, Bajpai A, Chandra R, Kalim S, Kumar R. Somatic embryogenesis for crop improvement. GERF Bull Biosci. 2011; 2(1):54–59 p.
22. Price HJ and Smith RH. Somatic embryogenesis in suspension cultures of *Gossypium klotzschiaanum* Anderss. Planta. 1979; 145: 305-307.
23. Davidonis GH and Hamilton RH. Plant regeneration from callus tissue of *Gossypium hirsutum* L. Plant Sci. Lett. 1983; 32: 89-93.
24. Méndez-Natera JR, Rondón A, Hernández J, Merazo-Pinto JF. Genetic studies in upland cotton (*Gossypium hirsutum* L.) I. heterotic effects, Pak. J. Bot. 2007; 39(2): 385-395
25. Chitra Devi B, Narmathabai V. Somatic embryogenesis in the medicinal legume *Desmodium motorium* (Houtt.) Merr. Plant Cell Tiss. Organ Cult., 2011;106: 409-418.
26. Quiroz FR, Rojas R, Galaz RM, Loyola VM. Embryo production through somatic embryogenesis can be used to study cell differentiation in plants. Plant Cell Tiss. Organ Cult. 2006; 86: 285-301.
27. Bian FH, Qu FN, Zheng CX, You CR, Gong XQ. Recent advances in *Cyclamen persicum* Mill. Somatic embryogenesis. Northern Horticult. 2007; 8:70-72.
28. Rodríguez Beraud MM, Latsague MI, Chacón MA, Astorga PK. Inducción *in vitro* de callogénesis y organogénesis indirecta a partir de explantes de cotiledón, hipocótilo y

hoja en *Ugni molinae*. Bosque, 2014; 35(1): 111-118. DOI: http://doi.org/10.4067/S0717-92002014000100011

29. Rojas C, Cuzquén C, Delgado GE. *In vitro* clonal propagation and cutting rooting of native cotton (*Gossypium barbadense* L.). Acta Agron. 2013;62(4): 312-320. ISSN 0120-2812
30. Petrone S. Variación funcional relacionada con la tolerancia al estrés salino de *Gossypium hirsutum* en México. Tesis que para obtener el título de Bióloga. Universidad Nacional Autónoma de México. 2015, 103 p.
31. Dunstan DI, Tautorus TE, Thorpe TA. Somatic embryogenesis in woody plants. In: Thorpe TA (ed) In vitro embryogenesis in plants. Kluwer Academic Publishers, Dordrecht. 1995; 471-538.
32. Wu JY, She JM, Cai XN, Bajaj YPS. Establishment of callus culture, somatic embryogenesis, and the regeneration of cotton plants. In: Bajaj YPS. (ed.) Cotton. Biotechnology in agriculture and forestry, Vol. 42. Berlin: Springer. 1998; 37-47.
33. Nedd LL, González ME, Martínez SJ. Efecto del 2,4-d y ácido ascórbico en la formación de callos embriogénicos en *Gossypium barbadense*L. cultivar 'MSI'. Biotecnología Vegetal, 2022:
34. Hossein A, Aydin M, Haliloglu K. Plant regeneration system in recalcitrantrye (*Secale cereale* L.)Arash Hossein Pour, Murat Aydin &Kamil Haliloglu. Biologia. 2019, 75(7):1017-1028 DOI http://doi.org/10.2478/s11756-019-00395-
35. Maura Isabel Díaz MI, Rodas JM, Luis Roberto González LR, Vera M. Establecimiento *in vitro* de segmentos nodales de *Handroanthus heptaphyllus* de flores blancas. Biotecnología Vegetal. 2020; 20(3): 203 – 210. SSN 2074-8647, RNPS: 2154
36. Devasigaman L, Devarajan R, Loganathan R, Rafath H, Padman M, Govinda MV, Giridhar L, Chetan HC . Devasigamani N. *Lavandula angustifolia* L. plants regeneration from *in vitro* leaf explants-derived callus as conservation strategy. Biotecnología Vegetal. 2020; 20(2): 75 – 82. ISSN 2074-8647, RNPS: 2154
37. Azofeifa A. Problemas de oxidación y oscurecimiento de explantes cultivados *in vitro.* Agronomía Mesoamericana. 2009; 20(1): 153-175. issn: 1021-7444
38. Mora ME, Peralta J, López HA, García R, González JG. Efecto del ácido ascórbico sobre crecimiento, pigmentos fotosintéticos y actividad peroxidasa en plantas de crisantemo Revista Chapingo Serie Horticultura. 2011; XVII (2): 73-81.
39. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant. 1962; 15:473–497.
40. Sharry S, Adema A, Abedini W. Plantas de probeta: Manual para la propagación de plantas por cultivo de tejidos *in vitro*. Editorial de la Universidad de La Plata, Argentina. 2015; 234 p. ISBN 978-950-34-1254-1
41. Rao AQ, Hussain SS, Shahzad MS, Bokhari SYA, Raza MH, Rakha A. Somatic embryogenesis in wild relatives of cotton (*Gossypium spp*.). J Zhejiang Univ-SCI B. 2006; 7(4):291–298.
42. Rajeswari S, Muthuramu S, Chandirakala R, Thiruvengadam V, Raveendran T. Callus induction, somatic embryogenesis and plant regeneration in cotton (*Gossypium hirsutum* L.). Electronic Journal of Plant Breeding. 2010;1(4):1186–1190
43. Han G, Wang X, Zhang G, Ma Z. Somatic embryogenesis and plant regeneration of recalcitrant cottons (*Gossypium hirsutum*). Afr J Biotechnol. 2009; 8(3):432– 437.
44. von Arnold S, Sabala I, Bozhkov P, Dyachok J, Filonova L. Developmental pathways of somatic embryogenesis. Plant Cell Tiss Organ Cult. 2002; 69: 233-249.
45. Yumbla M, Ferreira AC, Marques MV, Rocha DI, Silva D, Dias A, Barbosa LG, Campos Otoni W. Somatic embryogenesis and de novo shoot organogenesis can be alternatively induced by reactivating pericycle cells in Lisianthus (*Eustoma grandiflorum* (Raf.) Shinners) root explants. Available from: In *Vitro Cell Dev Biol – Plant.* 2017, 50:738–745. DOI http://doi.org/10.1007/s11627-017-9800-2
46. Khan T, Singh AK, Pant R. Regeneration via somatic embryogenesis and organogenesis in different cultivars of cotton (*Gossypium* spp.). *In Vitro* Cell Dev Biol-Plant. 2006; 42:498–501.
47. Zouzou M, Kouakou TH, Koné M, Georges AN, Justin KY. Effect of genotype, explants, growth regulators and sugars on callus induction in cotton (*Gossypium hirsutum* L.). Aust J Crop Sci. 2008; 2(1):1–9
48. Sun Y, Zhang X, Jin S, Liang S, Nie Y. Somatic embryogenesis and plant regeneration in wild cotton (*Gossypium klotzschianum*). Plant Cell Tiss Org. 2003; 75:247–253.
49. Han G, Wang X, Zhang G, Ma Z. Somatic embryogenesis and plant regeneration of recalcitrant cottons (*Gossypium hirsutum*). Afr J Biotechnol. 2009; 8(3):432– 437.
50. Upland (*Gossypium hirsutum* L.) and Pima (Gossypium barbadense L.) cottons. Crop Sci. 2001; 41:1235–1240.
51. Abdellatef E, Khalafallah M. Influence of growth regulators on callus induction from hypocotyls of medium staple cotton (*Gossypium hirsutum* L.) Cultivar barac B-67. J. Soil Nature. 2008; 2(1):17–22.
52. Hirimburegama K, Ga~mage N. *In vitro* callus and cell cultures of *Gossypium hirsutum* L. (cotton). J Natn Sci Coun Sri Lanka. 1994; 22(4):305–312.
53. González-Benito M, Carvalho J, Pérez C. Somatic embryogenesis of an early cotton cultivar. Pesq agropec bras. 1997; 32(5):485–488
54. Zhang B-H, Feng R, Liu F, Wang Q. High frequency somatic embryogenesis and plant regeneration of an elite Chinese cotton variety. Bot Bull Acad Sin. 2001; 42:9–16.
55. Ghaemi M, Majd A, Fallahian F, Bezdi G. Comparison of callus induction and somatic embryogenesis of some Iranian cottons (*Gossypium Spp*.) with Coker 312 and histology of somatic embryogenesis. African Journal of Biotechnology. 2013; 10(15):2915–2922.
56. Surgun Y, Yilmaz E, Çöl B, Bürün B. Callus induction, *In vitro* shoot development and somaclonal variations in cotton (*Gossypium hirsutum* L.). J Appl Biol Sci. 2014; 8(2):62–68.

57. Sanghera GS, Gill MS, Sandhu JS, Gosal SS. Effects of genotype, plant growth regulators and explant source on callus induction in cotton (*Gossypium hirsutum* L.). Asian Australas J Plant Sci Biotechnol. 2009; 3:37–42.
58. Efe L. Callus formation and plant regeneration from two cotton species (*Gossypium hirsutum* L. and *G. barbadense* L.). Pak J Bot. 2005; 37(2):227–236.
59. Martínez P, Cabrera JL, Herrera L. Las plantas transgénicas: una visión integral. Genosis [online]. 2004, 2:28 p.
60. Rao AQ, Ali MA, Khan MAU, Bajwa KS, Iqbal A, Iqbal T, Shahid AA, Nasir IA and Husnain T. Science Behind Cotton Transformation. Chapter from the book Cotton Research, Editado por: INTECH, 2016; 209-229. Downloaded from: http://www.intechopen.com/books/cotton-resear
61. Smith EF, Townsend CO. A plant-tumor of bacterial origin. Science 25, 671–673.doi: http://doi.org/10.1126/science. 1907; 25:643.671.
62. Binns A, Campbell A. *Agrobacterium tumefaciens*-mediated transformation of plant cells. Encyclopedia of Life Sciences. Nature Pub. Group. 2001; 1-6.
63. Chilton MD. *Agrobacterium*. A memoir. Plant Physiol. 2001; 125: 9-14.
64. Crouzet P, Hohn B. Transgenic plants. Encyclopedia of Life Sciences. Nature Publishing Group. 2002, 1
65. Hamilton CM, Frary A, Lewis C, Tanksley SD. Stable transfer of intact high molecular weight DNA into plant chromosomes. Proc. Natl. Acad. Sci. USA. 1996, 93: 997-9979.
66. Trieu AT, Burleigh SH. Kardailsky IV, Maldonado-Mendoza IE, Versaw WK, Blaylock LA, Shin H, Chiou TJ, Katagi H, Dewbre GR, Weigel D, Harrison MJ. Transformation of *Medicago truncatula* via infiltration of seedlings or flowering plants with *Agrobacterium*. Plant J. 2000; 22: 531-541
67. Umbeck P, Barton KA, Norheim EV, McCarty JC, Parrot WL, Jennings JC. Degree of pollen dispersal by insects from a field test of genetically engineered cotton. J Econ Entomol. 1991; 84: 1943-1950.
68. Trolider NL, Berlin JD, Goodin JR. 2,4-D resistant transgenic cotton. Proceedings Beltwide Production Research Conference. National Cotton, Council, Mephis, Tennesse, 1988; 840 p.
69. de Framond AJ, Barton KA, Chilton MD. Mini-Ti: a new vector strategy for plant genetic engineering. Biotechnology (N Y). 1983; 5 262–269
70. Finer JF, Vain P, Jones MW, McMullen MA. Development of the particle inflow gun for DNA delivery to plant cells. Plant Cell Reports. 1993; 11:323–328
71. Firoozababy E, Deboer DL, Merlod DJ, Halh EL, Rahska KL, Murray EE. Transformation of *Gossypium hirsotun* L. by *Agrobacterium tumefaciens* and regeneratión of trangenic plants. Plant Molecular Biology. 1987, 10 : 105-116
72. Cousins YL, Lyon BR and Llewellyn DJ. Transformation of an Australian cotton cultivar: Prospects for cotton through genetic engineering. Australian Journal of Plant Physiology. 1991, 18: 481-494.
73. Rajasekaran K, Grula, JW, Hudspeth, RL, Pofelis S, Anderson DM. Herbicide-resistant Acala and Coker cottons transformed with a native gene encoding mutant forms of acetohydroxyacid synthase. Molecular Breeding. 1996, 2: 307–319
74. Wendt-Gallitelli MF, Dobrigkeit I. Investigations implying the invalidity of octopine as a marker for transformation by *Agrobacterium tumefaciens*. Z. Naturforschg. 1973; 28,768–771.
75. Cousins YL, Lyon BR and Llewellyn DJ. Transformation of an Australian cotton cultivar: Prospects for cotton through genetic engineering. Australian Journal of Plant Physiology. 1991; 18: 481-494.
76. Keller K, Melillo J, de mello W. “Trace Gas Emissions from Ecosystems of the Amazon Basin”. En: Ciencia e Cultura. Journal of the Brazilian Association for the Advancement of Science. 1997, 49(01):87-97.
77. Nadolska-Orczyk A, Orczyk W, Przetakiewicz A. *Agrobacterium*-mediated transformation of cereals—from technique development to its application. Acta Physiologiae Plantarum. 2000; 22:77-88. DOI: http://doi.org/10.1007/s11738-000-0011-8
78. Thomas JC, Adams DG, Keppenne VD, Wasmann CC, Brown JK, Kanost MR. Protease inhibitors of *Manduca sexta* expressed in transgenic cotton. Plant Cell Reports. 1995; 14:758-762. DOI: http://doi.org/10.1007/BF00232917.
79. Dickens JC. Green Leaf Volatiles Enhance Aggregation Pheromone of Boll Weevil, *Anthonomus grandis*. Entomol. Exp. Appl., 1989; 52(3), 191-203.
80. Moffat AS. Transposons Help Sculpt a Dynamic Genome. Science, 2000; 289(5484), 1455-1457.
81. Makarova KS, Grishin NV, Shabalina SA, Wolf YI, Koonin EV. A Putative rna-interference-based Immune System in Prokaryotes: Computational Analysis of the Predicted Enzymatic Machinery, Functional Analogies with Eukaryotic rnai, and Hypothetical Mechanisms of Action. Biol. Direct, 2006; 1, 7.
82. Díaz C, Chaparro A. Métodos de transforMación Genética de plantas. Revista U.D.C.A Actualidad & Divulgación Científica. 2004, 15 (1): 49 – 61
83. Fundación Antama. El algodón transgénico ocupa alrededor del 70% de la superficie algodonera mundial. 2021. Consultado en: https://twitter.com/fundacionantama/status/1429461923867893762
84. FAO, Día mundial del algodón 2021. Consultado en: https://docs.wto.org/dol2fe/Pages/SS/directdoc.aspx?filename=q:/WT/CFMC/W93-03.pdf&Open=True.
85. Agrogebio. Los cultivos transgénicos en el mundo. 2019. Consultado en: https://www.argenbio.org/cultivos-transgenicos/12549
86. FAO. Perspectivas Agrícolas 2013-2022. 2013. Consultado en: http://www.oeidrus-bc.gob.mx/sispro/algodonbc/PRODUCCION/Mundial/Situacion%20Actual%20del%20mercado%20Internacional%20de%20Algodon.pdf
87. Shukla V, Devi P, Baghel S. Isolation, characterization and biomass production of *Trichoderma* spp. A review. Research in Environment and Life Sciences. 2016; 9(7): 889-894

88. Veluthambi K, Krishnan M, Gould JH, Smith RH, Gelvin SB. Opines stimulate induction of the *vir-genes* of the *Agrobacterium tumefaciens* Ti plasmid. J Bateriol. 1989; 171(7):3696-3703.
89. Rugini E, Mariotti D. *Agrobacterium* Rhizogenes T-DNA genes and rooting in woody species. Acta Horticulturae. 1999; 300: 301-308.
90. Sanford JC. The biolistic process. Trends Biotechnol. 1988, 6: 299-302.
91. Hansen G, Wright MS. Recent advances in the transformation of plants. Trends Plant Sci. 1999; 4: 226-231
92. Kohli A, Leech M, Vain P, Laurie DA, Christou P. Transgene organization in rice engineered through direct DNA transfer supports a two fase integration mechanism mediated by the establishment of integration hot spots. Proc. Natl. Acad. Sci. USA. 1998; 95: 7203-7208.
93. Gutiérrez A, Santacruz F, Cabrera JL, Rodríguez B. Mejoramiento genético vegetal *in vitro*. e-Gnosis, [online]. 2003; 1: 4. www.e-gnosis.udg.mx/vol1/art4
94. Reddy, MS, Dinkins RD, Collins GB. Gene silencing in transgenic soybean plants transformed via particle bombardment. Plant Cell Report. 2003; 21: 676-683.
95. McCabe DE, Martinell BJ. Transformation of elite cotton cultivars via particle bombardment of meristems. Bio/Technology. 1993; 11:596-598.
96. Maskin L, Turica M, Nakaya P, González A, Lewi DM. Técnicas aplicadas en la transgénesis en algodón (*Gossipyum hirsutum* L.). Trabajo presentado en el 1º Congreso Internacional de Algodón realizado el 27/10 en Presidencia Roque Sáenz Peña, Chaco. Argentina. 2018.

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El cultivo de tejidos y la transformación genética en Gossypium spp
The tissue culture and genetic transformation in Gossypium spp

Cultivos Tropicales
vol. 46, no. 1, e12, 2025
Ediciones INCA,
ISSN-E: 1819-4087