Plant tissue culture: Current status, opportunities and challenges

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Abstract

R. García-González, K. Quiroz, B. Carrasco, and P.D.S. Caligari. 2010. Plant tissue culture: Current status, opportunities and challenges. *Cien. Inv. Agr.* 37(3): 5-30. In the last two decades plant biotechnology applications have been widely developed and incorporated into the agricultural systems of many countries worldwide. Tissue culture tools have been a key factor to support such outcomes. Current results have allowed plant biotechnology and its products –including transgenic plants with several traits– to be the most assimilated technology for farmers and companies, representing several benefits such as: 125 millions ha of transgenic crops in 2008, the reduction of pesticides application by up to 9% in the last ten years, transgenic plants with a better nutritional quality, mass propagation of selected and healthy plants, and the production of proteins for industrial or therapeutic use. The rapid and extensive assimilation for this technology has improved the competences of the agricultural systems both in industrial and in developing countries, based on the proper application of research programs. Several theoretical and practical aspects supporting plant tissue culture applications, as well as the main results and current status of the technology are discussed in this review. The reader will find key elements to evaluate the potential of plant tissue culture tools for the development of agriculture, livestock, human health and nutrition, and human well being in general.

Key words: *in vitro*; micropropagation; organogenesis; plant bioreactors; somatic embryogenesis; transgenic plants.

Introduction

The increase in food demand worldwide, associated with unequal distribution and the disequilibrium in the distribution of wealth, has caused an increasingly important pressure on food producers who, in parallel, have increased

their requirements for new technologies that allow greater yields and better quality of the products that they offer (Christou and Twyman, 2004). While at the same time, there has been an increasing consumer-led demand for lower environmental damage and greater sustainability in the food production chain.

During the second half of the last century the development of genetic engineering and molecular biology techniques allowed the appearance of improved and new agricultural products which

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have occupied an increasing in the productive systems of several countries worldwide (Vasil, 1994a; Christou *et al.*, 2006; Navarro Mastache, 2007; James, 2008). Nevertheless, these would have been impossible without the development of tissue culture techniques, which provided the tools for the introduction of genetic information into plant cells, the selection of the plants, which carried the genes of interest and at the same time, the massive and rapid multiplication of the genotypes that finally could be introduced into the production systems.

The technology of transgenic plants is only one of the diverse applications that tissue culture has in plants because it is a tool of great versatility, which can contribute to solve various problems that affect humanity, not only necessarily related with food production (Pareek, 2005).

Throughout this review, the basic principles over which tissue culture techniques and the practical applications of the technology rest upon will be approached, together with the advances made to date.

Plant tissue culture background

Tissue culture may be defined as the aseptic culture of cells, tissues, organs or whole plants under controlled nutritional and environmental conditions (Thorpe, 2007). The first reports regarding tissue culture date back to the beginning of the 20th century when Gottlieb Haberlandt (Haberlandt, 1902) developed experiments to maintain mesophyll cells in culture based on postulates which established the “totipotentiality of plant cells”. From this moment on, development has been constant and every year hundreds of results and reports regarding the application of tissue culture techniques, applied to breeding programs, genetic biodiversity conservation and biopharmaceutical production are documented.

The development of tissue culture techniques rest upon two properties of plant cells: cell totipotency (Vasil and Hildebrandt, 1965) and cell plasticity (Thorpe, 2007). Cell totipotentiality

is the genetically retained capacity that all living cells possess to originate a new genetically identical cell, and, after cellular division and differentiation processes, to be able to form tissues, organs, systems and complete individuals (Haberlandt, 1921; Takebe *et al.*, 1971). Cellular plasticity is the characteristic which marks the difference between plant and animal cells in their capacity of multiplication, division, differentiation and formation of a new individual. As opposed to animals, plants are sessile organisms often with long life cycles which has meant that they have been forced to develop defense and survival mechanisms in order to face different negative biotic as well as abiotic factors. This capacity of modifying response allows plant cells to respond to external stimuli directed towards the achievement of a determined response.

From a practical point of view, the mechanisms which trigger the development of a plant from a cell or a tissue section depend on factors which vary according to the specie, the type and the age of the tissue, the environmental conditions and the composition of the culture media, which are generally managed empirically on a case case basis.

The development of tissue culture techniques

Plant tissue culture can begin once a genotype is selected on the basis of having identified a problem to be solved and the appropriate type of protocol to deal with it. Tissue culture techniques for plant micropropagation, genetic transformation, biotech assisted selection, mutagenesis, etc, rest on two fundamental morphogenesis processes: organogenesis and somatic embryogenesis.

Organogenesis is the formation of plant organs from a determined tissue in order to form complete plants, characterized by being polar, which means that only one aerial organ or root is emitted and from this a new complete plant is regenerated. At the same time, organogenesis may be direct, if the organogenic shoot is directly obtained from the explants, or indirect, if

the organogenic process occurs from previously formed callus in the initial explants (Vijaya and Giri, 2003).

Somatic embryogenesis is the production of embryos from somatic plant cells (any non-sexual cell) to obtain a complete plant. Unlike organogenesis, this is a polar process where the aerial structures and roots of the plants are obtained from the somatic embryo. It can also be direct or indirect, if the process originates from the initial explants or from previously induced callus. Somatic embryogenesis consists of four fundamental stages: A) Callus induction; B) Embryo formation and proliferation; C) Embryo maturation; and D) Embryo germination. At the same time, the embryos may pass through four stages in their development, the globular form, the heart form, the torpedo and the cotyledonary forms (Ammirato, 1983). Each one of the stages of somatic embryogenesis, just as the different phases of normal embryo development, depend on the species and on the genotypes which are being cultured.

Usually it is said that tissue culture is composed of four stages (Thorpe, 2007), but this concept limits the use of this technique to massive multiplication and today it is known that tissue culture is a much wider reaching technology. Because of this, we consider that only the micropropagation of plants through *in vitro* culture can be defined in this way and, it has five fundamental stages.

Stage 0 Preparation of donor plant: Any plant tissue can be introduced *in vitro*. Nevertheless, it has been established that success in the *in vitro* introduction and establishment depends on the physiological and phytosanitary qualities of the plant, which are in part determined by the environmental conditions under which the plant is being grown (George and Debergh, 2008).

To increase the probability of success it is suggested that during this stage, the plants used as explant donors should be cultivated under optimal conditions, with irrigation, nutrition and temperature control (Cassels and Doyle, 2005). Fungicide bactericide and insecticide treatments can be applied in order to diminish the

level of infestation by insects and infection, either by microorganism infecting the plant or by the amount of endophytic microorganisms that circulate throughout the tissues. In the same way, pretreatments with plant growth regulators may be carried out to improve the morphogenic response of the plant tissues during the *in vitro* establishment (García *et al.*, 1999; 2000).

Stage I Introduction and establishment: This is the most difficult stage of an *in vitro* propagation system, because the later steps depend on the phytosanitary and morphofisiological status and quality of the established explants (George and Debergh, 2008). The introduction of plant tissues into an *in vitro* culture is carried out through superficial disinfection with chemical products. Generally, the combined application of bactericide and fungicide products is suggested. The selection of products to carry out the superficial disinfection of the tissues depends on the type of explant to be introduced. The most commonly used disinfectants are sodium hypochlorite (Tilkat *et al.*, 2009; Marana *et al.*, 2009), calcium hypochlorite (García *et al.*, 1999) and ethanol (Singh and Gurung, 2009). Nevertheless, some tissues with high lignin or cellulose content, such as woody plants and tissues of organs developed in the soil, need more drastic disinfection treatments such as short immersion in mercuric (II) chloride (HgCl₂) (Hussain and Anis, 2009).

At this stage, it is also important to control the emission of phenolic compounds by the tissues and its oxidation. This is a defensive reaction of wounded plant tissues and is induced by plant age, the cutting of the tissue segment, the application of chemicals, simple manipulation or even from over rigorous washing with water and detergents (Shekhawat *et al.*, 1993; Hussain and Anis, 2009). It is not only important to minimize the production of such phenolic compounds but to minimize their oxidation in the explants by, for example, reduced light intensity (the explants may even be cultivated in darkness during the first days of culture) or lower culture temperatures can be used. Another approach is the addition of antioxidant compounds such as activated charcoal, citric acid, ascorbic acid, nicotinic acid and L-cysteine to

the media is also effective in reducing phenolic oxidation in tissues (Shekhawat *et al.*, 1993). It was found that antioxidant compounds like glutathione can induce a massive and selective induction of defensive genes that can protect plant tissues against different stresses (Wingate *et al.*, 1988). In tomato plants, the addition of activated charcoal and ascorbic acid at high concentrations improved shoot emission length (Bhatia and Ashwath, 2008). It has also been found that addition of L-glutamine into the co-cultivation medium allowed the efficient *Agrobacterium tumefaciens* mediated transformation of tea by avoiding the bactericidal effect of leaf polyphenols produced by leaf tissues (Sandal *et al.*, 2007). Addition of silver nitrate (AgNO_3), also known as an ethylene inhibitor, into the basal medium has significantly enhanced shoot production (Memon *et al.*, 2009) and rooting (Dai *et al.*, 2009); however, it has also been found that it can increase ethylene synthesis under *in vitro* cultures with several morphogenic responses but without basic molecular explanations (Zhang *et al.*, 1998; Kumar *et al.*, 2009).

The activity of antioxidant enzymes during somatic embryogenesis has been documented (Shohael *et al.*, 2007) and it has supported the role of natural antioxidants as glutathione and ascorbic acid in plant morphogenesis (Shohael *et al.*, 2007; Belmonte and Stasolla, 2009). Belmonte *et al.* (2005) found that glutathione addition into the media increased meristem differentiation, cellular organization and lower production of ethylene.

In general, antioxidant compounds can facilitate *in vitro* cultures by their protective effect against oxidative stress (Zsarka *et al.*, 2007) and/or by improving the cell growth (Bhatia and Ashwath, 2008; García *et al.*, 2008; Poleschuk and Gordabenko, 1995).

Hyperhydricity (formerly called “vitrification”, but it has become a term used to characterize cryoperserved tissues) of tissues is another physiological effect very common in plant tissue culture (Gaspar *et al.*, 1985; Ziv, 1991; Kevers *et al.*, 2004). Tissue culture vessels are generally tightly closed to avoid or reduce evaporation

and to keep cultures aseptic, but this environment also creates chemical conditions that affect morphological and physiological processes (Ogasawara, 2003). When plants are placed in sealed vessels, they show reduced growth, increased branching and reduced elongation (Sha *et al.*, 1985; Bairu *et al.*, 2009). Another constrain of hyperhydricity is the low survival in the *ex-vitro* step, as demonstrated by Marin *et al.* (2003) improving the acclimatization of tree rootstocks by reducing relative humidity in the culture flasks (Marin, 2003). Hyperhydricity is also linked to shoot-tip necrosis, a physiological disorder showed by *in vitro* plants as a consequence of high relative humidity, transpiration rate, Calcium availability in the medium and plant growth regulators (Bairu *et al.*, 2009).

Gelling agents can also induce hyperhydricity in plant tissue (Debergh *et al.*, 1981). Recovery of non-hyperhydric plants from *Carica papaya* somatic embryos was obtained by modifying agar concentration in the germination medium and exposure of the embryos to different light sources (Ascencio-Cabral *et al.*, 2008). Addition of phloridzin also enhanced plant recovery (Ascencio-Cabral *et al.*, 2008). In pear (*Pyrus communis* L. cv. ‘Durondeau’), the addition of guar gum (galactomannans obtained from *Cassia fastuosa* (cassia) or *Cyamopsis tetragonolobus*) mixed with agar enhanced the production of non-hyperhydric shoots (Lucyszyn *et al.*, 2006). Addition of certain commercial anti-hyperhydricity agents (i.e. EM2), as well as pectin or polysaccharides extract, was effective in avoiding the induction of hyperhydric shoots in *Eucaliptus* sp. hybrids (Whitehouse *et al.*, 2002).

Plant growth regulators can induce the formation of hyperhydricity in tissue cultures of several species (Ziv, 1991; Fraguas *et al.*, 2004; Toth *et al.*, 2004; Fraguas *et al.*, 2009). In *Ficus carica*, the addition of BA or GA3 in the shooting medium promoted the formation and elongation of hyperhydric shoots (Fraguas *et al.*, 2004); however, the authors reported that it was possible to avoid this response by supplementing the media with activated charcoal. Inoculation of a *Pseudomonas* sp. strain in oregano plants cultivated *in vitro* was effective to elimi-

nate and prevent hyperhydration (Perry *et al.*, 1999), but this practice must be done with care because *Pseudomonas* sp. can become a very fastidious contaminant for the *in vitro* aseptic tissues.

Closure materials can have a significant influence on the hyperhydric explant responses during *in vitro* culture. It has been found the plants derived from vessels with different ventilation rates showed different production of hyperhydric tissues mainly associated morphological changes, such as: density and size of epidermal cell and stomata, size of guard cells, and stomata aperture (Chen *et al.*, 2006). It was also found that hyperhydric tissues had a higher concentration of epidermal cells, as well as larger stomatal cells, but only 7% of the hyperhydric plants survived in the *ex vitro* step while 67% of survival was obtained from normal plants (Chen *et al.*, 2006).

A plant tissue is considered to be “introduced and established” to the *in vitro* culture when explants are not only free from superficial or visible contaminants, which interfere with the morphogenic response, but also when it shows a morphogenic response. This morphogenic response is characterized by multiplication and/or differentiation of the plant tissues such as: shoots, roots, leaves or production of calli (Noshad *et al.*, 2009; Christensen *et al.*, 2008).

Stage II Propagation of plants: The aim of this phase is to increase the number of units in the tissue culture system until the desired number is obtained (Saini and Jaiwal, 2002). The selection of the propagation technique and protocols depends on the specie or the genotypes. In some cases, as in sweet potato (García *et al.*, 2000), organogenic propagation by axillary shoots is recommended (Benedicic *et al.*, 1997), while in the case of coffee, the use of somatic embryogenesis is more suitable and efficient (Boxtel and Berthouly, 1996). In blueberry, for example, the response to citoquinins is cultivar dependent (Debnath, 2007) and this species can also be induced to form shoots or calli, depending on the explant management and the basal medium (Ostroloucka *et al.*, 2004). To enhance the mor-

phogenic responses from any explant cultivated *in vitro*, it is necessary to study and establish the effect of plant growth regulators like auxins and citoquinins interactions (García *et al.*, 1999; Tilkat *et al.*, 2009). Explant management and position onto the culture medium can also play a key role to improve the morphogenic response (García *et al.*, 1999; Papafotiou and Martini, 2009).

Stage III Rooting and explant preparation for the ex vitro conditions: The rooting stage may occur simultaneously to propagation in the same culture media used for multiplication of the explants. However, in some cases it is necessary to carry out media changes, including nutritional modification and growth regulator composition to induce rooting and the development of strong root growth. In this stage it is also necessary to prepare the plants for the *ex vitro* phase by modifying media composition and the gas exchange inside the culture vessels. Rooting efficiency may be related with the auxin:cytokinin rates (Leonardi *et al.*, 2001); the type of explant (Hiregoudar *et al.*, 2005); the number of subcultures (Rios *et al.*, 2005); the pH of the culture media; and the concentration of sucrose (Newell *et al.*, 2005).

Stage IV Ex vitro adaptation or plant acclimatization: At this point *in vitro* plants are adapted to the environment outside the laboratory conditions. Management of light intensity, substrate moisture and temperature at the leaf and root level are strongly recommended and can influence plant survival at this stage. At the beginning of the adaptation phase, a constant high relative humidity is recommended to facilitate the formation of active roots and to reduce water lost due of leave transpiration, but as plants start to adapt it is recommended to decrease the humidity in order to facilitate a better adaptation to field conditions (Pospíšilová *et al.*, 1999). The substrate characteristics are also important considerations during this phase, because they can influence the general physiological characteristics of the plants, as demonstrated by Rocha *et al.* (2008) who found that the organic substrate Ecoterra® produced a better root system and a higher quality of the aerial part in *Genipa americana*.

Tissue culture applications in agriculture worldwide

Biotechnology has been introduced into agricultural practice at a rate without precedent (James, 2008). Actually, the growth of the global area cultivated with transgenic plants continues to increase worldwide, and together with the massive propagation of plants, has turned it into a key technology for agriculture worldwide (Read, 2007; Navarro-Mastache, 2007).

Massive clonal propagation of genotypes of interest

The traditional propagation methods allow the clonal multiplication of genotypes of interest but at relatively low propagation rates, which explains why the introduction of a new genotype into agricultural practices may take a number of years. Tissue culture allows the rapid production of a large number of plants, even where normally the species has low multiplication rates. At the same time, the space requirement for such multiplication is considerably smaller. In order to have an idea of the plant volumes that may be obtained let us consider the example of a specific genotype which *in vitro* has a rate of multiplication of 3 (which in *in vitro* terms is considered low), a propagation cycle of 4 weeks, and an efficiency of propagation of the system of 90% - due to losses from contamination or handling error. Then just over 1 million plants would be obtained in only 12 months following the establishment of a single unique explant. This application is therefore ideal for the massive multiplication of species or genotypes with commercial potential, whether these are plants of agriculture or horticulture, such as fresh cut flowers, ornamental plants or fruit trees (Thorpe, 2007).

It is estimated that the actual area of plants produced by biotechnology has now reached about 800 millions of hectares worldwide from 1996 to 2008 (James, 2008), but this data only included GM crops without considering non-GM technologies such as micropagation. By 1994, it was estimated that 600 companies produced around 500 million tissue cultured plants from 50,000 species (Vasil, 1994b). In 2005, it was

estimated that there were about 196 laboratories destined to carry out work related to different fields of plant biotechnology in Latin America, from which around 25% were dedicated to *in vitro* plant propagation (Dlahmini *et al.*, 2005), with an estimated capacity of 75 million *in vitro* plants that could be produced per year. The average number of plants produced per laboratory was at that time of the order of 300,000 plants (Sasson, 2001). The countries with greater investment in the area of biotechnology in Latin America are Mexico, Argentina, Brazil, Cuba, Costa Rica, Colombia, Peru and Chile. In the same way, technologies for massive propagation are available in Chile for species of economical importance, such as: European hazelnuts (Berros *et al.*, 2005); strawberries (Debnath, 2005); grapevines (Zlentko *et al.*, 2002); blueberries (Debnath, 2007); cherries (Espinosa *et al.*, 2006); tulips (Ptak and Bach, 2007); avocados (Márquez Martín *et al.*, 2009; Zulfikar *et al.*, 2009); peaches (Zhou *et al.*, 2010); sweet cherries (Staniene *et al.*, 2009); grapes (Tapia *et al.*, 2007); Chilean strawberries (Rojas *et al.*, 2007) and Chilean endemic orchids *Chloraea crispata* (Quiroz *et al.*, 2007).

The massive propagation of plants has traditionally been carried out in solid medium (Pérez Ponce, 1998; Debnath, 2007), nevertheless during the last few years, cultures in liquid medium with the objective of massive plant propagation have appeared as an alternative, which allows reduced costs in relation to numbers of subculture and losses associated with manipulation and contamination, but also because of space reduction.

Systems of propagation with temporary immersion (TIS) provide an interesting alternative technology that consists in the immersion of plant tissues during periods of time with a determined frequency in the culture medium (Etienne and Berthouly, 2002). This procedure allows the maximization of the efficiency in the absorption of nutrients and water by the cells in order to optimize the morphogenic tissue responses.

TIS technology has been developed using a wide number of designs (Table 1) and a large number of species, but two TIS designs have be-

come more popular, the Temporary Immersion Biorreactor (TIB) and the Recipient for Automated Temporary Immersion (RATI). As any tissue culture tool, TIS can induce undesirable physiological or morphological disorders (Yang *et al.*, 2008), such as: hyperhydricity; low rooting ability; damages at the cellular level; reduction in the chlorophyll content; and variation in the amount of reducing and non reducing sugars (Sreedhar *et al.*, 2009). However, constrained morphophysiological responses can depend on the species, explant source, media composition, plant regulators concentration and interaction and environmental conditions (Welander *et al.*, 2007). In raspberry, shoot induction was more efficient with addition of Thidiazuron, but elongation of the plantlets was higher in the presence of 6-benzyladenine and rooting behaviour better in the growth regulators free medium (Debnath, 2010). Other examples in species like Eucalyptus (McAlister *et al.*, 2005) and pineapple (Escalona *et al.*, 2003) support the fact that it is necessary to broaden the evaluation of explant, media composition and environmental conditions to optimize the multiplication rates and the quality of the propagated plants.

Production and propagation of disease-free plants by tissue culture

Tissue culture allows the production and propagation of genetically homogeneous, disease-free plant material. For these, “cleanup” techniques to eliminate plant pathogenic organisms have been developed, such as meristem cultures or explant disinfection treatments through chemical or physical methods (Chatenet *et al.*, 2001).

Meristems are the growing points of the plants and are located in the apices, lateral buds and roots. Meristems have low development of vascular tissues which means that virus, bacteria or fungi presences in this tissue are lower compared to other tissues in the plant. Using meristem isolation culture in nutritive medium it has been possible to obtain a high percentage of plants free of bacterial or fungal diseases, which can then be propagated disease free (Rzepka-Plevnes *et al.*, 2009). In the case of berries, specially *Fragaria* sp., this is a very useful tech-

nique in order to eliminate viral, -i.e. *Fragaria* sp.: Strawberry mild yellow-edge virus (SMY-EV), Strawberry vein banding virus (SVBV), Strawberry crinkle virus (SCV), or fungal diseases -i.e. *Fragaria* sp.: *V. dahliae*, *Sphaeroteca macularis*, *S. humili*, *Botrytis cinerea*, relevant to Chilean farmers by allowing the production and large scale propagation of disease-free plants (McInnes *et al.*, 1992). For woody species, meristem cultures have also been efficient for the elimination of virus and the consequent massive multiplication of disease-free plants (Popesku *et al.*, 2010; Tan *et al.*, 2010).

However, the technology does have some limitations since it has been found that meristems can be strongly dependent on the cytokinin: gibberellin ratio as demonstrated for beans *cv.* Zorin (*Phaseolus vulgaris* L.). In this case, plant growth regulators in the basal medium had a significant influence on plant survival during *ex vitro* acclimatization - also called acclimation-producing more vigorous plants (Benedicic *et al.*, 1997). Thus it means the techniques sometimes take longer to develop initially.

Somaclonal variation

Cell and tissue *in vitro* culture is a useful tool for the induction of somaclonal variation because the tissues may be cultured with little differentiation or allows the culture of isolated cells (Marino and Battistini, 1990). This makes it easier to correctly dose the concentrations of the mutagenic agents, locate the mutagenic activity in the tissues with greater potential to be mutated, prolong the exposure to mutagens and regenerate plants with high levels of efficiency (Ravindra *et al.*, 2004). The downside is that some tissues more readily mutate even when simply exposed to plant growth regulators or other “normal” media components (Rzepka-Plevnes *et al.*, 2009).

Whether genetic variability induced by tissue cultures could be used as a source of variability to obtain new genotypes, or whether this variation can be disadvantageous for keeping the genetic fidelity of the *in vitro* propagated material, it is very important to assess the genetic stability of the plants during or after tissue culture.

Table 1. Use of different Temporary Immersion Systems (TIS) for propagation of plant species.

Species	Reference	TIS technology employed by the authors
<i>Alocasia amazonica</i>	Jo <i>et al.</i> , 2008	Temporary Immersion Biorreactor (TIB) Air-lift-balloon type bubble bioreactor (BTBB)
<i>Ananas comosus</i> L. Merr	González-Olmedo <i>et al.</i> , 2005	TIB
<i>Ananas</i> sp. cv. MD2	Pérez <i>et al.</i> , 2004	TIB
Apple (<i>Malus domestica</i>) rootstock M26	Zhu <i>et al.</i> , 2005	RITA™
Apple (<i>Malus domestica</i>)	Chakrabarty <i>et al.</i> , 2003	BTBB.
<i>Artemisia judaica</i> L.	Liu <i>et al.</i> , 2004	RITA™ Airlift bioreactor Flask (Erlenmeyer) 250 ml
<i>Artocarpus altilis</i>	Murch <i>et al.</i> , 2008	Liquid Lab™ Rocker system
<i>Chloraea crispa</i>	Quiroz <i>et al.</i> , 2007.	TIB and RITA™
<i>Coffea arabica</i>	Albarrán <i>et al.</i> , 2005	TIB
<i>Coffea canephora</i>	Ducos <i>et al.</i> , 2007	TIB
<i>Cucumis sativus</i> L.	Konstas and Kintzios, 2003	Plastic airlift bioreactors (Osmotek Lifereactors)
<i>Cucumis sativus</i> L.	Matakiadis and Kintzios, 2005	LifeReactor™
<i>Eucalyptus</i> sp.*	Castro, 2002	TIB
<i>Euphorbia millii</i>	Dewi <i>et al.</i> , 2006	BTBB
<i>Gladiolus</i> sp.*	Ruffoni <i>et al.</i> , 2008	RITA™
<i>Hosta tokudama</i> cv. 'Striptease', 'Minuteman', and 'Stiletto'	Adelberg, 2004	Thin-film rocker system.
<i>Ilex dumosa</i> var. <i>dumosa</i>	Luna <i>et al.</i> , 2008	RITA™
<i>Leucojum aestivum</i>	Ptak and Gadek, 2009	RITA™
<i>Musa</i> AAB, c.v. CEMSA ¾	Aragón <i>et al.</i> , 2010	TIB
Raspberry (<i>Rubus idaeus</i> L.)	Debnath, 2010.	TIB
<i>Rhodophiala</i> sp.*	Muñoz <i>et al.</i> , 2009.	TIB
<i>Rubus chamaemorus</i> L.	Debnath, 2007	Plastic airlift bioreactors (LifeReactor™, Osmotek, Rehovot, Israel)
<i>Rubus fruticosus</i> , <i>Arbutus unedo</i> and <i>Myrtus communis</i>	Damiano <i>et al.</i> , 2007	TIB
<i>Saccharum</i> sp.	Mordocco <i>et al.</i> , 2009	RITA™
<i>Spathiphyllum cannifolium</i>	Dewir <i>et al.</i> , 2006	BTBB
<i>Spathiphyllum cannifolium</i> cv. SunnySails	Dewir <i>et al.</i> , 2007	Air-lift BTBB
<i>Stevia rebaudiana</i>	Sreedhar <i>et al.</i> , 2008	BIOFLO III
Strawberry (<i>Fragaria annanasa</i>).	Debnath, 2008	RITA™
Strawberry (<i>Fragaria</i> sp.)	Debnath., 2009	RITA™
Strawberry (<i>Fragaria x ananassa</i>) cvs. Bounty, Jonsok, Korona, Polka, and Zephyr	Hanhineva <i>et al.</i> , 2005	RITA™
<i>Theobroma cacao</i> clone Scavina-6	Niemenak <i>et al.</i> , 2008	TIB

The induction of somaclonal variation during tissue culture can be detected by using cytogenetic, biochemical and molecular methods (Rani *et al.*, 2000; Kumar *et al.*, 2007; Minano *et al.*, 2009). It has been determined that several factors produce somaclonal variation during *in vitro* culture like disorganized meristematic growth, the genetic background of the cell (ploidy level and/or genotype), composition of the culture medium, concentration of plant growth regulators, the type of explant sources, and methylation pattern of DNA (Karp, 1991, 1995; Brar and Jain, 1998; Rani *et al.*, 2000; Aversano *et al.*, 2009).

In consequence, genetic stability under *in vitro* culture depends on the species and methods used. In potatoes, it has been demonstrated that tissue culture induced variability at the nuclear DNA level and it is strongly linked with the genetic background and the ploidy level, but no variability was induced in the plasmidic DNA (Aversano *et al.*, 2009). However, for *Coffea arabica* L. derived from somatic embryos, the results were different since the mitochondrial DNA showed higher variation (41%) than nuclear DNA (4,36%) (Rani *et al.*, 2000).

Management and modification of the ploidy levels

Ploidy level in plants is variable and can determine the expression of quantitative or qualitative traits. Another culture allows the generation of haploids which on doubling give homozygous lines that can be exploited in breeding (Morrison and Evans, 1988), hybridization (Rodrigues *et al.*, 2005) or genetic transformation programs (Coronado *et al.*, 2005).

On the other hand, the induction of polyploidy has been widely held out as a method to increase productivity of plants, as well as to stabilize interspecific hybrids. In ornamental species, it has been used to modify flower morphology, color or other desired traits and is made simpler by tissue culture (Liu *et al.*, 2007). Colchicine and oryzalin, two powerful antimitotic agents, have been widely used to increase the number of chromosomes in ornamental (Stanys *et al.*, 2006), crops (Broughton *et al.*, 2005), fruit spe-

cies (Bouvier, 2002) or species for industrial purposes (Yang *et al.*, 2006).

Embryo rescue

The technique of embryo rescue has application in breeding programs and genotype selection for individuals that present early abortion after fertilization (Sharma *et al.*, 1996). It is also useful for the rescue of species that have lost the capacity for sexual reproduction due to biotic or abiotic factors that prevent seed germination (Mohanty *et al.*, 2009).

In vitro cultures of mature and/or immature embryos are applied to recover plants obtained from inter-generic crosses that do not produce fertile seeds (Ahmadi *et al.*, 2010). For instance, this tissue culture tool has a strong application in Breeding Programs, especially considering that the technique has been demonstrated to be very efficient in a wide range of plant species such as grasses (Genovesi *et al.*, 2009); ornamental plants (Deng *et al.*, 2010) and tree species (Bagniewska-Zadworna *et al.*, 2010).

Germplasm conservation

Germplasm conservation worldwide is increasingly becoming an essential activity due to the high rate of disappearance of plant species, and the increased awareness of the need for safeguarding the floristic patrimony of the countries (Filho *et al.*, 2005).

Several plant species produce recalcitrant seeds that can not be stored for long time and in this case tissue culture can be used for plant conservation in vegetative state, often under conditions of slow growth (Lambardi *et al.*, 2002) or for cryopreservation (Halmagyi and Pinker, 2006) because of the advantages of relatively low costs and reduced space usage (Tyagi *et al.*, 2007). On the other hand tissue culture protocols can be also using for conservation of vegetative tissues when the target for conservation are clones instead of seeds, to keep the genetic background. Biotechnology offers the advantage of being more readily able to avoid the loss

of the conserved patrimony due to natural disasters, whether biotic (such as pests and diseases) or abiotic (such as fire, drought and flooding) which is especially threatening in conditions of climate change.

Genetic transformation

Probably, one of the most relevant applications of tissue culture, and not just because of the media exposure arising from the results, but, as was mentioned initially, because of the quantity of results and the practical application obtained during the last two decades, is genetic transformation.

The morphogenic response of tissues to determined stimulus, resulting in the production of organs or complete plants, is known as plant regeneration (Tisserat, 1985). Plant regeneration is one of the *sine qua nom* factors from which the establishment of a genetic transformation protocol depends. It is therefore important to have: 1) An efficient regeneration protocol; 2) The availability of an efficient method that allows the introduction, integration and the stable expression of exogenous genetic information in the plant cell; and 3) An efficient system for selection of the transgenic plants.

Genetic transformation systems may be classified into two great groups, based on methodology:

1-. Direct methods, where the desired genetic information is introduced into the plant cell without the need of using any biological vector that carries and introduces the genetic information.

2-. Indirect methods, where the desired information is introduced into the cell through a vector of biological origin that carries the desired information and through natural mechanisms it is capable of introducing this information into the plant cell and allow its integration it in the plant's genome (Potrykus, 1991).

Among the direct transformation methods, electroporation of protoplasts (Fromm *et al.*, 1987) and intact cell (Arencibia *et al.*, 1995), micro-injection (Nehaus *et al.*, 1987), polyethylene

glycol (Shillito *et al.*, 1985), liposome mediated transformation (Caboche, 1990) and the micro-projectile bombardment or *gene gun* (Sanford, 1988) have been used to introduce alien genetic information into plant cells.

In relation to indirect plant transformation methods, the most widely used has been via *Agrobacterium tumefaciens* (Hooykaas and Schilperoort, 1992), but also *Agrobacterium rhizogenes* - (Tepfer, 1990) and various viral vectors have been used to introduce foreign genes into plant cells (Brisson *et al.*, 1984).

Transformation mediated by *A. tumefaciens*, a ubiquitous plant pathogenic bacteria that causes the crown galls commonly seen on many trees, has turned into one of the most used technologies to introduce foreign genes in plant cells and the subsequent regeneration of transgenic plants (Nester *et al.*, 2004). *A. tumefaciens*, is capable of naturally infecting dicotyledonous plants, causing the formation of a neoplasm known as crown gall (Smith and Townsend, 1907). *A. tumefaciens* has the property of transferring a DNA (T-DNA) segment, that is found in the Ti plasmid, into the nucleus of the infected cells. Plasmids are extra chromosomal DNA sequences present in bacteria that have the property of auto replication and producing proteins from the genetic information present in them. The mechanism for T-DNA transfer from the bacterium to the plant cells is very well described, though it is still under strong research because the deep complexity of the whole mechanism (Pitzschke and Hirt, 2010). However, it is established that T-DNA transfer from *A. tumefaciens* occurs when another group of genes harbored into the Ti plasmid is activated by plant signals. The signals induce in the bacteria the expression virulence proteins and the formation of a type IV secretion system (T4SS). The virulence is known as *vir* region and its products drive the whole transfer mechanism of T-DNA, from the formation of the transfer intermediate (T-complex) to the movement into the plant cell and subsequent integration into the plant nucleus. The *vir* region is able to recognize and process the T-DNA that is also flanked by 25 bp (imperfect) direct repeats (also known as right and left borders) (Binns and Thomashow, 1988;

Torisky *et al.*, 1997). Recent findings have also shown the role of specific vir proteins (VirD2 and VirE2) on the exit of the T-DNA complex from the plant cell, trafficking inside the plant cell cytoplasm and nuclear targeting (Gelvin, 2010; Van Kregten *et al.*, 2009; Bhattacharjee *et al.*, 2008; Lacriox *et al.*, 2008; Tao *et al.*, 2004).

Once the T-DNA complex is in the plant cell, it is imported into the nucleus and stably integrated into the host chromosomes (Christie, 1997). Integration of T-DNA occurs in random and sometimes small fragments outside the T-DNA borders that can be inserted into the plant nucleus (Smith, 1998) and it has also been detected that large fragments of chromosomal *A. tumefaciens* genes can be also inserted into the plant nucleus (Ulker *et al.*, 2009). These findings could have relevant implications regarding the co-evolutionary processes of plant-bacterial interaction, but also for considering as a relevant aspect for releasing of transgenic plants into the field (Gelvin, 2008).

The results of the studies related to the process of T-DNA transfer to the plant cells have demonstrated three facts of great practical importance. Firstly, the tumor formation is the final result of the transference and integration of the T-DNA and its consequent expression in the plant cell. Secondly, the T-DNA is only submitted to the transcription process in plant cells and plays no important role during the transference process. Third, any foreign DNA that is inserted between two 25 bp repeated sequences, which border the T-DNA, may be introduced into the plant cells, independent of its donor (Torisky *et al.*, 1997; Akhond and Machray, 2009).

Though transgenic plant technologies have become a main technology for farmers there is still a long way to go in Chile to take advantage of them. In the last years, a range of plant species have been transformed through *A. tumefaciens*, including many species of economical importance to Chile, such as: 1) Apples (Flachowsky and Hanke, 2006); grapevines (Iocco *et al.*, 2001); blueberries (Song and Sing, 2004); and pears (Padilla *et al.*, 2006). Nevertheless, efficient and reproducible transformation of monocot species was obtained later but is now

becoming quite common in important species such as rice (Katiyar-Agarwal *et al.*, 2002), corn (Opabode, 2006), wheat (Jones 2005) and barley (Sharma *et al.*, 2005). However, the commercial application of national projects is forced to wait due the lack of a legal frame that allows the extensive use of transgenic plants in the Chilean agriculture.

In this context, it will be possible to increase the competitiveness of Chilean agricultural export products, such as grapes, wine and apples. Recently, it was demonstrated that transgenic grape plants (*cvs.* Silcora and Thompson Seedless) expressing an ovule-specific auxin-synthesizing (DefH9-iaaM) transgene that increases the indole-3-acetic acid content in the berry could increase fruit productivity. Transgenic Thompson Seedless plants expressing the foreign gene double the number of inflorescences per shoot, while berry number per bunch was increased in both cultivars expressing the transgenes. No modification of nutritional value and fruit quality were observed in the transgenic plants for both cultivars when compared to the non-transgenic plants. Therefore, the results indicate that modifying the auxin content could enhance fecundity and lead to increase yield with lower costs (Costantini *et al.*, 2007).

Transgenic approaches could also have a strong impact on the commercial value of apples. It is widely accepted that red coloration of apple (*Malus x domestica*) skin is a key determinant for consumers in several important markets for this fruit. The accumulation of anthocyanins in the skin is regulated by several genes that have been well characterized and it is thought that these genes are regulated by MYB transcription factors. A gene encoding for a transcription factor was isolated and characterized from apple skin (MdMYBA) and it was confirmed that its expression was specifically regulated depending on the tissue and cultivar/ species. However, it was also demonstrated that transient expression of the gene in cotyledonary tissues produced colored red spots (Ban *et al.*, 2007).

Another impacting application of the transgenic technology in the wine industry could be significant for reducing the production costs and

improving the competitiveness of the Chilean wine industry. Herbicide application in vineyards is quite expensive since they have to be selective and could produce plant losses if they are not properly applied. This problem could be reduced by introducing transgenic plants resistant to broad spectrum herbicide. Transgenic grape wine plants (cv. Chancellor) expressing the bacterial *tfdA* gene proved to be highly resistant to high doses of herbicides, reducing plant injury and survival of plants after application (Mulwa *et al.*, 2007).

Practical applications of genetic transformation and transgenic plants

Since the first transgenic plants were obtained and the development of efficient procedures to transfer exogenous information to plants, there has been a tremendous increase in the use of molecular biology in order to generate new cultivars with specific desired traits. In 2008, the cultivated area with transgenic plants worldwide was 125 millions of hectares, representing a 9.4% annual increase compared to 2007 (James, 2008).

The number of farmers who have incorporated transgenic plants into their production systems in 2008 was 13.3 million, in comparison to the 11 millions that cultivated them in 2007 (James, 2008). It is expected that in the next years these technologies will be even more accepted and that the number of humans benefitting will increase in consequence.

Among the most widely recognized benefits stemming from having transgenic plants in their productive systems are: a greater flexibility within the productive systems, a reduced use of chemical (especially herbicides and pesticides), the potential to use degraded soil, the reduction of production costs and consequently a reduction of prices to the consumers, a lower environmental impact and greater sustainability in the system (James, 2008). Currently, the newer varieties being released include ones with greater drought tolerance and better fertilizer use efficiency – which again will mean real advantages of applying such molecular technology.

Biotic and abiotic stress resistance

Transgenic plants with disease and pest resistance have offered an additional and very efficient option for farmers in developing countries. The use of transgenic plants of papaya (*Carica papaya* L.), resistant to the papaya ringspot virus (PRSV), in Hawaii has considerably increased the yields and diminished the costs associated with the pest control (Gonsalves, 1998). In the same way it is considered that transgenic plants that carry *Bacillus thuringiensis* genes (Bt plants) can reduce the use of chemical pesticides by 10% (Brookes and Barfoot, 2006).

The development of “self-protected” transgenic plants against biotic and abiotic stresses may help stabilize yields and productivity for crops of major economical importance worldwide. For example, the Rice Yellow Mottle Virus (RYMV) devastates African rice fields, directly affecting the plants or through its association with opportunist fungi, considerably limiting the yields. Traditional improvement has not been capable of creating resistance against this disease whereas in the last decade the use of a procedure denominated “genetic immunization” has created transgenic rice plants which are resistant to RYMV (Pinto *et al.*, 1999). The term “genetic immunization” has been adapted from animal biotechnology in regards of the delivery to a host organism of a cloned gene that encodes an antigen. After the cloned gene is expressed, it elicits an antibody response that protects the organism from infection by a virus, bacterium or other disease causing organism. In plants, the term is referred to resistance derived from an RNA based mechanism associated with post-transcriptional gene silencing (Pinto *et al.*, 1999). Cultivars resistant to this virus have been evaluated at field level in sub-Saharan Africa, and promise to help solve the cultivation of this grain by a great number of producers. After three sexual generations the plants have shown high levels of genetic resistance to viruses (Pinto *et al.*, 1999).

Other examples may be used in order to illustrate the actual investigations, including the virus resistance in papaya (Tennant *et al.*, 2001) and the resistance to bacteria in potato and rice (Torres *et al.*, 1999; Zhai *et al.*, 2000).

Annually millions of hectares of cultivable lands are lost worldwide by salinization and alkalization of the soil. Plant salinity resistance genes that present greater levels of tolerance towards salt stress, have been identified, isolated and transferred (for more detailed information see Kolodyazhnaya, 2009). To increase salt tolerance, the *gutD* gene of *Escherichia coli* was used to confer salt resistance in corn plants (Liu *et al.*, 1999). It has also been demonstrated that transgenic tomato plants accumulating polyamines are capable to tolerate high temperature stress (Cheng *et al.*, 2009).

Drought tolerance is another relevant target for plant breeding due the large rate of desertification that reduces agricultural lands every year. Seven candidate genes (CBF3, SOS2, NCED2, NPK1, LOS5, ZAT10, and NHX1) regulated by two different promoters are being now tested under field conditions to improve drought tolerance in rice. The first results demonstrated that two genes (LOS5 and ZAT10) protected the plants against drought stress (Xiao *et al.*, 2009).

Increases to the nutritional quality of crops

Annually, half a million children present vision problems due to vitamin A deficiency (Coanway and Toennissen, 1999) and the traditional methods of Genetic Improvement have had no success in the generation of commercial cultivars which are rich in this vitamin (Aluru *et al.*, 2008). For this reason, the main treatments against this nutritional deficit are based on therapies with vitamin supplements in tablet form. Transgenic rice plants have been produced and demonstrated to have a greater concentration of β -carotene, a main precursor of vitamin A, giving a yellowish color in their grains (Ye *et al.*, 2000). This helps in partly constituting a solution as a nutritional supplement in tropical areas. Several years after, this plant served as the prototype basis for the creation of the "Golden-Rice" plants (Al-Babili and Beyer, 2005). Similarly, transgenic maize by expressing the bacterial genes *crtB* (for phytoene synthase) and *crtI* (for the four desaturation steps of the carotenoid pathway catalysed by phytoene desaturase and ζ -carotene desaturase in plants), under the con-

trol of a 'super γ -zein promoter' for endosperm-specific expression, increased the amount of carotenoids (specially β -carotene) in the endosperm tissues (Aluru *et al.*, 2008).

Another problem which affects a significant fraction of humanity, is anemia. More than 400 million women of fertile age suffer of anemia, particularly related with maternity. In Africa and Asia more than 20% of the maternal deaths are related to anemia (Coanway and Toennissen, 1999). A transgenic rice cultivar with high levels of iron was obtained through the expression of a protein that associates to the iron molecules and the expression of an enzyme which facilitates iron availability in the diet (Ye *et al.*, 2000). The transgenic plants presented between 2 to 4 times more iron than the non transgenic controls.

Use of plants as biorreactors

The use of transgenic plants for the production of recombinant proteins for pharmaceutical use has led to one of the technologies with considerable potential for future development, this technology is known as "Molecular farming". The production of proteins of pharmaceutical and industrial interest form an industry that is actually valued at 40,000 million dollars annually, with a growth potential that depends on the innovative capacity of companies, universities and research centers (Howard, 2005).

The proteins of pharmaceutical interest may be expressed in a stable way in transgenic plants or through the transitory expression in tissues infected with virus, transfected with the coding genes for the proteins of interest (Daniell *et al.*, 2001).

Vaccines for human and animal use, are fundamentally produced on the basis of attenuated or biologically dead, but still immunologically active, pathogens, or in systems of recombinant expression such as yeast, bacteria or animal cells. In general, vaccines for human use utilize potentially toxic preservatives that may trigger immunological response in humans and cause secondary allergenic reactions. For this reason, this type of vaccine require expensive

and well established purification processes, that guarantee the security of the vaccination programs.

On the other hand, it is expected that any effective vaccine is secure, of sustainable action in time, stable, easy to administrate, of low cost and of reduced collateral effects. In this sense the expression systems of plant origin have appeared as a secure and inexpensive strategy for obtaining vaccines because they can produce recombinant proteins capable of triggering the immune response in mammals and because they are able to express, process and glycosylate correctly the proteins of interest, stably maintaining their biological activity (Glenz and Warzecha, 2006).

During 1990's the idea of using plant models as vaccines was firstly proposed, when Arntzen and his collaborators referred to the effectiveness of the system and to the low costs of the immunization based on transgenic plants, which would express antigens of interest. This technology could reduce the costs of vaccination programs and at the same time be useful to fight diseases that affect developing countries (Sala *et al.*, 2003). It was proposed that it was possible to produce specific proteins in fresh or processed plant parts consumed by humans such as fruits, seeds, tubers, leaves and petioles, allowing direct immunization and eliminating the purification, conservation and transport requirements of traditional forms of vaccines (Sala *et al.*, 2003). In the same way, it would be possible to produce hundreds of tons of biomass of species for extraction of vaccination programs.

The technology saw its first results in 1990 when a surface antigen of *Streptococcus mutans* was expressed in tobacco plants and it was demonstrated that transgenic plants generated immunological response in mice (Curtiss and Cardineau, 1990).

The concept of oral vaccine was established at the moment in which it was demonstrated that the surface antigen of Hepatitis B expressed in transgenic plants was capable of triggering immune response in animals that consumed it in their diet (Mason, 2002). Since then, the expression of new antigens in several plant species, with variable expression levels, has been

reported (Carter and Langridge, 2002). A series of reviews regarding the topic are available which allow a clearer picture of the magnitude of the research work that is being carried out, but at the same time indicate opportunities to develop research projects in innovative areas which have a major social impact (Sala *et al.*, 2003; Howard, 2005).

The expression of antibodies from plants has been another result of the application of molecular biology techniques, immunology and plant tissue culture (Wycoff, 2005). Antibodies are proteins which are part of the humoral immune response of mammals and are responsible for maintaining the defenses against certain epitopes (molecules, ions) which are known as antigens. Antigens, which may be of organic or inorganic nature, may enter the organism in a direct way through an infectious agent, which is also recognized by the immune system. They are recognized and neutralized by antibodies and "labeled" for their elimination by the immune defense cellular machinery of the organism. The IgG are the principal antibodies produced by the immune system which also produces IgA, IgM, IgD and IgE. These present two pairs of identical polypeptides called the light and heavy chain which form a "Y" structure, with very conserved regions in the base (Fc) and variable in the extreme of the arms (Fv). The light chains are linked to the arms of the heavy chains by disulphide bonds. The Fv region is responsible of the antigen-antibody union. The heavy and light chains are combined in pairs allowing the union of two antigen molecules to each antibody molecule and that the antigen-antibody union is specific. As the constant regions are not necessary for the antigen-antibody union, it is possible to express small sections of the variable regions and maintain the antibody activity (Fischer *et al.*, 2003).

The plant systems offer several advantages over the natural systems of antibody production, such as: low investment, production and purification costs, easy process scale-up, the non-existence of contaminant proteins or blood pathogens and the separate expression of the heavy and light chains with the consequent *in vivo* or *in vitro* assembly of the cellular organelles, as

it occurs with the secreting antibodies in mammals (Gargouri-Bouزيد *et al.*, 2006). The versatility of plants in order to express the proteins of different organisms (prokaryotes, animals) distinguishes them with respect to the rest of the known models. Besides, plants are able to generate large quantities of biomass and the production-purification costs tend to be lower than the rest of the animal or bacterial expression systems (Wycoff, 2005).

The concept of plants for expressing pharmacologically interesting proteins is enriched when considering the possibility of expressing proteins in plants which may improve the nutritional value of food or cosmetic quality. For example, antioxidants and flavonoids that are beneficial for human health for their antioxidant capacity have been a target in work related with the modification of their biosynthetic pathways (Kovacs *et al.*, 2007).

Conclusions

Plant tissue culture techniques have made significant contributions to the advance of agricultural sciences in recent times and today they constitute an indispensable tool in modern agriculture. The access to technology is no longer the exclusive of developed countries and so it is necessary that we all recognize the potentialities and that we exploit the technology in all

of its dimensions. The benefits already have moved from being regarded as merely part of the agricultural production. The plants and the productive systems based on modern agriculture are quickly becoming major revenue earners, but at the same time: guaranteeing food security worldwide and helping provide a better standard of living for each one of the inhabitants of the planet. The technology has demonstrated its usefulness and is available, now it's our turn to use it on a massive but responsible scale. In Chile, the time is right to invigorate the debate and renew the legal framework by considering that it is already permitted to grow transgenic plants and to import products which contain transgenic ingredients. Some sectors of our agriculture are under a growing pressure from Brazil and Argentina farmers, our main direct competitors in the region. This discussion has to be carried out with a clear vision of scientific elements, the market forces and the social reality of our country.

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Resumen

R. García-González, K. Quiroz, B. Carrasco y P.D.S. Caligari. 2010. Cultivo de tejidos de plantas: estado actual, oportunidades y desafíos. Cien. Inv. Agr. 37(3): 5-30. La aplicación de la biotecnología vegetal en la agricultura mundial ha experimentado un avance importante en los últimos veinte años y las técnicas de cultivo de tejidos vegetales han sido un soporte fundamental para estos avances. Los resultados actuales han propiciado que la biotecnología vegetal y sus productos –incluido el uso de las plantas transgénicas– sea la tecnología de más rápida asimilación por los agricultores y que los beneficios se hayan materializado en 125 millones de hectáreas de cultivos transgénicos cultivados en 2008, la reducción en un 9% de la aplicación de pesticidas en los últimos diez años, la creación de plantas transgénicas con mejor calidad nutritiva, la propagación masiva de plantas y genotipos élitos y la producción de proteínas de interés farmacéutico e industrial en células vegetales. La rápida asimilación de técnicas biotecnológicas ha mejorado la competitividad tanto de países industrializados como de países en desarrollo, gracias a la correcta aplicación de programas gubernamentales, regulaciones y el estímulo a la investigación. En este trabajo de revisión se abordan aspectos teórico-prácticos que sustentan el desarrollo de las técnicas de cultivo de tejidos, así como los principales resultados y avances en este campo para que el lector cuente con elementos que le permitan evaluar la potencialidad de estas tecnologías en el desarrollo agropecuario, la industria farmacéutica y la mejora de la calidad de vida de la población.

Palabras claves: biorreactores vegetales; embriogénesis somática; *in vitro*; micropropagación; organogénesis; plantas transgénicas.

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