Chapter 1
Somatic Embryogenesis. An Overview

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Abstract Somatic embryogenesis is one of the most powerful tools in plant biotechnology. It can be used to produce plants commercially, or to carry out basic studies of cell differentiation, gene expression, molecular genetics, and many others. We present here a compilation of the different chapters of this book.

1.1 Introduction

Initially, the necessity of solving important fundamental questions in plant biology, such as the cell theory and totipotency, led to the development of plant cell, tissue, and organ culture (PTC). However, nowadays PTC represents a set of very powerful biotechnological tools. The applications of PTC include commercial micropropagation of agronomically important plant species, production of haploid and double-haploid plants and disease-free plants, rescue of hybrid embryos or somatic cell fusion from intra- or inter-generic sources for the production of novel hybrid plants, induction of genetic or epigenetic variation for the production of variant plants, and more recently the genetic engineering of plants to produce new varieties, resistant to pests and diseases, as well to improve the quality and quantity of a particular product obtained from a plant. Other applications include genetic modification to produce plants that can remove toxic compounds or test its toxicity (bioremediation) (Hannink et al. 2001; Krämer and Chardonnens 2001), and the use

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of micropropagated shoots to maintain plant viruses. Root cultures can be used to study the interaction of roots with nematodes or mycorrhizas. Recently, plants have been modified by genetic engineers with the objective to increase the yield of cellulose or oil for the production of biofuels (Gressel 2008; Stokstad 2012; Takahashi and Takamizo 2012). Somatic embryogenesis is at the core of some of these biotechnological applications and is the focus of this book.

Gottlieb Haberlandt in 1902 (Haberlandt 1902) set the theoretical basis for plant tissue culture. He proposed that a single cell should eventually be capable of giving origin to a complete and functional plant. This theory has been proved to be right. At the core of this proof is the somatic embryogenesis.

1.2 Somatic Embryogenesis

Plant somatic embryogenesis (SE) is a biological process with both amazing basic and applied aspects. SE occurs in nature. At the edges of the leaves of several species of Kalanchoë appear small bipolar structures, some of them produce these structures constitutively, others by the action of environmental stress, and a third group is a combination of both (Garces and Sinha 2009).

In vitro plant cells can undergo dedifferentiation or redifferentiation to enter a new biological program that gives rise to somatic embryos. This process has raised one of the most important biological questions: which signals change the genetic program of a somatic cell and make it an embryogenic cell?

Numerous factors that affect SE have been investigated in order to understand the basis of this process and manipulate it to develop and establish efficient plant regeneration protocols as a fundamental step for its biotechnological application (Loyola-Vargas et al. 2008). A differentiated and specialized somatic plant cell or a group of somatic cells with specific functions must receive a stimulus from a set of plant growth regulators (PGRs), mainly auxins, perceive it, and then initiate the transduction to the nucleus where the specific regulatory and structural genes will be transcribed and subsequently will be translated into proteins involved in the differentiation that ultimately will lead to the formation of a new somatic embryo. All these changes can be followed at morphological, ultrastructural, genetic, physiological, biochemical, and molecular levels.

The idea of this book is to look somatic embryogenesis in an integrative way covering from the historical aspects of somatic embryogenesis to its applications. It is important to know about the history of those researchers whose contributions led to the development of this field. In Chap. 2 we describe the main facts that led to the historical first papers on SE (Miettinen and Waris 1958; Reinert 1959; Steward et al. 1958).

There are several pathways to initiate somatic embryogenesis (Chap. 3). Unlike the initial belief that all plant cells are totipotents, it has been seen that it is necessary to create appropriate conditions for some of them to regain totipotency. Among the several factors that play a role in the induction of SE are the plant

growth regulators, mainly auxins (Altamura et al. 2016). It is interesting that many species require an initial shot of auxins, but thereafter the auxin must be degraded for SE to proceed (Chap. 10) (Altamura et al. 2016). Clearly the onset of SE depends on a complex network of interactions among plant growth regulators, mainly auxins and cytokinins, during the early proembryogenic stages. Ethylene and gibberellic and abscisic acids pass to play a major role during the late stages of development. Together, the PGRs regulate multiple genes temporally and spatially which release the changes in the genetic program of somatic cells, as well as regulating the transition between each embryonic developmental stage.

In addition to phenotype, the origin of the explant, and genetic background of the plant, several stress treatments such as heavy metals, low or high temperature, osmotic shock, among others, might play a crucial role in SE induction, even in the absence of exogenous PGRs (Chap. 9) (Cabrera-Ponce et al. 2015; Ochatt and Revilla 2016; Salo et al. 2016).

An important concern is the fidelity of the somatic embryogenesis-regenerated plants (Chap. 8). There is an epigenetic reprogramming during the SE and the presence of somaclonal variation among the regenerated plants (De-la-Peña et al. 2015; Mahdavi-Darvari et al. 2015; Nic-Can et al. 2015; Solís et al. 2015). This variation can be the result of chromosomal aberrations, genetic alterations, epigenetic regulations, and transposable elements. The variation can be exploited for good, as selecting stress-tolerant somaclones (Bobadilla Landey et al. 2013; Us-Camas et al. 2014).

Beyond the biotechnological application of SE, it can be used to study the very different aspects of its induction and the development of somatic embryos. An aspect that is central to the study of SE is histology. SE has become an appropriate method for studying the morphophysiological and molecular aspects of cell differentiation (Chap. 26). The understanding of the developmental events during the induction phase as well as the development of somatic embryos is essential to regulate and improve each stage of the SE program efficiently. Anatomical and ultrastructural studies may be useful for the development of protocols more efficient for SE induction, as well as for the cellular mechanisms involved in the acquisition of competence for SE (Konieczny et al. 2012; Quiroz-Figueroa et al. 2002).

The molecular aspects of SE have been studied extensively. In this book, several authors have revised the most recent advances in the field. Transcriptomics of several species has been carried out during the induction of SE and the development of the somatic embryos (Chap. 4). Cotton is the species most studied, but the number of species investigated by this technique is growing every day. The pattern that is emerging from these studies suggests a predominant role of auxins during the induction of SE, as well as for genes like *LEC*, *WUS*, *FUS*, and a set of transcription factors (Shi et al. 2016; Tao et al. 2016; Trontin et al. 2016). The Next Generation Sequencing platforms of nucleic acids can be used together with techniques that allow the isolation of a specific cellular type, such as laser-assisted microdissection. Together these two techniques give us a closer approach to the state of the cell in determined space and time (Chap. 27).

The extreme changes required for the transcription of the genome during the change of a somatic cell to an embryogenic cell need a very active participation of transcription factors. In Chap. 5, authors made an extensive analysis of the state of the art in relation to the participation of transcription factors in this process. An interesting finding is that the most frequent transcription factors found active during the induction of SE belong to the pathways of the metabolism of growth regulators, stress, and flower development (El Ouakfaoui et al. 2010; Guan et al. 2009).

Among all the different mechanisms that regulate the expression of the genes, epigenetics also plays an important role (Chap. 6). Different reports suggest that auxins, in conjunction with the in vitro conditions modify the DNA methylation status in the embryogenic cells. These changes in DNA methylation patterns are associated with the regulation of several genes involved in SE, such as *WUS*, *BBM1*, *LEC,* and several others (De-la-Peña et al. 2015).

After the genes are expressed, all the weight of the process is on the proteins. Posttranslational modifications, protein turnover, and protein–protein interactions are common processes associated with the regulation of proteins. All of them are present during the induction of SE and development of somatic embryos. Proteomic studies carried out while the SE has begun to show the deep mechanism that works during the induction of SE (Chap. 7). One key question is if there is a common protein pattern among different species during the induction of SE (Campos et al. 2016; Mukul-López et al. 2012; Tchorbadjieva 2016).

SE has been induced in many different species; many of them crops of commercial interest. In the second part of the book, the SE of several important crops is analyzed: *Agave* spp., *Bixa orellana*, *Capsicum* spp., *Coffea* spp., *Curcuma*, *Musa* spp., *Zea mays*, lipid-producing plants like *Cocus nucifera* and *Jathropha curcas*, conifers such as *Pinus* spp., and model plants as *Arabidopsis thaliana* (Chaps. 11–22).

The two major applications of SE are scale-up propagation (Chap. 24) and genetic engineering (Chap. 23). Among the different systems to scale up the process of SE, the temporary immersion system has some advantages. It can be automatized to reduce labor and costs, and at the same time to produce high-quality plantlets (Fei and Weathers 2014, 2016; Ibaraki and Kurata 2001). The SE process is a very efficient method to regenerate transgenic plants. SE has been used in conjunction with *Agrobacterium* spp., particle bombardment, and chemical-mediated genetic transformation protocols. All the major annual and perennial crops, as well as model plants, have been transformed using efficient SE systems (Arroyo-Herrera et al. 2008; Bouchabké-Coussa et al. 2013; Canché-Moor et al. 2006; Palomo-Ríos et al. 2012).

Another application analyzed is the use of SE to produce secondary metabolites. The production of secondary metabolites by plants requires highly specialized tissues and a fine regulation and coordination in time and development by the plant (De Luca and St Pierre 2000). In nature, several plant species synthesize and store secondary metabolites in the zygotic seed, suggesting that somatic embryos can do it. In Chap. 25 the most recent discoveries related to the accumulation of secondary metabolites by somatic embryos are presented (Aslam et al. 2010, 2011; Sharma et al. 2015).

1.3 Concluding Remarks

PTC in general and SE in particular have turned into an invaluable tool to plant scientists. PTC has been commercialized around the world, and different companies are using plant tissue culture techniques for the massive propagation of plants. The use of PTC and the development of the omics and epigenetics have allowed the understanding of the basic biological process.

The use of SE leads to the understanding of differentiation, as well as to the genetic mechanisms involved in the transition from one stage to the next. Also it has led to the isolation of genes, proteins, and metabolites involved in the cell differentiation process. The combination of SE and genetic engineering will accelerate the discovery, isolation, and characterization of genes involved in different cellular processes.

From the agronomy side, the most important challenges ahead are the generation of resistant plants to pathogens and pests, as well as to abiotic stresses, increments

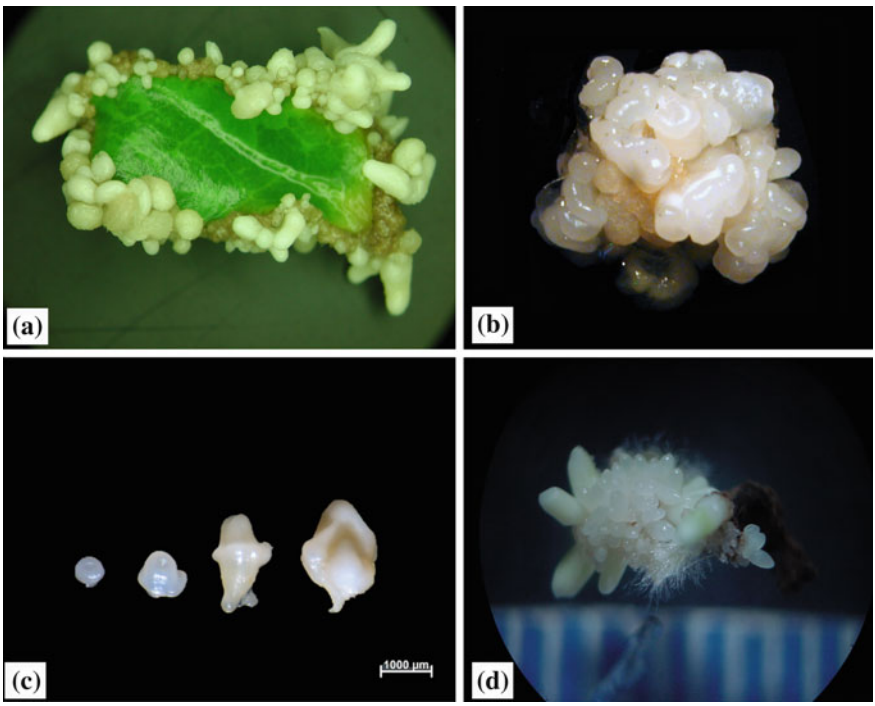

Fig. 1.1 Somatic embryogenesis process in different species. **a** Somatic embryogenesis in a leaf of *Coffea canephora*. **b** Embryogenic mass of *Cocus nucifera*. **c** Different developmental stages of *Musa acuminata* x *Musa balbisiana* genome AAB, subgroup Plantain. **d** Embryogenic mass of *Agave tequilana*. Picture **a** is from the laboratory of Víctor M. Loyola-Vargas. Pictures **b–d** are gifts from the laboratories of Carlos Oropeza-Salín, Rosa M. GraciaMedrano from Centro de Investigación Científica de Yucatán and Benjamín Rodríguez-Garay from Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, respectively

in yields in commercial crops, the production of better raw material for biofuel production, as well as the generation of plants capable of absorbing toxic compounds from the environment. In all these cases, SE will play an important role (Fig. 1.1).

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References

Altamura MM, Della Rovere F, Fattorini L et al (2016) Recent advances on genetic and physiological bases of in vitro somatic embryo formation. In: Germana MA, Lambardi M (eds) In Vitro embryogenesis in higher plants, Springer, New York, pp 47–85. doi:10.1007/978-1-4939-3061-6_3

Arroyo-Herrera A, Ku-Gonzalez A, Canche-Moo R et al (2008) Expression of *WUSCHEL* in *Coffea canephora* causes ectopic morphogenesis and increases somatic embryogenesis. Plant Cell Tiss Org 94:171–180. doi:10.1007/s11240-008-9401-1

Aslam J, Mujib A, Sharma M (2011) Influence of freezing and non-freezing temperature on somatic embryogenesis and vinblastine production in *Catharanthus roseus* (L.) G. Don Acta Physiol Plant 33:473–480. doi:10.1007/s11738-010-0569-8

Aslam J, Mujib A, Fatima Z et al (2010) Variations in vinblastine production at different stages of somatic embryogenesis, embryo, and field-grown plantlets of *Catharanthus roseus* L. (G) Don, as revealed by HPLC. In Vitro Cell Dev-Pl 46:348–353. doi:10.1007/s11627-010-9290-y

Bobadilla Landey R, Cenci A, Georget F et al (2013) High genetic and epigenetic stability in *Coffea arabica* plants derived from embryogenic suspensions and secondary embryogenesis as revealed by AFLP, MSAP and the phenotypic variation rate. PLoS ONE 8:e56372. doi:10.1371/journal.pone.0056372

Bouchabké-Coussa O, Obellianne M, Linderme D et al (2013) *Wuschel* overexpression promotes somatic embryogenesis and induces organogenesis in cotton (*Gossypium hirsutum* L.) tissues cultured in vitro. Plant Cell Rep 32:675–686. doi:10.1007/s00299-013-1402-9

Cabrera-Ponce JL, López L, León-Ramírez CG et al (2015) Stress induced acquisition of somatic embryogenesis in common bean *Phaseolus vulgaris* L. Protoplasma 252:559–570. doi:10.1007/s00709-014-0702-4

Campos NA, Paiva LV, Panis B et al (2016) The proteome profile of embryogenic cell suspensions of *Coffea arabica* L. Proteomics. doi:10.1002/pmic.201500399

Canché-Moor RLR, Kú-González A, Burgeff C et al (2006) Genetic transformation of *Coffea canephora* by vacuum infiltration. Plant Cell Tiss Org 84:373–377. doi:10.1007/s11240-005-9036-4

De Luca V, St Pierre B (2000) The cell and developmental biology of alkaloid biosynthesis. Trends Plant Sci 5:168–173. doi:10.1016/S1360-1385(00)01575-2

De-la-Peña C, Nic-Can GI, Galaz-Avalos RM et al (2015) The role of chromatin modications in somatic embryogenesis in plants. Front Plant Sci 6:635. doi:10.3389/fpls.2015.00635

El Ouakfaoui S, Schnell J, Abdeen A et al (2010) Control of somatic embryogenesis and embryo development by AP2 transcription factors. Plant Mol Biol 74:313–326. doi:10.1007/s11103-010-9674-8

Fei L, Weathers P (2016) Bioreactors for plant embryogenesis and beyond. In: Germana MA, Lambardi M (eds) In vitro embryogenesis in higher plants, Springer, New York, pp 245–259. doi:10.1007/978-1-4939-3061-6_10

Fei L, Weathers P (2014) From cells to embryos to rooted plantlets in a mist bioreactor. Plant Cell Tiss Org 116:37–46. doi:10.1007/s11240-013-0380-5

Garces H, Sinha N (2009) The 'mother of thousands' (Kalanchoë daigremontiana): A plant model for asexual reproduction and CAM studies. Cold Spring Harb Protoc 2009. doi:10.1101/pdb.emo133

Gressel J (2008) Transgenics are imperative for biofuel crops. Plant Sci 174:246–263. doi:10.1016/j.plantsci.2007.11.009

Guan Y, Ren H, Xie H et al (2009) Identification and characterization of bZIP-type transcription factors involved in carrot (*Daucus carota* L.) somatic embryogenesis. Plant J 60:207–217. doi:10.1111/j.1365-313X.2009.03948.x

Haberlandt G (1902) Kulturversuche mit isolierten pflanzenzellen. Sber Akad Wiss Wein 111:69–92

Hannink N, Rosser SJ, French CE et al (2001) Phytodetoxification of TNT by transgenic plants expressing a bacterial nitroreductase. Nat Biotechnol 19:1168–1172. doi:10.1038/nbt1201-1168

Ibaraki Y, Kurata K (2001) Automation of somatic embryo production. Plant Cell Tiss Org 65:179–199. doi:10.1023/A:1010636525315

Konieczny R, Sliwinska E, Pilarska M et al (2012) Morphohistological and flow cytometric analyses of somatic embryogenesis in *Trifolium nigrescens* Viv. Plant Cell Tiss Org 109:131–141. doi:10.1007/s11240-011-0081-x

Krämer U, Chardonnens AN (2001) The use of transgenic plants in the bioremediation of soils contaminated with trace elements. Appl Microbiol Biotechnol 55:661–672. doi:10.1007/s002530100631

Loyola-Vargas VM, De-la-Peña C, Galaz-Avalos RM et al (2008) Plant tissue culture. In: Walker JM, Rapley R (eds) Protein and cell biomethods handbook. Humana Press, Totowa, pp 875–904. doi:10.1007/978-1-60327-375-6_50

Mahdavi-Darvari F, Noor N, Ismanizan I (2015) Epigenetic regulation and gene markers as signals of early somatic embryogenesis. Plant Cell Tiss Org 120:407–422. doi:10.1007/s11240-014-0615-0

Miettinen JK, Waris H (1958) A chemical study of the neomorphosis induced by glycine in *Oenanthe aqnatica*. Physiol Plant 11:193–199. doi:10.1111/j.1399-3054.1958.tb08457.x

Mukul-López HG, De-la-Peña C, Galaz-Avalos RM et al (2012) Evaluation of the extracellular proteome profile during the somatic embryogenesis process of *Coffea* spp. J Mex Chem Soc 56:72–79

Nic-Can GI, Galaz-Avalos RM, De-la-Peña C et al (2015) Somatic embryogenesis: Identified factors that lead to embryogenic repression. A case of species of the same genus. PLoS ONE 10:e0126414. doi:10.1371/journal.pone.0126414

Ochatt S, Revilla M (2016) From stress to embryos: Some of the problems for induction and maturation of somatic embryos. In: Germana MA, Lambardi M (eds) in vitro embryogenesis in higher plants. Springer, New York, pp 523–536. doi:10.1007/978-1-4939-3061-6_31

Palomo-Ríos E, Barceló-Muñoz A, Mercado J et al (2012) Evaluation of key factors influencing *Agrobacterium*;-mediated transformation of somatic embryos of avocado (*Persea americana* Mill.). Plant Cell Tiss Org 109:201–211. doi:10.1007/s11240-011-0086-5

Quiroz-Figueroa FR, Fuentes-Cerda CFJ, Rojas-Herrera R et al (2002) Histological studies on the developmental stages and differentiation of two different somatic embryogenesis systems of *Coffea arabica*. Plant Cell Rep 20:1141–1149. doi:10.1007/s00299-002-0464-x

Reinert J (1959) Uber die kontrolle der morphogenese und die induktion von adventivembryonen an gewebekulturen aus karotten. Planta 53:318–333. doi:10.1007/BF01881795

Salo HM, Sarjala T, Jokela A et al (2016) Moderate stress responses and specific changes in polyamine metabolism characterize Scots pine somatic embryogenesis. Tree Physiol. doi:10.1093/treephys/tpv136

Sharma C, Kumari T, Pant G et al (2015) Plantlet formation via somatic embryogenesis and LC ESI Q-TOF MS determination of secondary metabolites in *Butea monosperma* (Lam.) Kuntze. Acta Physiol Plant 37:239. doi:10.1007/s11738-015-1973-x

Shi X, Zhang C, Liu Q et al (2016) De novo comparative transcriptome analysis provides new insights into sucrose induced somatic embryogenesis in camphor tree (*Cinnamomum camphora* L.). BMC Genom 17:26. doi:10.1186/s12864-015-2357-8

Solís MT, El-Tantawy AA, Cano V et al (2015) 5-azacytidine promotes microspore embryogenesis initiation by decreasing global DNA methylation, but prevents subsequent embryo development in rapeseed and barley. Front Plant Sci 6:472. doi:10.3389/fpls.2015.00472

Steward FC, Mapes MO, Mears K (1958) Growth and organized development of cultured cells. II. Organization in cultures grown from freely suspended cells. Am J Bot 45:705–708

Stokstad E (2012) Engineered superbugs boost hopes of turning seaweed into fuel. Science 335:273. doi:10.1126/science.335.6066.273

Takahashi W, Takamizo T (2012) Molecular breeding of grasses by transgenic approaches for biofuel production. In: Ciftci YÖ (ed) Transgenic Plants—Advances and limitations, In Tech, Rijeka, Croatia, pp 91–116. doi:10.5772/31889

Tao L, Zhao Y, Wu Y et al (2016) Transcriptome profiling and digital gene expression by deep sequencing in early somatic embryogenesis of endangered medicinal *Eleutherococcus senticosus* Maxim. Gene 578:17–24. doi:10.1016/j.gene.2015.11.050

Tchorbadjieva M (2016) Advances in proteomics of somatic embryogenesis. In: Mujib A (ed) Somatic embryogenesis in ornamentals and its applications. Springer 2016_SEOP_67_39983, India, pp 67–90. doi:10.1007/978-81-322-2683-3_5

Trontin JF, Klimaszewska K, Morel A et al (2016) Molecular aspects of conifer zygotic and somatic embryo development: A review of genome-wide approaches and recent insights. In: Germana MA, Lambardi M (eds) In Vitro Embryogenesis in Higher Plants, Springer, New York, pp 167–207. doi:10.1007/978-1-4939-3061-6_8

Us-Camas R, Rivera-Solís G, Duarte-Aké F et al (2014) In vitro culture: an epigenetic challenge for plants. Plant Cell Tiss Org 118:187–201. doi:10.1007/s11240-014-0482-8