An Overview on Stress and Somatic Embryogenesis

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Abstract- **Global climatic changes and growing demographic pressure have increased demand for agronomic resources, leading to an increasing lack of land suitable for agriculture; they have moreover provoked several abiotic stresses which, added to the biotic ones, result in physiological and metabolic disorders that ultimately impact on yield when it most needs to be improved. Understanding and resolving the impact of stress on yield is a major scientific and agronomic challenge and biotechnological breeding would be an efficient alternative. However, to reduce risks of soma clonal variations among regenerants and transformants, it is better to produce them through somatic embryogenesis. Somatic embryogenesis is a means by which plants can regenerate bipolar structures from a somatic cell. Somatic embryogenesis can be induced in-vitro by exposing explants to stress or growth regulator treatments. The ability to change their cell fate and generate somatic embryos is restricted to a discrete group of cells. Somatic embryogenesis is a complex phenomenon, and it is poorly understood. Somatic embryogenesis can start in various ways and the differential response among cells can be stimulated by several factors, among them highlight the genotype, the physiological state and the origin of the explants, as well as the medium culture or the plant growth regulators used for in-vitro culture. However, several stress treatments such as low or high temperature, heavy metals, osmotic shock, among others, might play a crucial role in somatic embryogenesis induction, even in the absence of exogenous plant growth regulators. Here in this narrative review we aimed to describe and delineate on recent perspectives on stress and their influence on somatic embryogenesis induction.**

Keywords: **Somatic embryogenesis, Stress, Signaling, Induction, Growth regulators.**

I. INTRODUCTION

Somatic embryogenesis represents a complete model of totipotency and involves the action of a complex signaling network, as well as the reprogramming of gene expression patterns that are regulated in a specific way. This gene regulation usually is in response to exogenous stimuli produced by the use of plant growth regulators (PGRs) or certain stress conditions, mainly low or high temperature, heavy metals, osmotic shock or drought [1]. The ability of an explant to undergo direct or indirect embryogenesis was historically thought to be determined by the age of the explant: the further the explant is from the zygotic embryo stage, the more reprogramming (callus formation) is required to convert the explant into a somatic embryo [2]. Although it is often more difficult to obtain somatic embryos from developmentally older tissues and organs, when somatic embryos develop, they can develop by either the direct route or the indirect route regardless of the age of the tissue [3]. It seems that the developmental context of a cell or tissue in combination with the culture environment is more important in defining whether embryogenesis is direct or indirect than its developmental distance from the embryonic state.

Higher plant embryogenesis is divided conceptually into two distinct phases: early morphogenetic processes that give rise to embryonic cell types, tissues, and organ systems, and late maturation events that allow the fully developed embryo to enter a desiccated and metabolically quiescent state [4]. Embryogenesis is the process by which embryo formation is initiated, either from a zygote viz. zygotic embryogenesis (ZE) or from somatic cells viz. somatic embryogenesis (SE). ZE is carried out after the fusion of gametes. However, the formation of asexual embryos can be induced in vitro from cells that come from an explant of vegetal tissue [5]. The SE process also occurs in nature. Under certain environmental conditions such as heat and drought, the plant Kalanchoë produces, around their leaves, small bipolar structures, which develop later in plantlets [6]. There are several other paths leading to the formation of an embryo. For instance, apomictic embryogenesis takes place in the seed primordium [ovule] and the embryos produced are genetically identical to the

mother plant. Microspores can also produce embryos, and the cells of the suspensor can change their identity to embryogenic cells when the original embryo loses its capacity to develop [7].

The induction of SE in vitro can be accomplished through two pathways. When SE is direct, somatic embryos are formed at the edge of an explant; when it is indirect, SE occurs through the proliferation of a disorganized and dedifferentiated tissue called callus [8]. SE can be induced in a wide range of explants, most commonly by treating them with plant growth regulators, usually the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) and/or abiotic stress treatments. The mechanism underlying auxin- and stress-induced SE is not known, but both treatments induce biosynthesis of endogenous auxins, which is thought to be an important early step in the switch to totipotent growth [9].

Somatic embryogenesis has several biological and scientific advantages. For instance, it has the potential for the improvement of plants of commercial importance, as well as for the study of the genetic and physiological changes that are related to the fate of a plant cell. Until now, most studies have examined the mechanisms involved in the induction of the SE process using model plant species, such as carrot, alfalfa, corn, and rice. However, other species, such as *Arabidopsis thaliana* and *Gossypium hirsutum*, have been used to study the signaling pathways of the PGR action leading to the development of plant cells [10].

II. EARLY SOMATIC EMBRYOGENESIS

Once the somatic cells are induced to generate cells with embryogenic capacity, the new cells can form structures capable of regenerating a complete plant. System suspensors are very noticeable in gymnosperm somatic embryos. However, in many angiosperms, suspensors are either absent or strongly reduced due to the absence of the hypophyseal cell [11].

It is unclear how cells initiate embryo formation. Nonetheless, it has been established that an irregular distribution of auxins must be established to initiate embryo formation. This asymmetrical auxin distribution results from differential transport [12]. In the case of ZE, an asymmetric cell division occurs, whereas in SE this is often not observed [13]. An asymmetric mitotic division of the zygote produces two different cells: one cell gives rise to the suspensor and the other to the embryo proper. At the octant and globular stage, protoderm formation and primordial initiation takes place [14]. The differential transport and asymmetrical auxin distribution continue during these stages, giving rise to the different tissues that will form the embryo. The transportation and accumulation of auxin produce the interaction with other factors, such as cytokinins (CKs), which leads to the expression of specific genes [15].

III. STAGES OF EMBRYO DEVELOPMENT

Although there is a morphological resemblance between somatic and zygotic embryos, their development is distinctive based on plant classification (angiosperms and gymnosperms). It is considered that zygotic embryos are nourished via the phloem tissue, whereas somatic embryos use an exogenous supply of carbohydrates and their morphological stages occur without vascular tissue connection [16].

Theoretically, plant development can be divided into two different phases: (1) embryogenesis, which begins with the formation of the zygote and concludes at the cotyledonary stage, and (2) the maturation of the seed [17]. The somatic and zygotic embryo developmental stages are divided into two main metabolic phases. The first is at a morphogenetic level, where the meristem activity is triggered at a physiological level and the process of growth, storage and maturation is initiated. The second is a metabolic stage that is characterized by biochemical activities and the preparation for desiccation to complete the seed formation process [16]. In this last phase, somatic embryos achieve both morphological and physiological maturity, which guarantees satisfactory post-embryonic performance. Therefore, the conversion potential is considered to be programmed during embryo maturation. However, somatic embryos do not require desiccation.

Somatic embryo development involves similar stages to ZE, such as the globular-shaped, heart-shaped, torpedo-shaped, and cotyledonal stages in the case of dicotyledonous species, and globular, scutellar, and coleoptile stages in the case of monocotyledonous species. Once the somatic embryos reach the cotyledonary stage, they initiate a shoot meristem, and seedling growth begins [18].

Somatic Embryogenesis Induction: Understanding the physiological and molecular mechanisms by which the induction (direct or indirect) of SE occurs is a crucial step for its manipulation. Several factors can induce SE. The conditions of the culture medium, the high concentrations of PGRs, and the wounding of explant are other types of stress that can cause plant cells to change their cellular and molecular programs. The type of explant, the age and the genotype of the mother plant, the physiological conditions of the incubation, and the cellular density in the case of suspension cultures, as well as the generation of homogeneous cell aggregates, are factors that must be considered in order to produce the acquisition of embryogenic potential [19].

The source of nitrogen, as well as its concentration in the culture medium, has been shown to be an essential element for the induction of SE. In different plant species, such as *Cucurbita pepo*, *Medicago sativa*, *Coffea arabica*, and *Daucus carota*, it has been determined that both nitrate and ammonium content in the culture medium have a significant effect on the response of the explants to the induction of SE. It has been proposed that stress is the switch that stimulates cellular reprogramming toward an embryogenic path [1]. However, the mechanism by which the nitrogen sources participate in the induction of embryogenic potential remains unknown.

Role of Plant Growth Regulators During the Induction of Somatic Embryogenesis: In plant culture systems, the addition of PGR to the culture medium plays an important role in inducing cell differentiation, in particular during the induction of SE. Most of the SE process depends on the concentration and kind of PGR used for each culture. Different plant species, such as C. canephora [12], A. thaliana [20], and Musa spp. [21] responded successfully to the SE induction using different explants, conditions, and concentrations of PGRs.

Many species that are able to produce somatic embryos from cell suspension cultures require the addition of auxins in the culture medium. The use of 2, 4-dichloroacetic acid (2, 4-D) has an essential role in the induction of SE and the initial stages of development of the somatic embryos [1]. For example, the productivity for embryogenic date palm crops increased 20 times by adding a low concentration of 2, 4-D. The use of auxins modified their endogenous metabolism in a significant way; for example, in carrots, the use of 2, 4-D in the culture medium induces an embryogenic response that is associated with the increase of the endogenous levels of indole-3-acetic acid (IAA). The pre-treatment of plants before the induction of SE in C. canephora also modified the endogenous metabolism of IAA [22].

Other PGRs, such as CKs, also participate in the development of the plants, promoting the formation of buds, delaying the aging of the leaves and, together with the auxins, stimulating cell division; both regulators are known to act synergistically. A high ratio between CKs and auxins stimulates the formation of shoots while that a low ratio induces the regeneration of roots and the proper establishment of meristems in Pisum sativum. These two PGR can act either synergistically or antagonistically during the induction of SE. Recent studies using synthetic reporter genes such as DR5 for auxins and a two-component system (TCSv2) for CKs have opened a window into the molecular mechanisms by which such interaction occurs during biosynthesis, transport and signaling [22].

In recent years there has been a significant increase in the knowledge of the signals that gives rise to the SE process, but it is still unknown if auxins are the primary signal that initiates the changes in the genetic program that leads to the production of somatic embryos. In *C. canephora*, it has been shown that polar transport of the IAA is needed for the formation of the apical-basal axis [22]. It has also been reported that CKs are essential to maintaining basal levels of auxin biosynthesis during root and shoot development, suggesting that there is a homeostatic regulatory network to support adequate concentrations between auxins and CKs in the development of the plant. It is possible that a similar system is operating during the induction of SE. However, this must be tested.

Plant Growth Regulator Response Genes During the Induction of Somatic Embryogenesis: The SE process implies the integration of endogenous signals and gene reprogramming, which unchains the signal that initiates the embryogenic process. The use of exogenous auxins, either alone or in combination with other PGRs or stress, induces the expression of different genes, which modify the genetic program of the somatic cells and regulate the transition to each of the stages during the development of SE [19]. Most of

these genes belong to one of these four categories: transcription factors (TFs), proteins that act in the cell cycle, biosynthesis of PGR, mainly auxins, as well as proteins involved in the signaling pathway.

It is generally accepted that the SE process involves three phases: the induction of SE, the formation of the meristematic centers, and the development of the somatic embryo. Each stage comprises the interaction of multiple factors, e.g., external signals, changes in the endogenous concentrations of different PGRs, and the expression of numerous genes. Molecular studies of the induction of SE are challenging since it is difficult to identify the cells that will become new somatic embryos. However, it is possible to carry out bioinformatics analysis from transcriptomic studies gain a better picture of the candidate genes involved in the initiation of the process [23].

Production of the signal that leads to the changes in the genetic program requires the participation of several metabolic pathways. However, there is a consensus that auxins play a critical role in the SE process [1]. It is known that auxin plays a crucial role in the formation of embryo patterns in angiosperms and in gymnosperms [24]. During the induction of SE in *C. canephora*, there is an increase in the content of endogenous IAA and in the expression of the genes that code for the enzyme tryptophan aminotransferase, and for the enzyme flavin mono-oxygenase. Both are involved in the biosynthesis of IAA [25].

The response of the explant is not confined to the increase in the IAA levels [1]. Differential gene expression can modulate the embryogenic capacity of cells, and the number of genes turned off in somatic cells to allow for the change from a somatic to an embryogenic state is higher than the number of genes that are turned on [15]. In the SE of Arabidopsis, the modulation of several auxin response factors (ARF) transcripts suggest the extensive participation of auxin signaling during the process. Almost half of the 23 ARF genes are transcribed during SE in Arabidopsis; six of them are upregulated and five are down-regulated. Other members of the auxin signal transduction pathway, like the putative Aux/IAA gene from *Elaeis guineensis*, EgIAA9, or cotton, are also involved in the induction of SE. An extensive analysis of gene expression during the induction of SE in cotton shows that more than 80 genes related to the metabolism of auxins are differentially expressed [22].

Transcription Factors and Signal Transduction Involved in Somatic Embryogenesis: There is very little current information on whether the genes involved in the induction of SE work independently or in a network-like structure. However, the analysis of the interaction among different clusters of genes shows that they can act in parallel or in sequence. The use of transcriptomics has provided valuable. Indicates that the genes expressed during the induction of SE are divided into the categories of stress-related genes, PGR-related genes, and TFs [22].

The changes in the genetic program of the cells that lead to the induction of SE require the regulation of several genes. In both angiosperms and gymnosperms, little is known about gene expression, the early stages of embryogenesis, which is crucial for the later development of the embryo. For example, it has been reported that in conifers such as *Araucaria angustifolia* that the expression patterns of AaSERK1 during SE are very similar to SERK1 homologs of angiosperms. These changes require the substantial participation of TFs. Plant genomes contain a large number (6–10%) of TFs-coding genes. In conifers, several homologs of important genes that participate during ES have been found, such as SERK1, LEC1, and OX2, but it is still unknown whether they present patterns and expression functions similar to angiosperms. Several of these genes are also expressed during the formation of zygotic embryos. The application of auxins or their analogs, like 2, 4-D, enhances the expression of several TFs, such as BBM, WUS, and VP1 during the induction of SE [21].

In some cases, like the SE induced in wounded tissues, there is a signal that occurs before to the expression of the TFs. The expression of wound induced differentiation1 [WIND1] TF, from the AP2/ERF family, is required before the expression of leafy cotyledon2 [LEC2] takes place. The expression of some TFs is specific to particular species; however, several others are expressed in all the systems of induction of SE studied. The roles of these TFs in the signaling process are discussed below [22].

Somatic Embryogenesis Receptor Kinases [SERK]: Among the different genes that increase their expression during the induction of SE, SERK is the most relevant. This family of TFs is involved in a range of developmental processes that include differentiation/trans differentiation and cellular

totipotency. The first SERK gene was identified in D. carota. It was detected in embryogenic cultures in the early days of culture in the presence of 2, 4-D. This gene is expressed in cells that develop in somatic embryos until the globular stage, just before the transition from the differentiation state to the development state. The expression of SERK increases several times in the embryogenic cells of A. thaliana, *Citrus unshiu, Dactylis glomerata, G. hirsutum, Helianthus annuus, Medicago truncatula, Solanum tuberosum, Vitis vinifera, Cocos nucifera, Oryza sativa, Theobroma cacao, Triticum aestivum, Zea mays, Cyrtochilum loxense*, and *A. angustifolia* [22].

The evidence of the participation of SERK in the induction of SE has emerged from the analysis of gene expression. For example, SERK1 is highly expressed during the formation of embryogenic cells in in vitro culture of *A. thaliana* and in all of the cells of the developing embryo during early SE, up until the heart stage of the somatic embryo. After this stage, the expression of SERK1 is no longer detectable in the embryo. However, in seedlings that over-expressed SERK1, the mRNA exhibited a 300–400% increase in the efficiency of the initiation of SE. These results suggest that an increase in the expression levels of SERK1 confers embryogenic competence to cells in culture. In *O. sativa*, SERK2 is expressed almost three times more in the embryogenic callus and maturation stage than in the non-embryogenic callus. These results suggest that different members of the SERK family have unique functions. Similar results have been found in T. aestivum. In this plant, members of the SERK family are expressed differentially in response to different PGR sensitivities; i.e., SERK2 and SERK3 elicit auxin-specific responses while SERK1 and SERK5 may be mediated by the signaling pathway of brassinosteroids [22].

In addition to auxins, other factors modified the expression of SERK. In M. truncatula, the expression of SERK1 is stimulated by the presence of auxin, but not by CKs. However, when the CKs are co-administered with auxin, the level of expression of SERK1 increases synergistically compared to the up-regulation of auxin alone. In response to a higher level of expression of SERK, the number of embryogenic calluses increase as well as the formation of somatic embryos [22].

Leafy Cotyledon [LEC]: Another important participant in the regulation of SE and plant embryo development is the LEC family of TFs. LEC1 has an essential role in ZE and has been suggested to control diverse processes in seed development, including embryo morphogenesis, maturation phases, germination, and early and late embryogenesis; it also appears to allow the formation of the embryo by establishing an embryonic environment. LEC1 is also involved in photosynthesis and chloroplast biogenesis early in seed development, and seed maturation late in the development of zygotic embryos. This gene network regulated by LEC1 has been conserved in dicotyledonous plants that diverged tens of millions of years ago [22].

LEC1 and LEC2 were the first TFs shown to induce SE when ectopically expressed in seedlings. The auxin-dependent upregulation of LEC2 has been associated with the induction of SE, whereas LEC2 expression was markedly lower in non-embryogenic callus of *A. thaliana*, suggesting that LEC2 mediates the increase in the endogenous auxins observed during the induction of SE. Similar results were found in T. cacao, where LEC2 is highly expressed in the embryogenic callus and its overexpression in cotyledon explants increased the embryogenic response. The ectopic overexpression of LEC2 from *Ricinus communis* in *A. thaliana* induces the expression of TFs such as LEC1, L1L, FUS3, ABI3, and WRINKELED1 (WRI1). Also, the expression of the fatty acid elongase 1 (FAE1) and, in consequence, an accumulation of triacylglycerols, especially those containing the seed-specific fatty acid, eicosenoic acid (20:1 Δ11), in vegetative tissues was observed [22].

WUSCHEL (WUS): The establishment of the shoot apical meristem (SAM) is essential for SE and for shoot regeneration. These processes require the expression of WUS, which encodes a bifunctional homeodomain TF. WUS contains a highly conserved homeobox domain, and at the conserved C terminal region it has three functional domains: an acidic domain, a WUS-box (TLPLFPMH), and an EAR-like motif [22]. A very important characteristic of WUS is its ability to move from one tissue to another. It can move from its biosynthesis site, the central zone (CZ), into the daughter cells in the peripheral zone, where it activates the transcription of CLAVATA3 (CVL3), a negative regulator. CLV3 moves into the extracellular

space and binds to CLV1, which in turn inhibits the transcription of WUS. This WUS-CLV feedback system establishment maintains the stem cell pool and the development of SAM. Therefore, WUS has been proposed to be essential for SE and in-vitro shoot regeneration [22].

WUSCHEL, like LEC2, responds to the presence of auxins. Auxins trigger a signaling cascade that initiates the vegetative-to-embryogenic transition, and this transition is mediated by WUS. The gradient of auxins that is detected during the pre-treatment of C. canephora plantlets and later during the initial phases of SE correlates with the induced WUS expression during SE in A. thaliana [22].

Baby Boom (BBM): Another key regulator of plant cell totipotency is BBM. BBM can induce embryogenesis in differentiated cells and could be a vital factor in plant embryogenesis development. BBM triggers a set of genes like LEC1 and LEC2, as well as ABI3 and the FUS3 network, which together activate SE. The induction of SE by BBM is a dose-dependent mechanism and regulates the transcription of significant embryo identity genes. The BBM family encodes APETALA 2/ETHYLENE RESPONSE FACTOR (AP2/ERF) DNA-binding type TFs identified in the gymnosperms, angiosperms, algae, and mosses, these TFs act as a network regulation in response to biotic and abiotic stress. The AP2/ERF domain can bind to a GCC box, a DNA sequence involved in the ethylene response. AP2/ERF are divided according to the number of AP2 domains that they contain, which are classified into subfamilies as the Dehydration-responsive 427 element-binding [DREB], ERF, AP2, and RELATED TO ABI3/VP1 (RAV) genes. Because RAV genes include another DNA-binding domain, B3, RAV genes are sometimes treated as a third group in the AP2/ERF family. The distinct feature of the BBM and BBM-like proteins is the presence of a conserved bbm-1 motif that is absent in other proteins of the euANT lineage. BBM activated the expression of a broad set of genes encoding proteins with potential roles in transcription, cellular signaling, cell wall biosynthesis and targeted protein turnover, such as the actin depolymerizing factor9 (ADF9) [22].

In *A. thaliana* and *B. napus*, BBM changes its spatial-temporal expression in the early stages of embryogenesis. Some reports show that BBM is expressed in the heart state of an embryo and root development and enhances the proliferation of somatic embryos. This response is also produced by ectopic expression of BBM, which changes from vegetative to embryonic growth and induces spontaneous SE in these two species. The heterologous expression of BBM from A. thaliana and B. napus in Nicotiana tabacum produced an increase in the regeneration capability. In *Capsicum annum*, both LEC1 and BBM are expressed and show high levels of expression in the different phases of development of the somatic embryo [22].

On the other hand, it is worth highlighting that BBM can show differential expression depending on the species and the embryogenic protocol. In a study using two species of the genus Coffea, it was found that while in C. arabica a BBM-like gene showed a twofold change in expression in embryogenic cell suspension in comparison to embryogenic calli, in C. canephora BBM1 expression was only observed after SE induction. It has been found that the BBM gene is expressed at higher levels during SE in comparison to ZE in *T. cacao*, and its overexpression in *A. thaliana* and *T. cacao* led to phenotypes associated with SE that did not require exogenous hormones. However, BBM overexpression can inhibit the subsequent development of the somatic embryos in *T. cacao*, while the BBM overexpression in Populus tomentosa induced SE [22].

IV. OTHER FACTORS INVOLVED IN SIGNAL TRANSDUCTION DURING THE INDUCTION OF SOMATIC EMBRYOGENESIS

Somatic embryogenesis signaling is a very complex process where several molecular players are involved; it would be tedious to list them all. However, there are two other major factors that need to be mentioned. One is the intervention of 14-3-3 proteins, which participate in several processes such as the development of the seeds, and during the induction of SE in *Carica papaya*. The other factor actively involved during the SE induction, process, and development is epigenetic [22].

14-3-3 Adaptor Proteins: 14-3-3 adaptor proteins are a group of proteins involved in the signal transduction pathway that is shared by several PGRs involved in SE induction. These proteins are highly conserved

phosphoserine-/phosphothreonine-binding proteins, discovered in the brain of mammals in 1967, with a subunit mass of 30 kDa. In plants the number of members of these proteins is variable. There are 13 14-3-3 adaptor proteins in Arabidopsis, six in cotton, 17 in tobacco, ten in tomato, five in barley, and eight in rice. The use of proteomics techniques has illuminated the changes in hundreds of proteins, including the family 14-3-3, during the induction of SE. Some 14-3-3 proteins are abundant in the embryogenic tissues of *Cyclamen persicum*, and Larix principis. In oak, these proteins are more abundant in proliferating embryos than in mature embryos [22].

An excellent example that shows the role of 14-3-3 proteins in the induction of SE is protein phosphatase 2A (PP2A). This enzyme consists of a catalytic subunit and a regulatory A subunit together with a third variable B subunit. The B subunit is the component that determines the substrate specificity and subcellular localization of PP2As. PP2A is a complex enzyme. In *A. thaliana*, there are 25 genes involved in the transcription of PP2A three subunits. The catalytic subunit (PP2Ac) is coded by five genes, three other genes encoding A subunits and seventeen different genes encoding B subunits. The subunit A is essential for auxin transport, while the 65 kDa regulatory subunit of PP2A has regulatory functions. The subunit A has been associated with the SE process. There is a noticeable increase in phosphorylation of specific proteins in embryogenic cultures compared to the non-embryogenic cells of *C. persicum*, which has been correlated with higher levels of PP2A and a 14-3-3-like protein. Other components of the signal transduction cascade, such as G proteins and calreticulin, increased during cyclamen SE. It has been suggested that the increase in the regulatory subunit of PP2A and 14-3-3 proteins during the induction of SE is related to the stress conditions produced by the in vitro culturing of *C. persicum* and *L. principis* embryogenic cultures [22].

Epigenetics: In recent years, epigenetic mechanisms during chromatin remodeling have emerged as critical factors in SE. Epigenetic modifications are an essential part of the signaling pathway that leads to changes in the genetic program of the cells and the development of somatic embryos. There is evidence that shows that changes in the chromatin are able to control totipotency in plant cells. The level to which chromatin reprogramming is required before SE induction depends on several factors, such as origin of the explant, the culture medium, the genetic background of the mother plant, and especially the amount of PGR used.

Plants are developmentally plastic organisms. Not only do they continually differentiate new organs from the stem cell niche throughout their lifespan, but they also regenerate new cells and organs after wounding or during in vitro culture (pluripotency). Plant cells also show an extraordinary capacity for totipotent growth, the ability to produce a new organism through embryogenesis. During sexual reproduction, a diploid zygote is formed upon fusion of two haploid gametes, an egg cell and a sperm cell, and goes on to form the embryo and eventually a new plant. In flowering plants, the embryo develops together with the endosperm, and both are surrounded by the maternally derived seed coat. Together these tissues constitute a seed. During germination, the embryo breaks out of the seed coat and develops further to produce the different organs that make up the plant body. Thus, the single-celled zygote has the capacity to form a whole plant and is therefore totipotent. A number of plants produce embryos in the absence of egg cell fertilization as part of their natural reproductive cycle. In apomictic plants, embryos develop spontaneously from the sporophytic tissues of the seed coat precursor or from an unreduced gametophytic cell. Adventitious plantlets also form on the leaf margins of some plants, e.g., Kalanchoe spp., also known as 'mother of thousands.' In *K. daigremontiana* these plantlets initiate through adventitious embryogenesis and then complete their development through organogenesis. The capacity for totipotent growth reaches its maximum potential during in vitro tissue culture, where an even wider range of explants can be induced to undergo embryogenesis, including haploid cells of the male and female gametophyte (gametophytic embryogenesis) and vegetative cells of the sporophyte (somatic embryogenesis, SE) [22].

Ectopic Expression of Embryo and Meristem Identity Genes Can Induce Somatic Embryogenesis: The LEC proteins LEC1 and LEC2 were the first TFs shown to induce SE when ectopically expressed in seedlings. LEC1, which encodes subunit B9 of a nuclear factor Y protein (NF-YB9), and the B3 domain protein LEC2 are part of a larger network of "LAFL" proteins (for

LEC1/LEC1-LIKE (L1L), ABSCISIC ACID (ABA) INSENSITIVE 3 (ABI3), FUSCA3 (FUS3) and LEC2 that regulate embryo identity and maturation. Loss-of-function mutations in LAFL genes result in defects in cotyledon development, storage macromolecule accumulation, and desiccation tolerance in zygotic embryos. In contrast, ectopic expression of LEC1 and LEC2 induces somatic embryo formation on the cotyledons and leaves of arabidopsis seedlings. Later it was found that L1L/NUCLEAR FACTOR Y subunit B6 [NF-YB6] and three other NF-Y subunits, A1, 5 and 9, with roles in embryo development, drought resistance, and ABA perception, also induce spontaneous SE in seedlings when overexpressed. The remaining two LAFL genes, FUS3 and ABI3, do not induce SE when overexpressed, but do confer cotyledon identity to leaves.

Another embryo-expressed TF that can induce SE is RWP-RK DOMAIN-CONTAINING 4 (RKD4)/GROUNDED (GRD). RKD4 is expressed throughout early embryos and in suspensors. While mutation of RKD4 leads to short suspensors and embryo arrest, induced overexpression of RKD4 in seedlings causes over proliferation of root cells, from which somatic embryos developed. In line with its unique role during early embryogenesis—other RKDs only affect embryo sac development—RKD4 is the only RKD factor that induces SE [22].

BBM is a member of the AINTEGUMENTA-LIKE (AIL) clade of AP2/ERF TFs that was initially identified as a marker for the induction of haploid embryo development from Brassica napus immature pollen grains. Ectopic expression of BBM is sufficient to induce SE on the leaves and cotyledons of arabidopsis seedlings without exogenous hormone application. BBM overexpression also induces other types of regeneration, including callus and adventitious shoot and root formation. This property has been exploited to improve transformation in crop and model plants [22].

BBM belongs to a gene clade that also includes AINTEGUMENTA (ANT) and six other AIL/PLETHORA (PLT) genes. Arabidopsis BBM and the other arabidopsis AIL/PLT genes are expressed in the embryo and the root and/or shoot meristems, where they act redundantly to maintain embryo growth and to define and maintain the stem cell niches. Overexpression of AIL5 also triggers somatic embryo and adventitious organ formation. Recently, it became clear that overexpression of all AIL proteins, except the phylogenetically distinct AIL1 and ANT, induces SE. This shows that the embryo-inducing capacity of AIL proteins is not limited to embryo-expressed AILs, and suggests that AIL proteins can regulate similar target genes. Overexpression of another member of the AP2/ERF TF family, WIND1 or RAP2.4, also induces SE. WIND1 and its close homologs WIND2−4 are induced by wounding and stimulate callus proliferation after tissue damage. Ectopic WIND1 expression is sufficient to promote callus formation from shoots, hypocotyls, and roots, which can then give rise to shoots, roots, or somatic embryos.

WUS is a homeodomain TF that is expressed in flower and shoot meristems, where it induces stem cell fate in a non-cell-autonomous manner. Overexpression of WUS in arabidopsis is sufficient to induce organogenesis and SE in the shoot and root tip. WUS was also identified as plant growth activator 6 (PGA6) in an activation tagging screen for genes that induce somatic embryo formation from root callus. The above studies show that SE can be induced by ectopic expression of TFs from several different classes, with different roles during plant development. Some of these TFs have roles in early embryo development or in maintaining embryo identity, but non-embryo-expressed stem cell regulators can also induce SE. Below we compare the regeneration pathways that are induced by overexpression of these different TFs [22].

Regeneration pathways: Hormone- or stress-induced SE from cultured explants follows two routes depending on the stage of the explant and the tissue culture conditions. Somatic embryos develop either directly from the explant or indirectly from callus, but it is not always clear why somatic embryos form via one route or the other. These two routes are also observed in TF-induced SE and studies on these pathways have shed light on this phenomenon.

V. STRESS AND SOMATIC EMBRYOGENESIS

Somatic embryogenesis is a multifactorial event, which is the result of a series of physiological, biochemical and molecular changes taking place in plant cells. SE requires embryogenic competence through dedifferentiation, chromatin remodeling, programming of gene expression, and stress events mentioned above [26]. In general, the SE induction includes a multitude of parallel signals that involve

alterations in the levels of endogenous PGR and stress factors [27]. Different studies support the theory that the first stages of SE are characterized by the induction of numerous genes related to stress such as those discussed later on this review [1]. Recent evidence in potato [28], *Pinus sylvestris* [29], *Picea asperata* [30], *Oldenlandia umbellata* [26], and *Cyathea delgadii* [31] has revealed that the presence of different types of stress plays an essential role in the induction of SE. The main stress for cells during the induction of SE is the presence of high auxin concentration in the culture medium. Other stresses used for the induction of SE are extreme pH, heat-shock exposure or treatment with various chemical substances.

Usually, the combination of physical stress with high auxin concentration in the culture medium improves the embryogenic response. This effect was observed in *Cattleya maxim* where the effect in the SE induction was evaluated using a combination of salt (0.3 M NaCl) or osmotic stress (sorbitol 0.4 M), and the culture in a medium supplemented with 2,4-D (0.45 μM) significantly increases the percentage of protocorms with embryogenic calli [32]. In some angiosperms such as Panax ginseng, the treatment of somatic embryos with abscisic acid (ABA) and polyethylene glycol (PEG) at a concentration of 20 μM and 3.75%, respectively, improve both the maturation and regeneration of somatic embryos compared to the untreated. However, in gymnosperms, the combined application of ABA and PEG has been shown to be necessary to stimulate the maturation and functional development of somatic embryos. For example, in Pinus sylvestris, embryo production is commonly induced by eliminating auxin from the culture medium, ABA addition and subsequently a PEG drying step [29]. In P. strobus, variable amounts of water at the beginning and during the cultivation phase influences the maturation response of the embryos [33]. Meanwhile, changes in water availability either by solutes or physical restriction can affect the maturation response in some conifers [34]. Other types of stress like heat-shock induce the SE in Gladiolus hybridus [35]. In cotton, several of the genes expressed during the induction of SE are related to the homeostasis of auxins and ethylene, as well as several related-stress TFs [36].

Although experimental data just started to be accumulated on this field, it already can be generally stated that mechanisms driving the responses of plants to environmental stresses often depend on post-translational histone odifications, dynamic DNA methylation, and ATP-dependent chromatin remodeling. The combined effects of epigenetic and hormonal pathways play an essential role in the regulation of stress adaptation including the adjustment of developmental pathways. Somatic embryogenesis is a still largely unexplored model to study and better understand the integration of stress and developmental pathways at the chromatin level in plants.

In-vitro somatic embryogenesis is a complex process which includes metabolic, genetic, epigenetic and developmental reprogramming of cells [37]. It is most frequently achieved by removing the explants from the plant body, from their developmental context, and culture them in synthetic media under the influence of unphysiological growth regulator concentrations and various stress factors. Due to the undefined but stressful in vitro conditions, the developmental control on cell fate is lost resulting in various possible outcomes, including dedifferentiation, death, cell proliferation, meristem formation, or embryogenesis, depending on the explant and the conditions applied. These developmental pathways are under strong hormonal, genetic, and epigenetic repression in differentiated vegetative cells [38]. The release from the repression can take place only under harsh environmental conditions which force the plant cells to follow an “erase and rebuild” strategy to ensure their survival. In parallel with the in vitro culture imposed developmental switches, these conditions are well known to result in epigenetic as well as heritable genetic alterations, generally referred as “somaclonal variation” [39]. Stress in general has a large impact on genetic as well as epigenetic stability that may improve the adaptability of plants towards adverse conditions in nature as well as in-vitro. Stress and plant hormones are implicated in the regulation of genome stability that not only provides a more consistent defense response against stress, but also increase mutation frequency driven by conformational variations in DNA structure and chromatin organization in order to accelerate the adaptive processes. The role of stress-induced epigenetic chromatin reorganization on the genetic instability of in vitro cultured plants received considerable attention during the past year. Dedifferentiation is hypothesized to represent a cell state that favors DNA

recombinations and transpositions to take place due to genome wide epigenetic reorganization. Stress-induced DNA methylation as well as histone acetylation changes evoked by tissue culture can result in the activation of transposon. Histone methylation was also shown to be involved in genetic variation driven by DNA recombination as demonstrated by the telomerase-independent telomere lengthening in dedifferentiating protoplast. Based on the overall stress that in vitro culture can impose on the plant genome it was claimed that plants obtained through in vitro culture including dedifferentiation, proliferation, redifferentiation, or transdifferentiation, each possesses a genotype, which is not exactly like the genotype of the original differentiated cells. Genetic instability therefore seems to be an inherent feature of in-vitro plant regeneration including somatic embryogenesis and that should be taken into account in case of practical applications [9].

VI. STRESS TREATMENTS FOR THE INDUCTION OF SOMATIC EMBRYOGENESIS

Osmotic Shock: Abiotic stress, especially high saline concentrations, can stimulate the correct development of somatic embryos. During SE induction in Triticum aestivum, the formation of somatic embryos was incomplete due to a precocious germination during the early phases of SE and high cell proliferation. These defects were corrected by adding 40 mM NaCl in the culture medium, which suppressed the precocious germination and increased somatic embryos production. Stress treatment has also been applied to plant cells to determine the first changes related to embryogenic induction. In Daucus carota, somatic embryos development can be induced by culturing shoot apical meristems on PGRs-free medium with a chemical stressor. Changes in sucrose concentration (0.7 and 0.1 M, respectively) induced an important modification in the cell fate that promoted the generation of numerous somatic embryos directly on the surface of shoot apical meristem explants. In Arachis hypogaea, a high concentration of sucrose (0.5–0.7 M) triggered the accumulation of triglycerides, the increase of these lipids did not improve the embryogenic system and the embryos became necrosed. The supply of 20 μM abscisic acid (ABA) to the medium, partially improved the morphology of the embryos. Neither the osmotic, or desiccation treatments, stimulated the conversion of embryos into plantlet. In callus cultures of Hevea brasiliensis, application of exogenous ABA (1 × 10−5M) only stimulated the formation of globular embryos. The use of an osmotic agent, such as polyethylene glycol (PEG; 140 g L−1), improved the conversion of pro-embryogenic mass into torpedo-shaped embryos and allowed the proper establishment of morphological appearance of embryos and contributed to decrease the secondary SE [1].

Application of osmotic treatments with different sources of carbohydrate also impacts on somatic embryo generation. The better embryogenic responses were reached using sucrose 350 mM, followed by glucose 89 mM, being the osmotic potential of –1.42 and –1.30 MPa, respectively, for each osmotic. These data suggest that the frequency of somatic embryos enhanced with the increase of osmotic potential. Unlike *E. europaeus*, the embryogenic aggregates of Gentiana lutea required a moderate osmotic stress to produce all embryogenic stages. This stress can be produced by sugar alcohols such as mannitol or sorbitol (0.16 M) in a PGR-free medium. On the other hand, Phaseolus vulgaris, considered as a recalcitrant species, can produce somatic embryos under osmotic stress. The application of 0.5 M sucrose by 48 h to zygotic embryos, used as explants, in combination with benzyladenine. The Relationship between stress and somatic embryogenesis 157 (BA) (44.3 μM) and adenine free base, led to the induction of direct SE. These data suggest that SE response could be related to the adaptation to osmotic stress and the internal pool of cytokinins; a mechanism that needs further investigations [1].

Heavy Metal Ion: Similar to osmotic stress generated by sucrose in carrot cells, heavy metal ions can act as stress inducers in plants and can also promote SE in the absence of PGRs treatment. In carrot, more than 40 % explants treated with 0.5 mM CdCl2 during two weeks were able to produce healthy embryos. Other heavy metal ions, such as cobalt, nickel, zinc, silver, and copper have also been used for SE induction. However, they only induced a low rate of somatic embryos in carrot or exhibit SE in T. aestivum. Nevertheless, leaf explants of wheat treated with cadmium [0.5 mM] and cultured in the absence of 2,4-D produced somatic embryos. Taken together all these results, it would be interesting to investigate how and

when the cadmium ion induces the cell reprograming in the somatic cells and the acquisition of cellular totipotency since these plant species (carrot and wheat) did not require the exogenous stimulus of PGRs. Using similar stress treatments as in carrot, in the model plant Arabidopsis thaliana positive results were obtained during SE induction. However, shoot apical meristem explants cultured on PGRs-free solid medium and exposed, either to osmotic treatment (0.7 M mannitol, 0.7 M sorbitol, 0.7 M sucrose, or 0.3 NaCl), or heavy metal ion (0.6 mM CdCl2) required an additional treatment with 2,4-D to stimulate embryo development [1].

Temperature Stress: Another treatment able to induce embryogenic development is temperature shock. It has been observed that exposition of explant tissues for suitable periods, either low or high temperatures can induce the acquisition of cell totipotency and even mimic the early ZE development. Brassica napus was one of the first plants in which temperature-treatment was used to stimulate the embryogenic program. Before the induction of SE in B. napus, the plants were grown at 25°C Day/16°C night until plants generated the first flower buds. After that, the temperature was decreased 12–15°C Day/7–10 °C night, until bud collection, and only then, the heat shock treatment was imposed. In this model system, it is well established that treatments at high temperature (32°C) during 8–72 h was a necessary step for both anthers and microspores to change their gametophytic program 158 G.I. Nic-Can et al. by an embryogenic pathway [23]. The embryogenic pattern might be different, depending on treatment duration. For instance, microspores pretreated at 32°C for 24 h tended to develop a suspensor-like structure as occurs during ZE, whereas a prolonged treatment up to five days prevented the formation of suspensor in the embryos. This response could be due to prolonged heat-treatment that affected the polar auxin distribution in the uninucleate microspores which prevented the proper establishment of apical-basal polarity in the cells. In Cichorium intybus x Cichorium endivia SE process is also temperature-dependent. When leaf explants were cultured in the presence of PGRs [0.1 μM naphthaleneacetic acid (NAA) and 0.1 μM isopentenyladenine (2iP) and exposed to several temperatures (20–35°C), the cells in the wounded leaves began to grow and proliferate, regardless the temperature conditions. However, the cells were directed to different type of morphogenesis. It is interesting to observe how temperature can impact the plant cell plasticity. Callus generation was predominant when the explants were incubated at 20°C, but the shoot formation was stimulated at 25°C, whereas the incubation at 35°C targeted the direct SE [1].

Direct SE was also induced in carrot when the apical tip explants were pretreated at 37 °C for three weeks before culture them in PGRs-free medium at 25°C. But, when temperature was decreased at 35°C, the explants did not generate somatic embryos; instead, apical explants developed into plantlets. In microspores of Nicotiana tabacum, the embryogenic response at normal temperatures was around 50 %. However, when they were exposed to heat treatment [33–37°C] for three days, followed by incubation at 25°C, their embryogenic capacity increased. The embryogenic response might increase even more, through the combination of sucrose starvation and heat shock treatment, independent of the heterogeneity of microspores. On the other hand, although the high temperatures can involve important cellular damages, in Capsicum annuum the heat shock treatment necessary to induce the embryogenesis was larger than in other species. In this crop, the low yield induction was improved using a pretreatment of anthers at 35°C for eight days and cultured in the presence of kinetin (4.64 μM). Not all plant species respond to heat treatment, in some cases the high temperatures can lead to an inhibition of SE [1].

In wheat, microspores isolated from tillers, did not show any response to embryogenesis induction when they were exposed to 33°C; instead that, the microspore embryogenesis could be accomplish without any stress pretreatment, but the rate of conversion into embryos was relatively small. On the other side, when microspores were pretreated at 4 or 25°C for 48 h, the percentage of induction of SE in the microspores showed a twofold increase respect to the response of microspores isolated from freshly tillers. This response was conditioned by the presence of some ovary´s in the culture medium.

In *B. napus*, high temperature treatments were a prerequisite to induce embryogenesis, but only under suitable exposition time (32°C, one day) the embryos developed suspensor-like structures. It has been also the relationship between stress and somatic

embryogenesis 159 shown that in this plant, the continued use of low temperature (18°C) induced microspore embryogenesis. The first division allows the establishment of asymmetric cell identities, early polarity, and the formation of suspensor-like structures, in a process resembling the ZE process. Even when the number of embryos decreased respect to those induced at 32°C, there was a synchrony and a homogeneity among the embryos generated. Additionally, more than 50 % embryos developed suspensor-like structure, which was very important to understand the early events that establish the polarity and developmental embryo patterning [1].

Nutrient Starvation: It is known that sucrose is frequently used as primary carbon source in the culture medium for several plant species; however, it is also known that high concentrations of sucrose suppress SE response. By contrast, it has been shown that nutrient starvation stress is another important factor for SE induction in some plant species. For instance, the sucrose and nitrogen starvation induced the embryogenic development of tobacco microspores, even better than heat stress. In wheat microspores, the first step for embryogenesis induction might require a nitrogen starvation in a medium with mannitol as the only carbon source for a two-day period, as well as high temperatures followed by a culture in an ovary conditioned medium. Under these conditions, microspores can develop multicellular structures; however, the carbon source must be changed by maltose to embryogenesis proceed. Whereas glucose or sucrose impairs wheat embryogenesis through the increase in size and the accumulation of starch, maltose can be used to induce embryogenesis. The effect of maltose on microspore embryogenesis can be attributed to a slow hydrolysis by plant cells, which exerts early starvation conditions in the medium culture and a stable osmolarity later on. In carrot, the embryo production increased up to 4.5-fold when embryogenic callus was cultured on MS basal medium in the absence of sucrose and reduced humidity respect to MS medium with sucrose. Embryogenic callus without medium for five days (stress starvation) showed a 20-fold increase in the production of somatic embryos. This result suggests that the absence of medium culture inhibited cellular dedifferentiation, but improved the production of somatic embryos [1].

Sucrose starvation in embryogenic callus of Phoenix dactylifera also caused a significant effect on somatic embryos production. It was found that reduction of MS at half-strength, in a liquid medium, and two weeks without sucrose were the best conditions to obtain embryogenic structures which reached their germination after culturing in 3 % sucrose. Gossypium hirsutum somatic embryogenesis, as for other species, is a genotype-dependent process and shows low frequency of somatic embryos formation. This SE response was modified when callus was transferred from 2,4-D to a 160 G.I. Nic-Can et al. PGRs-free liquid medium without myo-inositol by a single cycle of 10 days, followed by three subcultures on MS basal medium. This fact did not only improve the efficiency, but also induced SE synchronization in cotton. Myo-inositol starvation could stimulate the signaling pathways mediated by phosphoinositides through the release of intracellular Ca + and diacylglycerol to activate the protein kinase C [1].

Macro and Micronutrients: Inorganic nitrogen forms such as $NO3^{-}$, and $NH4^{+}$ also affect the SE response. For instance, the carrot callus cultured on medium containing $NH4^{+}$ as sole nitrogen source, improved the formation of embryogenic cells instead of a combination of both $NO3^{-}$ and $NH4^{+}$. Similar results were also observed in Cucurbita pepo when NH 4 Cl was used as the sole nitrogen source. NH4 Cl (1 mM) could replace the use of 2,4-D and the conversion of pro-embryos to globular stages was stimulated, while the addition of unreduced nitrogen improved the embryos maturation. These authors also showed that the presence of $NH4^{+}$ in the culture medium culture induced a high activity of glutamine synthetase and phenylalanine ammonia-lyase. This increment of enzymatic activity coincided with a higher activity of stress-related enzymes, such as superoxide dismutase and soluble peroxidase, suggesting that a burst of oxidative stress targeted the tissue when $NH4^{+}$ was the only nitrogen source [1].

In *Oryza sativa* as in wheat, sucrose stimulated the starch accumulation and cell death; sucrose starvation for three days considerably affected induction of cell division of immature pollen grains and allowed the formation of cellular mass. Also, it was observed that substitution of $NaH2PO4$ by $KH2PO4$ improved the frequency of embryogenic colony formation.

Furthermore, it was reported that when zygotic embryos of *Panax ginseng* were cultured on MS PGRs-free medium supplemented with high levels of NH4NO3 (61.8 mM), their growth was suppressed and the explants turned brown. A high formation of somatic embryos was observed from these abnormal zygotic explants, whereas the use of KNO3 as only nitrogen source decreased at half the embryogenic response. On the other hand, the micronutrient boron appears to be an important factor in the onset and development of SE. It was shown that the induction of SE from leaf explants of Cucumis sativus, only began in the absence of boron and PGRs in the culture medium. Also, it was found that boron in the medium influenced somatic embryos development of D. carota. For instance, without boron the apical shoot was suppressed, whereas that of roots was promoted. More recently, the importance of boron in the activation of several transcription factors was highlighted, and the impact of these transcription factors on the levels of endogenous PGRs as well as proteins related to the acquisition of embryogenic potential [1].

VII. EXPRESSION PATTERNS OF STRESS-RELATED GENES DURING SOMATIC EMBRYOGENESIS INDUCTION

Much work has been done to understand the molecular mechanisms by which plant cells retain their plasticity and enter the embryogenic pathway. Several reports indicate that some genes, suggested as indicators of SE, are conserved among species and their expression patterns are similar between somatic and zygotic embryogenesis. Also, it has been shown that their overexpression triggers the embryo formation from vegetative cells. It is important to note that a high number of stress-related genes show altered expression during the onset of embryogenesis, mainly influenced by both maternal and in vitro culture conditions, as well as by specific cell types. For instance, in immature cotyledons of Glycine max, the development of somatic embryos from the adaxial side of cotyledons was preceded by changes in the transcripts of genes associated to oxidative stress and cell division, suggesting a balance between cell proliferation and cell death. It was observed that several genes, related to the oxidative burst were upregulated during the first 14 days in the presence of 2,4-D, especially a large number of GLUTATIONE-S-TRANSFERASE genes (GST7, GST8, GST11, GST16, GST19). During the beginning of SE development there was a coordinate expression of several families of genes. Wound-induced genes [expansin, extensine], cell wall remodeling genes (pectinesterase, glucanase), as well as various genes encoding proteins associated to oxidative stress control (thaumatin, ACC oxidase) or in the programed cell death (adenosine-5′-adenylphosphosulfate reductase, endonucleases) showed an increase in their expression [1].

On the other hand, it was found that some stress-responsive genes are also upregulated during later stages of somatic embryos. For instance, during maturation of maize embryos, the transcript levels of genes encoding heat shock proteins and hydrolytic enzymes, such as nucleases, proteases and glucosidases showed an increase in their expression. In potato, as in soybean, an increase in the level of transcription of stress-related genes was observed during the induction of SE; however, the patterns were different. This suggests a species variation or differences due to the type of explant and the culture medium used for the induction or embryo maturation [1].

Other reports confirmed that the gene expression related to the oxidative stress and redox homeostasis is a common pathway that is triggered, independently of the SE system. For instance, GSTs are expressed during the onset of SE on oil palm, soybean, B. nupus, Pinus Pinaster and maize [40]. A coordinated correlation between gene expression of GST and auxin related genes, such as AUX/AIA was observed. Oxidative stress-related genes that encode thioredoxin H, cytosolic ascorbate peroxidase, glutamine peroxidase, among others peroxidases, also showed a high abundance during onset of oil palm somatic embryos. The same authors also found ESTs encoding several pathogenesis-related proteins [endo-β-1,3-glucanase, γ-thionin, and oil palm defensine EGAD1 that although were expressed in response to pathogen attack, they seemed to play a significant role during embryogenic callus generation and somatic embryo development [1].

Although SE and ZE development are similar in many aspects, recently it was highlighted an important difference between both processes. According to differential gene expression patterns, cotton SE process showed a high transcriptional activity of

stress-related genes in comparison to ZE. These genes included mainly GRTs-related genes [genes involved in ABA biosynthesis, as well as jasmonic acid]. Other genes are members of kinase family and downstream stress-responsive genes, particularly Late Embryogenesis Abundant [LEA], Early Responsive to Dehydration (ERD) and Responsive to Dehydration (RD), as well as at least 15 WRKY transcription factors family members. All these genes also showed an increase in their expression under stress treatment either by NaCl (75 mM) or ABA (0.5 mM). Both treatments were involved in the repression of cell proliferation of embryogenic callus and consequently accelerated the somatic embryos development. These data suggest that stress responses might regulate the balance between cell proliferation and differentiation. In maize around 2,000 genes were overexpressed 8-fold in only 24 h after SE induction. These genes were classified in diverse biological processes, such as oxidation-reduction, metabolic processes, protein phosphorylation, transmembrane transport and stress response, which were consistent with a complex coordination of multiple pathways involved in the transition of somatic cell into SE [40]. Two maize genes WOUND INDUCED PROTEIN 1 (WIP1) and CHITINASE A1 related with plant defense and stress response, respectively, were upregulated over 1,500-fold from 0 to 24 h. WIP1 is involved in the hypersensitive defense response; however, its role is unknown during SE. The expression of the chitinase gene has an important function during early stages of SE. Also, about 50 % of GSTs family members showed a high transcriptional activity during the onset of SE. Likewise, it was found that GSTs were coexpressed with BBM, WUS, SERK, PIN-FORMED (PIN), and GERMIN LIKE PROTEIN (GLP). Whereas PIN is involved in the auxin transport, GLPs affect the plant redox. These results are consistent with the fact that GST, indirectly influence the auxin transport, and might also promote the embryogenic environment for the expression of master transcriptional factors associated with SE [40]. Recently, the transcriptome dataset obtained from the SE process in Arabidopsis, revealed a much higher transcriptional activity in somatic embryos than that in actively dividing callus. These data are consistent with the fact that SE involves a highly stress function than their counterpart, the ZE, particularly during the first days of induction [40]. It was observed that SE development exhibited a higher level of expression of several genes encoding for oxidative stress, such as peroxidase superfamily members (AT1G68850, AT2G18980, AT5G14130, AT5G17820, one FAD-binding berberine family protein). Also, genes for salt stress (plant invertase/pectin methyl esterase inhibitor superfamily), and genes encoding several LEA proteins (AT3G19430, AT4G27400, AT5G54370, and AT5G60530). Interestingly, these last genes encoding LEA proteins were coexpressed with at least two genes that encode for auxin metabolism (flavin-binding monooxygenase and auxin-responsive GH3). However, further studies must be done to discard whether these results are specific to embryogenic process itself, or is a side effect of the 2,4-D used for the SE induction.

VIII. CONCLUSION AND FUTURE PERSPECTIVES

Plant cell totipotency has intrigued scientists for decades. It is well accepted that SE induction is promoted, particularly after exposing the cells to a high condition of stress. The first protocols used stress or growth factor treatments for SE induction and the process was studied at the histological level. SE can now be induced by embryo- and meristem-expressed TFs, as well as TFs involved in wound repair. The fact that different types of stresses, namely osmotic shock, application of high or low temperatures, starvation treatments, among others, can impact the cell fate and begin the embryogenic competence is really interesting for scientific research. The exposition to heavy metal ions, such as cadmium also induced cell reprograming and the acquisition of cellular totipotency. In other plant species, the SE can be induced by sugar alcohols, or by changing the composition of the nitrogen source by substitution of some macroelement, or by the absence of a carbon source and in some cases some microelements in the culture medium.

The process of in-vitro culturing plant cells, tissues and organs is, in itself, a stress already but it has become apparent over the years that stress can be both elicitor and a promoter of regeneration in different types of approaches exploited in-vitro. Several factors or different stress signals may be responsible for cell reprogramming, which triggers the same downstream route, suggesting that the embryogenesis initiation

may be induced through the convergence of cell signaling pathways. Thus, studies on biochemical and molecular aspects of the early stages of the somatic embryogenesis process may increase the understanding of the stresses associated with cellular dedifferentiation phase and provide increasingly clear solutions for the control of oxidative stress and the signaling of reactive oxygen species for the success of the technique of somatic embryogenesis for a greater number of species.

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