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REVIEW
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Signaling Overview of Plant Somatic Embryogenesis

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Somatic embryogenesis (SE) is a means by which plants can regenerate bipolar structures from a somatic cell. During the process of cell differentiation, the explant responds to endogenous stimuli, which trigger the induction of a signaling response and, consequently, modify the gene program of the cell. SE is probably the most studied plant regeneration model, but to date it is the least understood due to the unclear mechanisms that occur at a cellular level. In this review, the authors seek to emphasize the importance of signaling on plant SE, highlighting the interactions between the different plant growth regulators (PGR), mainly auxins, cytokinins (CKs), ethylene and abscisic acid (ABA), during the induction of SE. The role of signaling is examined from the start of cell differentiation through the early steps on the embryogenic pathway, as well as its relation to a plant's tolerance of different types of stress. Furthermore, the role of genes encoded to transcription factors (TFs) during the embryogenic process such as the *LEAFY COTYLEDON (LEC)*, *WUSCHEL (WUS)*, *BABY BOOM (BBM)* and *CLAVATA (CLV)* genes, Arabinogalactan-proteins (AGPs), *APETALA 2 (AP2)* and epigenetic factors is discussed.

Keywords: differentiation, growth regulators, signaling, somatic embryogenesis, totipotency, transcription factors

INTRODUCTION

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 (West and Harada, 1993; Goldberg et al., 1994). Embryogenesis is the process by which embryo formation is initiated, either from a zygote (zygotic embryogenesis, ZE) or from somatic cells (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 (Loyola-Vargas and Ochoa-Alejo, 2016). 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 (Garcês and Sinha, 2009). 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 (Radoeva and Weijers, 2014).

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 (PGR) or certain stress conditions, mainly low or high temperature, heavy metals, osmotic shock or drought (Nic-Can et al., 2016). 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 (Quiroz-Figueroa et al., 2006).

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 (Zhou et al., 2016).

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 (Smertenko and Bozhkov, 2014).

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 (Márquez-López et al., 2018; **Figure 1**). In the case of ZE, an asymmetric cell division occurs, whereas in SE this is often not observed (Toonen et al., 1994). 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 (Dodeman et al., 1997). 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 (Quiroz-Figueroa et al., 2002).

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 (Pila Quinga et al., 2018).

Theoretically, plant development can be divided into two different phases: (1) embryogenesis *sensu stricto*, which begins with the formation of the zygote and concludes at the cotyledonary stage, and (2) the maturation of the seed (Dodeman et al., 1997). 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 (Harada and Kwong, 2002; Pila Quinga et al., 2018). 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 (Smertenko and Bozhkov, 2014).

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 (Winkelmann, 2016), and globular, scutellar, and coleoptile stages in the case of monocotyledonous species (Zhao et al., 2017). Once the somatic embryos reach the cotyledonary stage, they initiate a shoot meristem, and seedling growth begins (Yang and Zhang, 2010).

FACTORS THAT INDUCE SOMATIC EMBRYOGENESIS

Understanding the physiological and molecular mechanisms by which the induction (direct or indirect) of SE occurs is a crucial step for its manipulation (Grzyb et al., 2018). 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 (Pencik et al., 2015; Loyola-Vargas and Ochoa-Alejo, 2016).

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 (Reinert et al., 1967). In different plant species, such as *Cucurbita pepo* (Pencik et al., 2015), *Medicago sativa* (Walker and Sato, 1981), *Coffea arabica* (Fuentes-Cerda et al., 2001), and *Daucus carota* (Kamada and Harada, 1979), it has been determined that both nitrate and ammonium content in the culture medium have a significant effect on the response

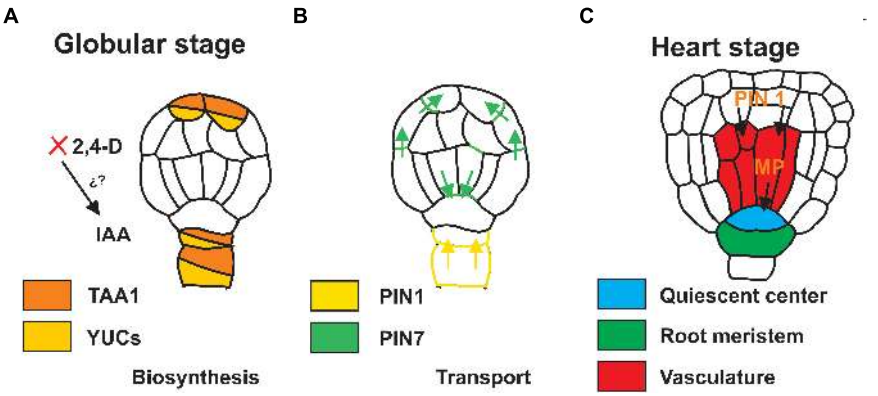

FIGURE 1 | Auxin biosynthesis during the induction of somatic embryogenesis. **(A)** Auxin transport during the development of somatic embryos, globular stage **(B)** and heart stage **(C)**. Colors indicate the localization of the expression of the genes. IAA, indole-3-acetic acid; 2, 4-D, 2, 4-Dichloroacetic acid; *TAA1*, *TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS 1*; *YUC*, *YUCCA*; *PIN*, *PIN-FORMED*; MP, monopteros.

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 (Nic-Can et al., 2016). However, the mechanism by which the nitrogen sources participate in the induction of embryogenic potential remains unknown.

THE 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* (Márquez-López et al., 2018), *A. thaliana* (Grzybkowska et al., 2018), and *Musa spp*. (Awasthi et al., 2017) responded successfully to the SE induction using different explants, conditions, and concentrations of PGR.

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 (Nic-Can and Loyola-Vargas, 2016). For example, the productivity for embryogenic date palm crops increased 20 times by adding a low concentration of 2, 4-D (Abohatem et al., 2017). 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) (Michalczuk et al., 1992). The pre-treatment of plants before the induction of SE in *C. canephora* also modified the endogenous metabolism of IAA (Ayil-Gutiérrez et al., 2013).

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 (Novák and Ljung, 2017; Singh and Sinha, 2017). 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* (Kotov and Kotova, 2018). 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 (Liao et al., 2015).

In recent years there has been a significant increase in the knowledge of the signal(s) 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 (Márquez-López et al., 2018). 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 (Jones et al., 2010). 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 (Loyola-Vargas and Ochoa-Alejo, 2016). 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 (Leljak-Levanic et al., 2015).

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 (Elhiti et al., 2013). 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 (Elhiti et al., 2013).

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 (Nic-Can and Loyola-Vargas, 2016). It is known that auxin plays a crucial role in the formation of embryo patterns in angiosperms and in gymnosperms (Larsson et al., 2008). 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 (*TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS 1*; *CcTAA1*), and for the enzyme flavin mono-oxygenase (*YUCCA*; *CcYUC1* and *CcYUC3*). Both are involved in the biosynthesis of IAA (Ayil-Gutiérrez et al., 2013).

The response of the explant is not confined to the increase in the IAA levels (Nic-Can and Loyola-Vargas, 2016). 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 (Quiroz-Figueroa et al., 2002). In the SE of *Arabidopsis*, the modulation of several *AUXIN RESPONSE FACTORS* (*ARF*) transcripts suggests the extensive participation of auxin signaling during the process (Wójcikowska and Gaj, 2017). 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*, *Eg*IAA9 (Ooi et al., 2012), or cotton (Yang et al., 2012), 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 (Yang et al., 2012).

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 (Krishnan and Siril, 2017). In general, the SE induction includes a multitude of parallel signals that involve alterations in the levels of endogenous PGR and stress factors (Mozgová et al., 2017).

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 (Nic-Can et al., 2016; Nowak and Gaj, 2016). Recent evidence in potato (Kaur et al., 2018), *Pinus sylvestris* (Salo et al., 2016), *Picea asperata* (Jing et al., 2017), *Oldenlandia umbellata* (Krishnan and Siril, 2017), and *Cyathea delgadii* (Grzyb and Mikula, 2019) 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 (Cueva Agila et al., 2015). 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 (Langhansová et al., 2004). 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 (Stasolla et al., 2002). 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 (Salo et al., 2016). In *P. strobus*, variable amounts of water at the beginning and during the cultivation phase influences the maturation response of the embryos (Klimaszewska et al., 2000). Meanwhile, changes in water availability either by solutes or physical restriction can affect the maturation response in some conifers (Montalbán and Moncaleán, 2018). Other types of stress like heat-shock induce the SE in *Gladiolus hybridus* (Kumar et al., 2002). 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 (Jin et al., 2014; Cao et al., 2017).

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 (Ikeuchi et al., 2018). 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 (Cetz-Chel and Loyola-Vargas, 2016; Chu et al., 2017).

The changes in the genetic program of the cells that lead to the induction of SE require the regulation of several genes (Riechmann et al., 2000). 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 (Trontin et al., 2016). 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 (Steiner et al., 2012). These changes require the substantial participation of TFs. Plant genomes contain a large number (6–10%) of TFs-coding genes (Riechmann et al., 2000). Some of these TFs are shared among a variety of plant species (**Supplementary Table S1**). Among the TFs that have been found during the induction of SE in different species are *ABAINSENSITIVE 3 (ABI3)* (Shiota et al., 1998), *AGAMOUS LIKE (AGL)* (Harding et al., 2003; Thakare et al., 2008; Zhai et al., 2016), *BABY BOOM (BBM)* (Florez et al., 2015), *CUP SHAPED COTYLEDONS (CUC), FUSCA3 (FUS3)* (Luerûen et al., 1998), *LEAFY COTYLEDON (LEC)* (Iwase et al., 2015), *LEAFY COTYLEDON LIKE (LIL)* (Kwong et al., 2003), *SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE1 (SERK1)* (Pérez-Pascual et al., 2018), *RWP-RK DOMAIN-CONTAINING 4 (RKD4)/GROUNDED (GRD)* (Waki et al., 2011). *VIVIPAROUS1 (VP1)* (Footitt et al., 2003), and *WUSCHEL (WUS)* (Arroyo-Herrera et al., 2008; Xiao et al., 2018). In conifers, several homologs of important genes that participate during ES have been found, such as *SERK1*, *LEC1*, and *WOX2*, but it is still unknown whether they present patterns and expression functions similar to angiosperms (Trontin et al., 2016). 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 (Awasthi et al., 2017).

In some cases, like the SE induced in wounded tissues, there is a signal that occurs before to the expression of the TFs listed in the last paragraph. The expression of *WOUND INDUCED DEDIFFERENTIATION1 (WIND1)* TF, from the AP2/ERF family, is required before the expression of *LEAFY COTYLEDON2 (LEC2)* takes place (Iwase et al., 2015). 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.

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/transdifferentiation and cellular totipotency (Pilarska et al., 2016).

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 (Schmidt et al., 1997), 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* (Hecht et al., 2001), *Citrus unshiu* (Shimada et al., 2005), *Dactylis glomerata* (Somleva et al., 2000), *G. hirsutum* (Pandey and Chaudhary, 2014), *Helianthus annuus* (Thomas et al., 2004), *Medicago truncatula* (Nolan et al., 2003), *Solanum tuberosum* (Sharma et al., 2008), *Vitis vinifera* (Maillot et al., 2009), *Cocos nucifera* (Pérez-Núñez et al., 2009), *Oryza sativa* (Hu et al., 2005; Ito et al., 2005), *Theobroma cacao* (de Oliveira Santos et al., 2005), *Triticum aestivum* (Singh and Khurana, 2017), *Zea mays* (Baudino et al., 2001), *Cyrtochilum loxense* (Cueva et al., 2012), and *A. angustifolia* (Steiner et al., 2012).

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 (Hecht et al., 2001). In *O. sativa*, *SERK2* is expressed almost three times more in the embryogenic callus and maturation stage than in the non-embryogenic callus (Singla et al., 2009). 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., *SERK*2 and *SERK3* elicit auxin-specific responses while *SERK1* and *SERK5* may be mediated by the signaling pathway of brassinosteroids (Singh and Khurana, 2017).

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 (Nolan et al., 2003).

Leafy Cotyledon (LEC)

Another important participant in the regulation of SE and plant embryo development is the *LEC* family of TFs (Guo et al., 2013). *LEC1* has an essential role in ZE and has been suggested to control diverse processes in seed development (Pelletier et al., 2017; Tvorogova and Lutova, 2018), including embryo morphogenesis, maturation phases (Guo et al., 2013), germination (Tvorogova and Lutova, 2018), and early and late

embryogenesis; it also appears to allow the formation of the embryo by establishing an embryonic environment (Harada, 1999). *LEC1* is also involved in photosynthesis and chloroplast biogenesis early in seed development, and seed maturation late in the development of zygotic embryos (Pelletier et al., 2017). This gene network regulated by *LEC1* has been conserved in dicotyledonous plants that diverged tens of millions of years ago (Pelletier et al., 2017).

LEC1 and *LEC2* were the first TFs shown to induce SE when ectopically expressed in seedlings (Stone et al., 2001). 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* (Ledwon and Gaj, 2009), suggesting that *LEC2* mediates the increase in the endogenous auxins observed during the induction of SE (Ayil-Gutiérrez et al., 2013). 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 (Zhang et al., 2014). 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*) (Kim et al., 2014). 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 (Kim et al., 2014).

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 (Ikeda et al., 2009). 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 (Yadav et al., 2011). 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 (Somssich et al., 2016; Negin et al., 2017; Zhang et al., 2017). Therefore, *WUS* has been proposed to be essential for SE (Xiao et al., 2018) and *in vitro* shoot regeneration (Zhang et al., 2017).

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* (Zuo et al., 2002). The gradient of auxins that is detected during the pre-treatment of *C. canephora* plantlets and later during the initial phases of SE (Márquez-López et al., 2018) correlates with the induced *WUS* expression during SE in *A. thaliana* (Su et al., 2009).

It has been observed that *WUS*-related genes are up-regulated during SE in different species, such as *Ocotea catharinensis* Santa-Catarina et al. (2012), *M. truncatula* (Chen et al., 2009), *G. hirsutum* (Zheng et al., 2014), and *C. canephora* (Arroyo-Herrera et al., 2008). In *C. canephora*, overexpression of *WUS* enhances SE in heterologous systems (Arroyo-Herrera et al., 2008), increasing the somatic embryo production by 400%. In *G. hirsutum*, the ectopic expression of *AtWUS* promotes the proliferation and differentiation of transgenic callus and positively regulates *LEC1*, *LEC2*, and *FUS3* (Zheng et al., 2014). *WUS* overexpression enhances the induction of SE and can improve regeneration in cotton (Bouchabke-Coussa et al., 2013), and its overexpression in *A. thaliana* roots, leaf petioles, stems, or leaves induces the formation of somatic embryos (Zuo et al., 2002).

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 (Irikova et al., 2012). *BBM* triggers a set of genes like *LEC1* and *LEC2*, as well as *ABI3* and the *FUS3* network, which together activate SE (Horstman et al., 2017). The induction of SE by *BBM* is a dose-dependent mechanism and regulates the transcription of significant embryo identity genes (Horstman et al., 2017).

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 (Kim et al., 2005). The AP2/ERF domain can bind to a GCC box, a DNA sequence involved in the ethylene response (Ohme-Takagi and Shinshi, 1995). *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 (Gutterson and Reuber, 2004). Because *RAV* genes include another DNA-binding domain, B3, *RAV* genes are sometimes treated as a third group in the AP2/ERF family (Kim et al., 2005). The distinct feature of the BBM and BBM-like proteins is the presence of a conserved bbm-1 motif (GLSMIKTW) that is absent in other proteins of the euANT lineage (Bilichak et al., 2018). 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) (Passarinho et al., 2008).

In *A. thaliana* and *B. napus*, *BBM* changes its spatial-temporal expression in the early stages of embryogenesis (Kulinska-Lukaszek et al., 2012). Some reports show that *BBM* is expressed in the heart state of an embryo and root development (Galinha et al., 2007) and enhances the proliferation of somatic embryos (Florez et al., 2015). 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 (Kulinska-Lukaszek et al., 2012). The heterologous expression of *BBM* from *A. thaliana* and *B. napus* in *Nicotiana tabacum* produced an increase in the regeneration capability (Srinivasan et al., 2007). In *Capsicum annum,* both *LEC1* and *BBM* are expressed and show high levels of expression in

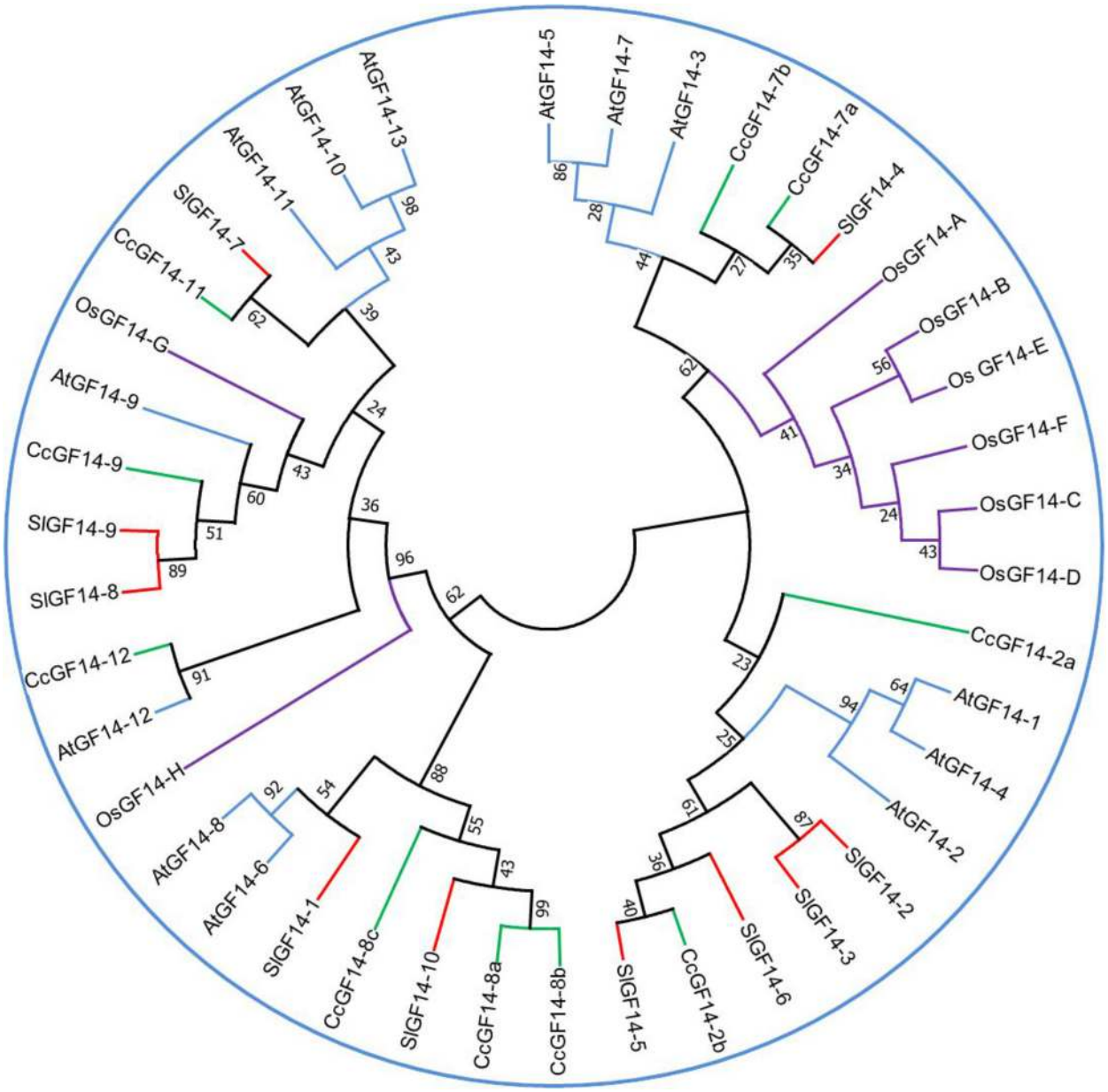

FIGURE 2 | Phylogenetic tree for 14-3-3 genes family in several species. The sequences of *Coffea canephora* GF14 were obtained from http://coffee-genome.org. Rice sequences were obtained in http://rice.plantbiology.msu.edu. Tomato sequences were obtained in https://solgenomics.net/. Arabidopsis sequences were obtained in https://www.arabidopsis.org/. The sequences were aligned in the software MEGA 7 (http://www.megasoftware.net/). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The analysis was conducted in MEGA7 using the Neighbor-Joining method. Abbreviations: Os, *Oryza sativa*; Sl, *Solanum lycopersicum*; Cc, *Coffea canephora*; At, *Arabidopsis thaliana*.

the different phases of development of the somatic embryo (Irikova et al., 2012).

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 (Silva et al., 2015), in *C. canephora* BBM1 expression was only observed after SE induction (Nic-Can et al., 2013). 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* (Florez et al., 2015), while the *BBM* overexpression in *Populus tomentosa* induced SE (Deng et al., 2009).

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 (Zhao et al., 2015) and during the induction of SE in *Carica papaya* (Vale Ede et al., 2014). The other factor actively involved during the SE induction, process, and development is epigenetic (Us-Camas et al., 2014; De-la-Peña et al., 2015; Duarte-Aké and De-la-Peña, 2016).

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 (Carlson and Perez, 1967).

In plants the number of members of these proteins is variable (**Figure 2**). There are 13 14-3-3 adaptor proteins in Arabidopsis (Rosenquist et al., 2000; DeLille et al., 2001), six in cotton (Zhang et al., 2010), 17 in tobacco (Konagaya et al., 2004), ten in tomato (Camoni et al., 2018), five in barley (Schoonheim et al., 2007), and eight in rice (Yao et al., 2007).

The use of proteomics techniques has illuminated the changes in hundreds of proteins, including the family 14-3-3, during the induction of SE (Zhao et al., 2015; Tchorbadjieva, 2016). Some 14-3-3 proteins are abundant in the embryogenic tissues of *Cyclamen persicum* (Lyngved et al., 2008), and *Larix principis* (Zhao et al., 2015). In oak, these proteins are more abundant in proliferating embryos than in mature embryos (Gomez-Garay et al., 2013).

An excellent example that shows the role of 14-3-3 proteins in the induction of SE is protein phosphatase 2A (PP2A) (Marsoni et al., 2008). This enzyme consists of a catalytic subunit and a regulatory A subunit together with a third variable B subunit (Janssens and Goris, 2001). 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 (Farkas et al., 2007). The subunit A is essential for auxin transport (Michniewicz et al., 2007), while the 65 kDa regulatory subunit of PP2A has regulatory functions. The subunit A has been associated with the SE process (Marsoni et al., 2008). 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 (Lyngved et al., 2008). Other components of the signal transduction cascade, such as G proteins and calreticulin, increased during cyclamen SE (Rensing et al., 2005). 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* (Lyngved et al., 2008) and *L. principis* embryogenic cultures (Zhao et al., 2015).

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 (Duarte-Aké and De-la-Peña, 2016; Kumar and van Staden, 2017). 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 (De-la-Peña et al., 2015).

DNA methylation is important for somatic embryo development (Nic-Can et al., 2013; Yakovlev et al., 2016). In general, higher global DNA methylation has been found in non-embryogenic cultures of *Pinus radiata* (Bravo et al., 2017), *P. nigra* (Noceda et al., 2009), *Rosa* x *hybrid* (Xu et al., 2004), and *Eleutherococcus senticosus* (Chakrabarty et al., 2003), while low global DNA methylation has been found in embryogenic cultures of several plants. In *Quercus alba* DNA is demethylation during the induction of SE (Corredoira et al., 2017), as well as during the generation of pro-embryogenic mass, but it gradually increases as the embryo is developing (LoSchiavo et al., 1989). Similar results were observed during the SE of *C. canephora* (Nic-Can et al., 2013), where the proembryogenic mass had lower DNA methylation, while the maturation of the embryos was marked by a gradual increase in the global levels of methylation.

In *A. thaliana* it was found that both *de novo* DNA methylation and maintenance of it are required for the regulation of SE (Grzybkowska et al., 2018), and similar results were found in *Picea abies* (Yakovlev et al., 2016). Changes in the global DNA methylation pattern during long-term subcultures could lead to the loss of the embryonic potential of proembryogenic masses (Fraga et al., 2016).

In order to prove that in fact DNA methylation is strongly related to SE, pharmacological experiments have been conducted in several plant species. The application of 5-azacitidine (5-AzaC; a demethylating agent) decreased the levels of global DNA methylation in *A. thaliana* and inhibited the induction of SE (Grzybkowska et al., 2018). Similar results have been found in *M. truncatula* (Santos and Fevereiro, 2002), *D. carota* (Yamamoto et al., 2005), and *C. canephora* (Nic-Can et al., 2013). Furthermore, *LEC1*, *LEC2*, and *BBM* genes were up-regulated in the *drm1drm2* and *drm1drm2cmt3* mutants, an upregulation that was related to an improvement in the SE response (Grzybkowska et al., 2018). In *T. cacao,* DNA methylation increased during the induction of SE, and treatment with 5-AzaC led to the recovery of SE potential in aged cultures (Pila Quinga et al., 2017). 5-AzaC

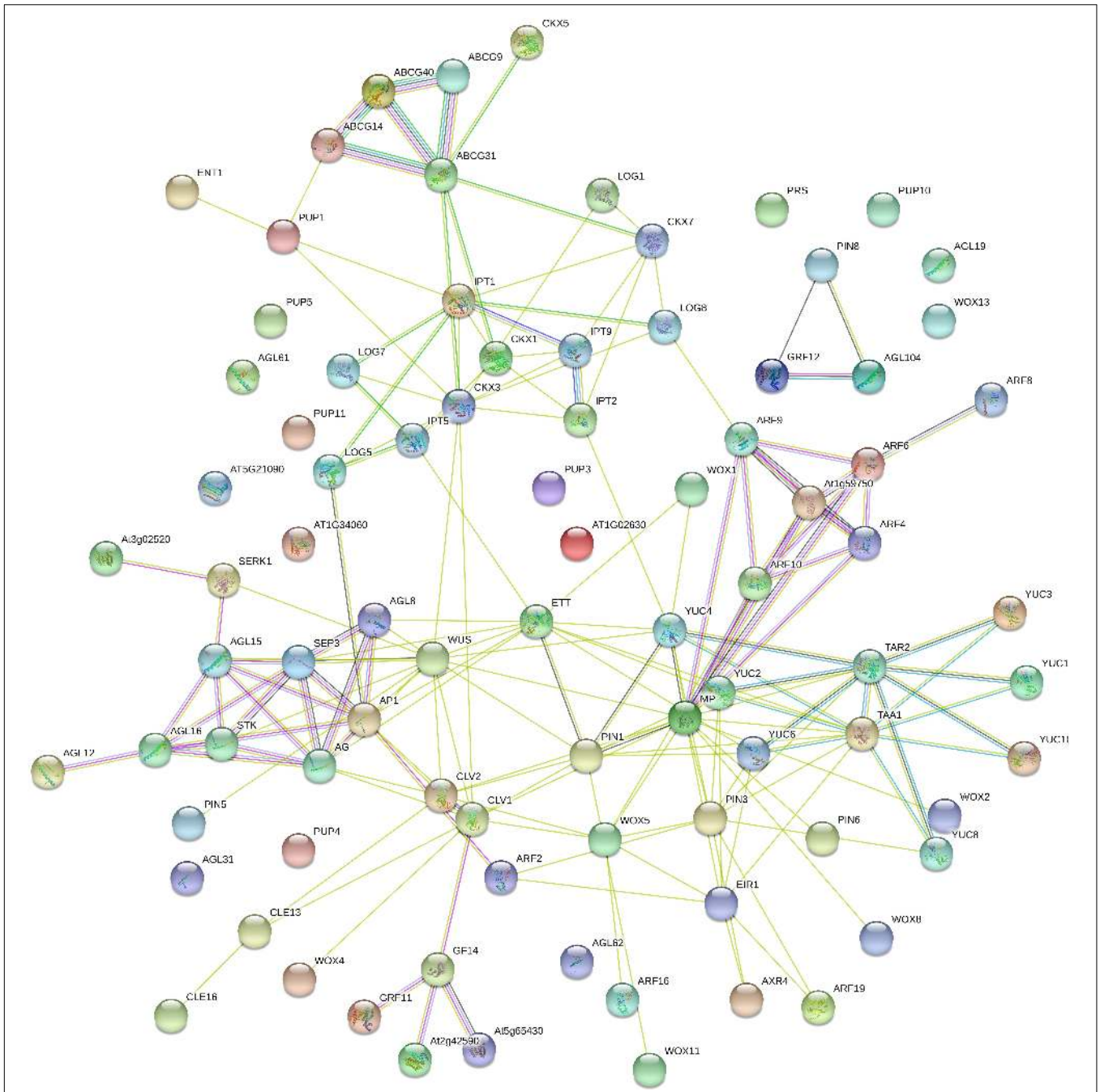

FIGURE 3 | Interactome of *Coffea canephora* proteins related to somatic embryogenesis. *C. canephora* proteins were compared with *Arabidopsis thaliana* proteins using STRING software (https://string-db.org/); max score and sequence coverage were the principal parameters in the identification and selection. Colored lines mean the following: Gene neighborhood (dark green), co-expression (black), experimentally determined (pink), Text-mining (light green), from a curated database (light blue), protein homology (gray), and gene co-occurrence (dark blue). The description of the roles of every gene in the interactome is listed in **Supplementary Table S2**.

is not the only drug used to disrupt epigenetic modifications; trichostatin A (TSA), the function of which is inhibiting histone deacetylases (HDACs), has a positive effect on gene expression. The inhibition of HDACs has also led to an increase in the number of haploid embryos produced by heat stress in *B. napus* (Li et al., 2014). In fact, the treatment with TSA of germinating spruce somatic embryos preserves their embryogenic nature (Uddenberg et al., 2011). In the double mutant hda6/hda19, the upregulation of *LEC1*, *FUS3*, and *ABI3* genes was evident in germinating Arabidopsis seeds (Tanaka et al., 2008). These double mutants also led to the production of somatic embryos in the leaves of Arabidopsis (Tanaka et al., 2008).

Histones' posttranslational modifications have been implicated in the formation of somatic embryos. Histone deacetylation may also play a role in the reprogramming of cells in the early stages of SE (De-la-Peña et al., 2015; Lee et al., 2016), since the levels of histone acetylation and the activity of HDACs change in response to the presence of exogenous PGR during the induction of SE.

There are several tissue-specific events involving H3K27me3. The loss of this mark upregulates the auxin pathway and its increase leads to the repression of leaf identity (He et al., 2012). Polycomb repressive complex 2 (PRC2) is involved in the methylation of lysine 27 in histone H3 (Molitor et al., 2014). Double mutants of the *PRC2* gene, which functions as a histone methyltransferase, *CLF* and *SWN* or *VERNALIZATION 2* (*VRN2*) and *EMBRYONIC FLOWER2* (*EMF2*) form callus on the shoot apex, lead to indirect somatic embryo formation and ectopic roots (Chanvivattana et al., 2004). A *PRC2* mutant root hairs fail to maintain their differentiated state and form unorganized cell masses and eventually somatic embryos from callus (Ikeuchi et al., 2015). The effect of silencing genes of the PCR2 family in inducing SE depends on the explant. In tissues where *PCR2* is scarcely active, the production of somatic embryos is efficient; however, in the tissues where it is highly expressed somatic embryos do not form (Liu et al., 2016; Mozgová et al., 2017).

CONCLUDING REMARKS

Since the 1950s, the research on the SE process has gone from empirical approaches to a more methodical investigation leading to the production of somatic embryos (Loyola-Vargas, 2016). We are well on the way to understanding the role of auxins and other PGRs, as well as stress, on the induction of SE (Nic-Can et al., 2016; Nic-Can and Loyola-Vargas, 2016). We now have a set of genes that, in some cases, can be used as markers of the initiation of SE. However, the signal pathway from the initial signal to the first steps of the development of the somatic embryo remains practically unknown.

Scientists have just begun to understand the complex network of interactions among a set of TFs, the endogenous concentrations of auxins, CKs, ABA, ethylene and salicylic acid, their transport and receptors, and the origin of the explant that lead to the establishment of a somatic embryo (**Figure 3** and **Supplementary Table S2**).

Current scientific knowledge lets us hypothesize that the initial signal, stress or the signals produced by the PGRs induce a change in the endogenous concentration of several PGRs, especially auxins and CKs. The differences in the relationship between the auxins and CKs lead to the expression of TFs and ARF, which in turn modify the cell wall, a vital component in the cell differentiation process. Once the cell(s) are settled into the SE pathway, the expression of TFs, such as *BBM*, *SERC*, and *LEC*, leads to downstream changes in the endogenous content of different compounds and produces a cascade of events, such as chromatin remolding, that drives the induction of SE. However, there are still many questions to answer to understand how the life of a somatic embryo begins. The roles of ethylene, salicylic acid, the organization of the cytoskeleton, brassinosteroids, and other compounds remain to be elucidated.

The overexpression of genes such as *WUS*, *BBM*, and *LEC* has been used to induce SE in different plant species. This approach has been instrumental in understanding the role of different genes during the induction of SE; however, under certain conditions, the overexpression also inhibits the induction of SE. This means that under the present state of the art, every gene and every plant species must be tested, before all of the pieces of the puzzle are in place.

Increasing knowledge of the induction of SE and of the development of somatic embryos will lead to the development of multiple biotechnological applications and new opportunities for the understanding of the fundamental aspects of SE. In particular, the alteration in the methylation or acetylation profile of DNA and/or histones by genome-editing techniques holds great promise to increase the production and to improve the quality of crops of agronomical importance (Karim et al., 2016).

AUTHOR CONTRIBUTIONS

VL-V developed the idea. All authors drafted the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2019.00077/full#supplementary-material

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