Chapter 5
Transcription Factors in the Regulation of Somatic Embryogenesis

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Abstract Somatic embryogenesis (SE), the process through which already differentiated cells reverse their developmental programme and become embryogenic, requires drastic changes in the transcriptome of the explant cells. Among the various factors that underlie this developmental switch, genes encoding transcription factors (TFs), which constitute the sequence-specific DNA-binding proteins, are widely accepted as playing a central function in the gene expression regulation. In recent years, intensive analysis of the global transcriptomes of plant cells that are undergoing embryogenic transition and the use of Arabidopsis (a model in plant genomics) in studies on the genetic control of SE have substantially contributed to the identification of SE regulators. A survey of SE-associated transcriptomes illustrated the combinational effects of stress and hormone signalling that are related to the in vitro environment that is imposed during a culture. Accordingly, among the TFs that are considered to be essential in SE induction, those that are involved in stress and hormone plant responses and especially flower development were found to be most frequent. This chapter provides a comprehensive review of the current knowledge about the TFs that are involved in the induction of SE in plant explants that are cultured in vitro. In addition to a general characterisation of the TF transcriptomes that are associated with SE induction in different plants, the individual TF genes with documented functions in the regulation of SE are presented with a special reference to their possible targets and the TF-controlled molecular mechanisms that underlie SE induction.

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5.1 Global Characteristics of the SE-Associated TF Transcriptomes

The reprogramming of already differentiated somatic cells towards embryonic development requires a substantial modification of a cell's transcriptomes. Embryogenic transition induced in somatic cells involves the repression or activation of numerous genes and thus transcription factors (TFs) that have a key function in the regulation of gene expression seem to play a crucial role in this process. The large number (6–10 %) of TF-coding genes that have been found in plant genomes imply the transcriptional regulation to play an even more important role in plant than in animal development (Riechmann et al. 2000).

Most of the available data on SE-involved transcriptomes was provided by global analytical approaches and among these microarray analysis has been intensively applied to investigate the embryogenic cultures of different plants including oil palm (Low et al. 2008), *Medicago truncatula* (Mantiri et al. 2008a), potato (Sharma et al. 2008), rice (Chakrabarty et al. 2010) and cucumber (Wiśniewska et al. 2012). Besides microarrays, EST sequencing in wheat (Singla et al. 2007) and RNA-seq in Arabidopsis (Wickramasuriya and Dunwell 2015) and cotton (Yang et al. 2012) have been applied in order to reveal SE-related transcriptomes. The microarray-based data showed that 1–12 % of all of the genes that were significantly modulated during embryogenic induction in different plants were found to encode TFs. However, the number of SE-involved TF genes based on microarray data appears to be seriously underestimated. A much more accurate evaluation of the TF genes that are involved in SE might provide approaches that are focused specifically on the analysis of TF transcriptomes. Accordingly, a multi-parallel qRT-PCR analysis of almost 1,900 TF genes of Arabidopsis showed that 1,768 (94 %) TF genes were expressed during SE induction and a large fraction of these (41 %) was found to have undergone a significant modulation of transcription (Gliwicka et al. 2013).

The examination of SE-related transcriptomes indicated that a common set of TF genes that encode the proteins representing MYB, MADS, AP2/ERF, bHLH, C2H2, WRKY, NAC and HB families is engaged in SE induction in different plants. Members of SE-involved TF families belong to various functional groups and the TF genes that are engaged in the transcriptional regulation of hormone and stress responses as well as those that control plant developmental processes, predominantly embryo and flower development, have been found to be the most frequent (Thibaud-Nissen et al. 2003; Che et al. 2006; Hosp et al. 2007; Sharma et al. 2008; Gliwicka et al. 2013; Wickramasuriya and Dunwell 2015). Interestingly, the *TF*s that control the development of the plant generative organs were found to be especially frequent among SE-modulated transcripts, thus suggesting that there are some similarities in the genetic regulation of generative and embryogenic transitions (Thibaud-Nissen et al. 2003; Sharma et al. 2008; Gliwicka et al. 2013).

The TFs that control zygotic embryogenesis (ZE) constitute the obvious candidates for SE regulators due to the anticipated similarities of an SE to its zygotic counterpart (Dodeman et al. 1997). Expression of at least 500 genes has been reported to control ZE in Arabidopsis and among the genes-encoded TFs, the members of ABI3VP1, AP2/ERF, ARF, C3H and Dof families have been indicated (Tzafrir et al. 2004), but these data also appear to be underestimated. A recent study on the ZE transcriptome of Arabidopsis showed that at least 60 % of Arabidopsis genes are expressed during seed development and up to 5 % of these were identified as TF-encoded (Belmonte et al. 2013). In support of the expected similarities in SE- and ZE-related transcriptomes, an analysis of an embryogenic culture in rice revealed that out of 242 rice homologues of the genes that are essential for ZE in Arabidopsis, 87 % were expressed during SE (Su et al. 2007). Further support for some type of convergence of the genetic determinants that control SE and ZE was provided by the observation that most of the genes that are differentially regulated during SE in various plants were revealed to represent the major TF families that are engaged in ZE (Singla et al. 2007; Imin et al. 2008; Chakrabarty et al. 2010; Wisniewska et al. 2012; Gliwicka et al. 2013; Wickramasuriya and Dunwell 2015).

In contrast to the numerous TF genes that have a differential expression in the embryogenic cultures of different plants and are thus assumed to contribute to SE induction (Singla et al. 2007; Sharma et al. 2008; Mantiri et al. 2008a; Chakrabarty et al. 2010; Wiśniewska et al. 2012), only a small number has been experimentally proven to control SE transition. Conclusively for the mechanism that is involved in SE induction, among the TF genes that have validated functions in SE, those that are related to hormone and stress responses have been indicted to be prevalent (Zavattieri et al. 2010; Fehér 2015).

The overrepresentation of the TFs that is related to hormone responses among the SE-modulated genes that have been reported in different plants reflects the common belief about the essential role of plant growth regulators in the control of the morphogenic pathways, including SE induced in plants in vitro (Jiménez 2005; Fehér et al. 2003). In Arabidopsis, 43 % of SE-modulated TF genes have been annotated to be hormone-related (Gliwicka et al. 2013). Besides the TFs that are involved in the metabolism and signalling of auxin, the genes that are related to cytokinin (CK), abscisic acid (ABA), jasmonic acid (JA), ethylene (ET), gibberellin (GA) and brassinosteroids (BR) have been reported in Arabidopsis and other plants (Singla et al. 2007; Imin et al. 2008; Chakrabarty et al. 2010; Wisniewska et al. 2012; Gliwicka et al. 2013; Wickramasuriya and Dunwell 2015). In support of the essential role of the genetic regulation of hormone metabolism and signalling in embryogenic transition, the mutations affected the level and sensitivity of different hormones (IAA, ABA, GA and ethylene) and the inhibitors of hormone metabolism or signalling have been shown to negatively impact SE induction in Arabidopsis (Gaj et al. 2006; Bai et al. 2013; Nowak et al. 2015).

Relevant to the common use of auxin treatment in SE induction in different plants (Gaj 2004) and the key role of IAA in the control of plant development (reviewed in Vanneste and Friml 2009), auxin signalling and metabolism have been postulated as being crucial for in vitro induced SE (Jiménez 2005; Fehér et al.

2003). In accordance, auxin-related TFs have been found to be the most frequent among the hormone-related genes that are involved in SE induction (Yang et al. 2012; Gliwicka et al. 2013; Wickramasuriya and Dunwell 2015). The TF genes that have a regulatory role in SE include the core regulators of auxin signalling, *AUX/IAA* and *ARF* genes (Rensing et al. 2005; Su et al. 2007; Wu et al. 2009; Yang et al. 2012; Gliwicka et al. 2013). A differential expression of *IAA16*, *IAA29*, *IAA30*, *IAA31* and *ARF1*, *ARF2*, *ARF3*, *ARF5*, *ARF6*, *ARF8* and *ARF11* was observed during SE induction in Arabidopsis and the mutants in these genes were substantially defective in the embryogenic response (Gliwicka et al. 2013; Wójcikowska and MDG, in preparation for publication). AUX/IAA–ARF-mediated auxin responses were also assumed to operate in the embryogenic cultures of other plants including cotton, rice, *Cyclamen persicum* and *Gossypium hirsutum* (Rensing et al. 2005; Su et al. 2007; Wu et al. 2009; Yang et al. 2012). This induction of *AUX/IAA* and *ARF* expression that is commonly associated with SE parallels a substantial function of auxin signalling in the control of zygotic embryo development (Sato and Yamamoto 2008; Rademacher et al. 2011, 2012).

In spite of the notable progress that has recently been made in deciphering the auxin-mediated regulation of plant development, our knowledge about the functions of *ARFs* in different developmental processes is still fragmentary and the results that have been obtained in various experimental approaches are frequently inconclusive (Rademacher et al. 2011). Within *ARF*s, the *ARF5* encoded so-called MONOPTEROS (MP) protein is functionally the best characterised and the role of MP in the mediation of auxin signal has been documented in a number of developmental processes including ZE (reviewed in Möller and Weijers 2009). In addition to auxin signalling, ARF5 was also reported to control the polar auxin transport by targeting the auxin efflux carrier, *PIN1* (*PIN-FORMED1*) (Wenzel et al. 2007). Our recent results provided some evidence about the engagement of *ARF5* in SE induction including the auxin-stimulated, strong accumulation of its transcripts in the explant parts that are involved in SE and the significantly impaired embryogenic potential of the *afr5* mutant and the overexpressor line (Wójcikowska and MDG, in preparation for publication). However, the mechanism of *ARF5* action in embryogenic transition remains to be demonstrated and a prerequisite for an understanding of the biological function of *ARFs* in somatic cells that are undergoing SE induction is the identification of their target genes, especially those that are directly controlled.

5.2 *LEAFY COTYLEDON* Genes—Master Regulators of the Embryogenic Development in Plants

The *LEC* group of genes includes the *LEC1*, *LEC2* and *FUS3*-encoded TFs that have a major role in the control of the morphogenesis and the maturation phases during ZE (Harada 2001). *LEC1* encodes the CCAAT box-binding factor HAP3 subunit (Lotan et al. 1998) while *LEC2* and *FUS3* encode proteins that have a

plant-specific B3 domain, which binds a highly conserved RY motif and regulates the expression of ZE-specific genes (Stone et al. 2001; Braybrook et al. 2006). The observation that the overexpression of *LEC2* and *LEC1* resulted in developmental disorders in plants that included the formation of callus and somatic embryos on seedlings suggested SE-related functions of *LEC*s (Lotan et al. 1998; Stone et al. 2001). In support of the proposed role of the *LECs* in the embryogenic transition of somatic cells, the *lec* mutants were found to be strongly defective in SE but not in the shoot organogenesis that was induced in vitro (Gaj et al. 2005). In addition, a key role of LEC TFs in the establishment of a cellular environment that promotes embryo development supported the activity of the LEC genes, which has commonly been observed in the embryogenic cultures of different plants (Zuo et al. 2002b; Harding et al. 2003; Yazawa et al. 2004; Ikeda et al. 2006; Fambrini et al. 2006; Guo et al. 2013; Zhang et al. 2014; Zhu et al. 2014a). The complex interactions between LEC genes and hormone metabolism that were revealed to control the maturation phase in zygotic embryo development (reviewed in Jia et al. 2014) provided a clue as to how LEC genes might support SE induction. The LEC-mediated control of auxin, ABA and GA metabolism observed during ZE seemed to especially be of importance for the promotion of SE (Braybrook and Harada 2008).

5.2.1 LEC2

Among the *LEC* genes, the *LEC2*-mediated mechanism of SE induction has been the most intensively investigated. As a result, the auxin-related functions of *LEC2* in the embryogenic transition of somatic cells have been documented. Similar to the regulatory link that was observed between the *LEC2* gene and *YUC* genes involved in auxin biosynthesis in Arabidopsis seedlings (Stone et al. 2008), *LEC2* was found to stimulate *YUC1*, *YUC4* and *YUC10* transcripts in in vitro cultured explants (Wójcikowska et al. 2013). As a result of the LEC2-mediated activation of the *YUC* pathway of auxin biosynthesis, a significant increase of IAA content was demonstrated in explant tissue that was undergoing embryogenic transition (Wójcikowska and Gaj 2015). The activation of *YUC* genes was also reported in the embryogenic callus of Arabidopsis in which somatic embryos were induced in response to the removal of auxin from a medium (Bai et al. 2013). *LEC2* was postulated to directly target *YUC4* in planta (Stone et al. 2008); however, further analyses are necessary to reveal the mode of regulatory interaction that has been observed between *LEC2* and *YUC* genes upon SE induction in vitro. Collectively, *LEC2* contributes to SE induction via an increase in the endogenous auxin levels that in turn results in the activation of the auxin-responsive genes that are operating in the SE-inductive network. Genetic components of this network and their complex interactions remain to be determined. Revealing how similar the molecular pathways that are triggered by endogenous versus exogenous auxin during SE induction will also be challenging.

In addition to the regulation of auxin metabolism, *LEC2* may be involved in the control of auxin signalling. To support this, the *LEC2*-mediated activation of the key components of the auxin-response pathway, members of *AUX/IAA* family (*IAA1*, *IAA17*, *IAA30* and *IAA31*), was reported in Arabidopsis seedlings (Braybrook et al. 2006; Stone et al. 2008). Relevant to these observations and important for the predicted functions of *AUX/IAA* genes in SE, mutations in two of these genes, *iaa30* and *iaa31,* were observed to seriously impair the embryogenic potential of in vitro cultured explants (Gliwicka et al. 2013). In addition to the postulated regulatory interactions with the auxin metabolism and signalling, the possible involvement of *LEC2* in auxin polar transport cannot be ruled out as the upregulation of auxin efflux facilitators, *PIN1* and *PIN2*, was observed in transgenic tobacco plants that overexpressed *LEC2* (Guo et al. 2013). PIN proteins are believed to direct plant developmental responses to environmental and endogenous signals through the control of the polar cell-to-cell transport of auxin (Habets and Offringa 2014), and relevantly, a key function of the auxin efflux carriers in ZE was documented (Friml et al. 2003). The findings that the explants of a *pin1* mutant were unable to undergo embryogenic induction in vitro (Su et al. 2009) and that the inhibitors of the auxin polar transport severely impaired the embryogenic response of explants in different plants (Venkatesh et al. 2009; Palovaara et al. 2010) provided further support for the involvement of *PINs* in SE induction.

The complex *LEC2*-mediated crosstalk between hormones is assumed to be associated with the mechanism of SE induction considering that in tissues that overexpress *LEC2*, the increase of auxin content has been related to the extensive changes in the accumulation of cytokinins, ABA and SA (Wójcikowska and Gaj 2015). Furthermore, a link between *LEC2* and ethylene may be also expected considering the *LEC2*-stimulated expression of the *ACS4* gene that is engaged in the synthesis of an ethylene precursor (Braybrook et al. 2006), and the regulatory relationship that has been indicated between the *LEC2* and *ERF022* genes involved in the ethylene biosynthesis/signalling (Nowak et al. 2015).

The observation that the overexpression of the *YUC* genes alone is not sufficient to induce SE provided some additional insight into the hormone-related functions of *LEC2* in SE (Zhao et al. 2001). This implies that only SE-competent cells can respond to the auxin signal. Thus, relevant to the *LEC2* function in the maturation phase of ZE, the gene was proposed to enable somatic cells to become capable of responding to the SE-inductive signal by lowering the GA content (Braybrook and Harada 2008). In this GA-related regulatory circuit, LEC2 directly activates *AGL15*, which in turn activates *GA2ox6* resulting in a reduced GA level coupled with the enhanced potential for the formation of somatic embryos (Wang et al. 2004). The report on the negative impact of exogenous GA_3 on the embryogenic response of Arabidopsis explants supports the inverse relation between the GA level and a tissue's capacity for SE (Gaj et al. 2006). The *LEC2*-mediated establishment of a proper balance between GA and ABA levels promotes the accumulation of the storage reserves that was proposed to enhance the embryogenic competence in cells (Braybrook and Harada 2008). The fact that the ectopic expression of *AtLEC2* has

been reported to induce the maturation processes in transgenic Arabidopsis, tobacco and *Theobroma cacao* tissue (Stone et al. 2008; Guo et al. 2013; Zhang et al. 2014) and that the high expression of the genes encoding storage proteins, including *CRA1* and *OLEO4*, was found to be associated with the embryogenic potential of Arabidopsis (Stone et al. 2008; Gliwicka et al. 2012) support this speculation.

Considering that *LEC2* positively impacts auxin accumulation and in turn, auxin activates its expression (Ledwoń and Gaj 2009; Wójcikowska et al. 2013), the revealing of the genetic components of a regulatory feedback loop that seems to operate between auxin and *LEC2* is required for the full understanding of the LEC2-controlled mechanism of embryogenic transition. In line with this notion, an auxin-responsive AuxRE element was identified in *LEC2* promoter region, which implies the involvement of ARFs in the regulation of *LEC2* expression. Among the potential regulators of *LEC2*, there are several *ARFs* (*ARF1*, *ARF2*, *ARF3*, *ARF5*, *ARF6*, *ARF8*, *ARF11*) that are differentially expressed in SE of Arabidopsis (Wójcikowska and MDG, in preparation for publication).

In the search for the genetic regulators of *LEC2* in SE, the proteins that are indicated to directly inhibit the *LEC2* expression in planta should be considered including, TT8 (TRANSPARENT TESTA8), ASIL1 (ARABIDOPSIS 6B-INTERACTING PROTEIN1-LIKE1) and PRC (POLYCOMB REPRESSIVE COMPLEXES) (Gao et al. 2009; He et al. 2013; Chen et al. 2014). In addition, miRNA166 was reported to indirectly control *LEC2* expression through the regulation of *PHABULOSA* (*PHB*) and *PHAVOLUTA* (*PHV*) (Tang et al. 2012). Whether similar regulatory interactions exist during SE induction is as yet unknown.

In summary, a possible model of the LEC2-controlled and hormone-related pathways that seem to underlie the embryogenic transition in somatic cells can be proposed (Fig. 5.1).

5.2.2 LEC1

Expression of *LEC1* has been associated with SE that is induced in vitro and in planta (Lotan et al. 1998; Yazawa et al. 2004; Garcês et al. 2007; Alemanno et al. 2008; Ledwoń and Gaj 2009; Guo et al. 2013; Nic-Can et al. 2013; Zhu et al. 2014a). Similar to *LEC2* and meaningful for the possible role of *LEC1* in the SE-inductive mechanism, the encoded TF is involved in auxin metabolism/signalling. Among the candidate targets of LEC1, the *YUC10* gene, which is involved in auxin biosynthesis and the members of the Aux/IAA family (*IAA5*, *IAA16*, *IAA19*), were postulated (Junker et al. 2012). In addition to auxin, the regulatory relations between *LEC1* and the metabolism/signalling of other hormones including ABA, JA and BR were implicated as underlying the function of *LEC1* in zygotic and somatic embryogenesis (reviewed in Junker and Bäumlein 2012; Junker et al. 2012).

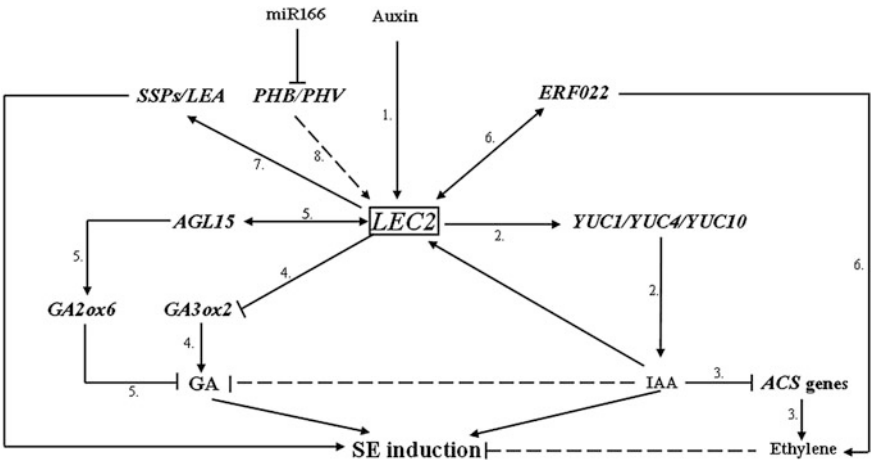

Fig. 5.1 Model for the *LEC2* role in SE induction through gene interactions with hormones. The interactions that need verification are indicated with dashed lines. *1* Ledwoń and Gaj (2009); *2* Wójcikowska et al. (2013); *3* Bai et al. (2013); *4* Curaba et al. (2004); *5* Braybrook et al. (2006); *6* Nowak et al. (2015); *7* Braybrook et al. (2006); *8* Tang et al. (2012). SSP-seed storage protein

In addition to hormones, the sugar-related functions of *LEC1* were reported to promote embryonic cell identity and in support of this, sucrose was demonstrated to modulate the penetrance of embryogenic traits in a *turnip* mutant that ectopically expressed *LEC1* (Casson and Lindsey 2006). Given the fact that sugars are proposed to act as morphogens that provide positional information in plant development, *LEC1* may exert its role in control of SE via the regulation of the sugar metabolism (Rolland et al. 2002). Another clue for the identification of the SE-promoting functions of *LEC1* was provided by the observation about the upregulation of the genes encoding the cell wall associated enzymes—hydrolase xyloglucan (XTH9) and expansine (EXP1B) in response to *LEC1* overexpression (Junker et al. 2012).

LEC1 is a member of the nuclear factor Y (NF-Y) family of TFs, which are highly conserved in all eukaryotic organisms. The NF-Y heterotrimer consists of three subunits NF-YA, NF-YB and NF-YC while *LEC1* represents NF-YB9 TF (Mu et al. 2008). Importantly, for the understanding of the *LEC1*-related regulatory interactions that can operate during SE, the overexpression of *LEC1* upregulates *NF-YA1*, *NF-YA5* and *NF-YA9* and, in turn, the overexpression of *NF-YA1* and *NF-YA9* positively regulates the expression of embryo- or seed-specific genes including *LEC1* (Mu et al. 2008, 2013). Similar to *LEC1*, the overexpression of *NF-YA1*, *NF-YA5*, *NF-YA6* and *NF-YA9* is sufficient to induce the formation of somatic embryos from vegetative tissues (Mu et al. 2013). Moreover, the cooperation of *NF-YA5* and *LEC1* is involved in the regulation of the genes that are responsible for the zygotic embryo development (Zhao et al. 2009) and whether similar interaction occurs during SE induction remains to be determined.

Collectively, some of the evidence presented above indicates the involvement of *LEC1* in SE induction; however, the impact of the encoded TF on the embryogenic response appears to be less pronounced in comparison to *LEC2*.

5.2.3 FUS3

In contrast to the other two *LEC* genes, *FUS3* is not upregulated in Arabidopsis explants induced towards SE, and overexpression of *FUS3* does not lead to the formation of somatic embryos (Ledwoń and Gaj 2011). However, the existence of a pathway that involves an LEC2-induced increase in auxin levels that promotes FUS3 activity was proposed (Braybrook and Harada 2008), thus suggesting that FUS3 might be involved in an LEC2-controlled mechanism of SE induction.

Numerous hormone-related genes are expressed in response to the activation of *FUS3* and among them the *YUC* genes of auxin biosynthesis, *AUX/IAAs* and *ARFs*, which encode the key components of auxin signalling, and the genes that are related to the biosynthesis of ABA, CK and BR were reported (Yamamoto et al. 2010; Wang and Perry 2013). Moreover, the relation of *FUS3* to GA was documented and the encoded TF, similar to two other LEC TFs, may enhance the competence for SE induction via the repression of *AtGA3ox2*, thereby resulting in a reduced level of bioactive GA (Curaba et al. 2004).

FUS3 was also reported to regulate vegetative phase transitions by negatively modulating ethylene-regulated genes in Arabidopsis, and among the downregulated genes those involved in ethylene biosynthesis (*ACS6*) and signalling (*ERF1*, *ERF104*, *ESE3*, *EDF4*) were reported (Lumba et al. 2012). In support of the ethylene-related function of *FUS3*, the ethylene level was found to correlate with the expression of *GmFUS3* in SE of soybean (Zheng et al. 2013). The role of the ethylene-associated activities of *FUS3* in SE induction requires further study.

5.3 SE-Related Functions of *AGL15*

AGL15 encodes one of the MADS domain proteins that it is believed to play key roles in the regulation of the developmental processes in eukaryotes (reviewed in Smaczniak et al. 2012). TF with MADS domain selectively binds to a consensus DNA sequence, the CArG (C-A/T rich-G) motif, to either activate or repress the expression of the targeted genes (West et al. 1997). The SE-related function of *AGL15* was postulated in Arabidopsis due to the somatic embryo-promoting effect of *AGL15* overexpression that was observed in the seedlings and in immature zygotic embryos that were cultured in vitro (Harding et al. 2003; Thakare et al. 2008). The AGL15 protein was found to accumulate during the early stages of ZE in *Brassica napus*, *Zea mays* and *A. thaliana*, in the somatic embryos of *Medicago sativa* and in a microspore culture of *B. napus* (Perry et al. 1999). The role of

AGL15 in the promotion of embryogenic responses was reported as being related to the GA metabolism and as a result, the *GA2ox6* that encodes gibberellin oxidase was identified among the targets of AGL15 (Thakare et al. 2008). It is postulated that AGL15 controls SE via the downregulation of the level of biologically active GA, and the inhibitory effect of GA on cell division may account for the requirement of a low level of this hormone during the early stages of SE (Wang et al. 2004). Besides GA, AGL15 seems to control the metabolism of ethylene. Recently, *At5g61590*, a member of AP2/ERF family and an orthologue of *MtSERF1*, which is involved in SE induction in *M. truncatula,* was identified as being a direct target of AGL15 (Zheng et al. 2013). It was shown that *At5g61590* (*DEWAX–Decrease Wax Biosynthesis*) acts as a repressor of the biosynthesis of cuticular wax (Go et al. 2014). In addition, the ethylene biosynthesis genes, *ACC SYNTHASE* (*ACS*) and *ACC OXIDASE* (*ACO*), are expressed in response to *AGL15* (Zheng et al. 2013). Stress-related functions of *AGL15* were postulated in soybean and the enhanced embryogenic response of explants that was observed upon the overexpression of *GmAGL15* was suggested to be the result of the activation of the genes that are involved in stress response (Zheng and Perry 2014).

AGL15 is believed to be a component of the SERK1 (SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE1) complex (Karlova et al. 2006), which was proposed to mark the cells that are competent in SE (Hecht et al. 2001). *SERK1* was assumed to interact with BRI1 (BRASINOSTEROID-INSENSITIVE1), and thus the function of AGL15 in BR signalling might be inferred (Aker and de Vries 2008).

AGL15 was also hypothesised to be involved in chromatin repression via its interaction with SIN3/HDAC (SWI-INDEPENDENT3/HISTONE DEACETY LASE), HDA (Hill et al. 2008) and topless (TPL) and topless-related (TPR) proteins (Causier et al. 2012). AGL15 has also been suggested to contribute to chromatin repression by increasing the efficiency of the formation of a repressive complex or the recruitment of a corepressor (Fernandez et al. 2015). Further analysis is needed to identify the targets of the epigenetic repression that is mediated by AGL15 during embryogenic induction.

Besides protein-encoded targets, the genes encoding microRNA156 (*MIR156a* and *MIR156c*), which are involved in the suppression of the SQUAMOSA promoter binding protein-like 3 (SPL3) transcription factor that promotes the floral transition in Arabidopsis, have recently been indicated to be AGL15-controlled (Wu and Poethig 2006; Serivichyaswat et al. 2015). Considering the apparent similarities in the genetic control of floral and embryogenic induction (El Ouakfaoui et al. 2010), a regulatory interaction between AGL15 and the floral suppressor, miR156, may be anticipated during SE induction. In support of this, the significant repression of the miR156 accumulation was observed during SE induction in Arabidopsis (Szyrajew and MDG, data unpublished). However, the existence of a regulatory link between the AGL15 and *MIR156* genes in an embryogenic culture remains to be validated, and the identification of the miR156-regulated mRNAs upon SE induction is required in order to reveal whether the mechanism of embryogenic transition is convergent with floral induction.

5.4 Stress-Related TFs

The embryogenic transition of the somatic cells was postulated to manifest a general response of the cultured tissue to stress conditions imposed in vitro such as wounding or the 2, 4-D treatment of explants (Fehér et al. 2003; Karami and Saidi 2010). In line with this postulate, various stress factors (osmotic, salt, water and heavy metals) were shown to replace or enhance the hormone treatment that was used for SE induction in different plant species (Kamada et al. 1993; Patnaik et al. 2005). Relevant to the assumed role of stress in SE induction, numerous (4–12 %) stress-related mRNAs have been found among those that were differentially expressed during SE induction (Legrand et al. 2007; Chakrabarty et al. 2010; Yang et al. 2012; Jin et al. 2014; Wickramasuriya and Dunwell 2015). In conjunction with the stress-regulated mechanism of SE induction, almost 40 % of the differentially expressed TF genes in an embryogenic culture of Arabidopsis were annotated to stress-related functions (Gliwicka et al. 2013). Some of these genes were subjected to closer inspection including *NTL8*, *DREB2F*, *ATHB-12*, *LBD20* and *MYB74*, and the SE-impaired phenotypes that were observed in the plants that carried mutations in these genes strongly support the notion that SE induction shares a mechanism at the molecular level that is relevant to general stress responses (Gliwicka et al. 2013).

5.4.1 ERF *Genes and the Ethylene Response*

In SE induced in Arabidopsis, *AP2/ERF* genes constituted a substantial part (10 %) of the stress-related TF genes that had a differential expression (Gliwicka et al. 2013). Similar to these data that are based on qRT-PCR analysis, RNA-seq analysis of the SE-related transcriptome of an embryogenic culture in Arabidopsis indicated that almost 40 % of the highly stimulated TF genes represented the genes encoding AP2/ERF TFs (Wickramasuriya and Dunwell 2015). In addition to Arabidopsis, the differential activity of numerous *ERF* genes was described in embryogenic cultures of wheat (Singla et al. 2007), *M. truncatula* (Imin et al. 2008), rice (Chakrabarty et al. 2010), cucumber (Wisniewska et al. 2012) and *Hevea brasiliensis* (Piyatrakul et al. 2012). Given that ERF TFs were assumed to regulate stress responses, and especially ethylene-related pathways (Nakano et al. 2006), the common expression of *ERF*s during SE induction implies the involvement of ethylene in SE induction.

The observed modulation of ethylene-related *ERF* genes during SE induction may result in part from the auxin treatment of explants. To support this assumption, auxin was documented as influencing ethylene signalling and metabolism and the complex interactions between auxin and ethylene were recently demonstrated in the regulation of plant development (Stepanova et al. 2007; reviewed in Muday et al. 2012). In accordance with in planta development, the ethylene level was also shown

to affect auxin biosynthesis and distribution in an embryogenic culture of Arabidopsis (Bai et al. 2013).

In contrast to dozens of the ethylene-related genes that are differentially expressed during embryogenic transition, the functions of only a few of them have been experimentally proven as being related to SE.

5.4.1.1 *MtSERF1*

MtSERF1 gene was identified as promoting SE induction in *M. truncatula* and its high expression, which was observed in globular somatic embryos, was found to be ethylene-induced (Mantiri et al. 2008a). The orthologues of *MtSERF1* were also reported to positively impact the embryogenic responses in cultures of Arabidopsis (Zheng et al. 2013) and *H. brasiliensis* (Piyatrakul et al. 2012). Importantly for the SERF1-mediated mechanism of SE induction, an Arabidopsis orthologue of *MtSERF1* (*At5g61590*) was recently identified as being a direct target of AGL15 (Zheng et al. 2013), which has an essential role in SE induction (see paragraph 3). In addition, interactions between *MtSERF1* and the members of HD-ZIP III family, PHB, PHV and REV (REVOLUTA), which are regulators of early zygotic embryo development in Arabidopsis, were suggested, and the encoded TF was proposed as linking the stress response to development during SE induction (Mantiri et al. 2008b).

5.4.1.2 *ERF022*

ERF022 expression was observed to be drastically inhibited during SE induction in Arabidopsis and a significantly impaired embryogenic response of the *ERF022* mutant was found to be associated with increased ethylene production (Gliwicka et al. 2013; Nowak et al. 2015). Further analysis indicated the negative impact of *ERF022* on the biosynthesis and signalling of ethylene and the candidate target genes including *ACS7* involved in ethylene biosynthesis, and *ERF1*, which is an essential element in the ethylene signal transduction pathway (Nowak et al. 2015). The inhibitory effect of ethylene on SE induction that was observed in Arabidopsis was postulated as resulting from the negative impact of ethylene on biosynthesis and the local distribution of auxin (Bai et al. 2013). In support of this postulate, auxin accumulation following the LEC2-mediated activation of the *YUC*-dependent pathway of IAA production was found to be required for SE induction in an Arabidopsis culture (Wójcikowska et al. 2013). The interactions between *ERF022* and *LEC2*, which is the key regulator of auxin-dependent embryogenic transition in Arabidopsis, were also demonstrated to be important for the role of *ERF022* in the genetic network that underlies SE induction (Nowak et al. 2015).

5.4.2 TFs that Control LEA Accumulation

LEA proteins are accumulated in the late stages of ZE and the increased expression of *LEA* and other genes that encode storage proteins was found to be associated with SE induced in numerous plants including soybean (Thibaud-Nissen et al. 2003), maize (Che et al. 2006), rape (Hosp et al. 2007), potato (Sharma et al. 2008) and Arabidopsis (Gliwicka et al. 2012). As a consequence, it was postulated that the increased tolerance to stress that is caused by an accumulation of storage proteins promotes the induction of embryogenic development (Stone et al. 2008).

Consistent with this hypothesis, several TF genes that are presented below appear to promote SE via accumulation of the storage proteins. Among such genes are those encoding the proteins of the MYB family that regulate the transcription of the target genes through a highly conserved DNA-binding domain, which is homologous to animal c-MYB (Dubos et al. 2010). The functions of two of the *MYB* genes, *MYB118* and *MYB115*, support the proposition that a storage protein-related mechanism might be considered in SE promotion. *MYB118* and *MYB115* were indicated to have stress-related functions, and their SE-promoting activity in the seedlings and root explants of Arabidopsis was reported (Wang et al. 2008). Relevant to the concept on the positive relation between the storage proteins and the embryogenic potential of tissue, *MYB118* and *MYB115* were documented to positively control *LEA* (*LATE EMBRYOGENESIS ABUNDANT*) genes, including *EM1*, *EM6*, *EM10*, *LEA76* and *ECP63* in ZE (Zhang et al. 2009). In addition to the stimulation of LEA production, *MYB118/MYB115* were recently reported to negatively control bezonyloxy glusosinolate biosynthesis, which is a secondary metabolite produced in response to stress (Zhang et al. 2015). This finding provides further support to the stress-related functions of *MYB115/118* in SE induction.

Another TF gene, *bHLH109*, which is a member of bHLH family, is also assumed to promote SE induction in Arabidopsis via the activation of *LEA* genes. The strong activation of *bHLH109* expression was found to be associated with SE induction, and the overexpression of this gene was indicated as enhancing the embryogenic response of Arabidopsis explants (Gliwicka et al. 2013). Recently, it was postulated that *bHLH109* might operate in SE as an activator of the *LEA* gene *ECP63*, and the TF genes that were annotated to stress-related functions (*At5g61620*, *bZIP4* and *bZIP43*) were indicated to be among the potential regulators of *bHLH109* (Nowak and Gaj 2016).

Collectively, the identification of the TF genes that control the LEA proteins of stress protective function among the SE regulators provided new evidence that the cell responses to stress that are imposed under in vitro conditions underlie the promotion of SE.

5.4.3 WIND1

WOUND INDUCED DEDIFFERENTIATION 1 (*WIND1/RAP2.4*) of the AP2/ERF TF superfamily, which positively regulates cell dedifferentiation in Arabidopsis, was found to be induced by wounding (Iwase et al. 2011). An elevated *WIND1* expression was demonstrated to be sufficient to promote unorganised cell proliferation and the redifferentiation of the callus into roots, shoots and embryos on a hormone-free medium. *WIND1*-overexpressing explant cells were demonstrated to reacquire pluripotency and the modulation of the cytokinin biosynthesis/signalling through ARR-dependent signalling pathway was proposed as being associated with the SE-promoting functions of *WIND1* (Iwase et al. 2011). Other molecular elements that link the WIND1-mediated initial wound response to the control of cell dedifferentiation needs to be revealed.

5.5 *PLETHORA* Genes—The Integrators of Hormonal Inputs

The AINTEGUMENTA-LIKE (AIL) family of TF genes, which have an AP2/ERF domain, includes the *AINTEGUMENTA* (*ANT*), *BABY BOOM* (*BBM/PLT4*) and *PLETHORA* genes. All of these are expressed in young, dividing tissue and play central roles in different developmental processes including embryogenesis (Elliott et al. 1996; Klucher et al. 1996; Boutilier et al. 2002; Aida et al. 2004; Galinha et al. 2007). The importance of the *AIL* function and its relation to auxin in zygotic embryo development was indicated (Aida et al. 2004; Blilou et al. 2005). Besides auxin, *AIL* genes were reported to be related to ABA, GA and JA signalling and thus, *PLT/BBM* genes were postulated to integrate multiple hormonal inputs in the plant development and to act as 'hubs in a plethora of networks' (Horstman et al. 2014).

Similar to *BBM/PLT4*, other *PLETHORA* TFs (*PLT1*, *PLT2*, *PLT3*, *EMK/PLT5* and *PLT7*) have also been indicted to exert the SE-related functions because the somatic embryo formation in response to their overexpression was observed (Tsuwamoto et al. 2010; Horstman 2015). Although our knowledge about the *PLT*-mediated induction of SE is rather fragmentary, a recent finding that *PLT3*, *EMK/PLT5* and *PLT7* stimulate auxin biosynthesis through the activation of *YUC* genes (*YUC1* and *YUC4*) to control phyllotaxis (Pinon et al. 2013) implies a possible role of *PLTs* in the auxin-related mechanism of SE induction. An *EMK/PLT5*-controlled induction of SE may also be related to GA and an encoded TF was reported to negatively impact GA biosynthesis in the control of the storage protein accumulation in Arabidopsis seeds (Sundaram et al. 2013).

Regulatory interactions between the *PLT* genes and other TFs that play key roles in SE may be expected. In support for this assumption, the activation of the *LEC*

genes (*LEC1*, *LEC2*, *FUS3*) that have essential functions in SE (Gaj et al. 2005) was found to be associated with the overexpression of *PLT2* and SE induction (Horstman 2015). The effect of *PLT2* overexpression is dose dependent, and its high expression exclusively leads to the formation of a somatic embryo (Horstman 2015). Along with the central role of *PLT2* in the embryonic root development during ZE (Horstman et al. 2014), the gene was recently shown to be involved in the formation of the root stem cell niche in the embryogenic callus (Su et al. 2015).

5.5.1 BBM

BBM/PLT4, the best characterised *PLT* gene, which was identified in the microspore-derived embryogenesis in *B. napus,* was found to produce somatic embryos on a hormone-free medium as a result of its overexpression, and relevant to this observation, it was suggested that the encoded TF may stimulate the production of auxin or increase a cell's sensitivity to this hormone (Boutilier et al. 2002). The identification of BBM-binding sequences during SE in Arabidopsis revealed the targets that are related to the biosynthesis (*YUC3*, *8*, *TAA1*), transport (*PIN1*, *4*) and signalling (*ARF2*, *10*, *IAA2*, *7*, *28*) of auxin. Among the direct targets of BBM during SE, the *LEC* genes that have documented SE-promoting functions were also proposed, which implies a linkage between the BBM- and LEC-mediated SE pathways (Horstman 2015).

The phenotypes that are related to *BBM* overexpression were indicated to be dosage- and context dependent, and accordingly, a model of AIL functions has recently been proposed (Horstman 2015). According to this model, SE induction requires a high level of AIL transcripts and the mode of the embryogenic pathway that is triggered depends on the developmental stage of the seedling. Direct SE is induced when *BBM* is activated before or during seed germination, whereas post-germination activation of the gene leads to the indirect pathway of SE induction. Analysis of BBM targets revealed the gene's involvement in the positive control of cell division, cell wall modification and the differentiation of plant organs (Passarinho et al. 2008; Nic-Can et al. 2013).

The *HD-ZIP IV/HOMEODOMAIN GLABROUS* (*HDG*) TFs, which are expressed in the L1 layer of meristems and specify an epidermis identity, were reported within the potential targets of BBM (Takada et al. 2013). *BBM* and *HDGs* are co-expressed during early ZE and their transcripts were found to promote cell divisions and differentiation, respectively (Horstman et al. 2015). The antagonistic functions of these genes are also observed in SE where the downregulation of *BBM* and the overexpression of *HDGs* result in a reduced embryogenic response of cultured explants. The overexpression of *HDG1* leads to the development of highly differentiated cells along the margin of the cotyledons and leaves due to the downregulation of cell proliferation genes including the D-type cyclin CYCD3;1. In contrast, the cotyledons of 35S::BBM transgenic seedlings consist of small,

undifferentiated cells that are able to produce somatic embryos. BBM and HDG1 have common target genes that might be antagonistically regulated or co-regulated, i.e. *PLT5* is activated by BBM in contrast to HGD1 (Horstman et al. 2015).

5.6 *WOX* Genes

The *WOX* (*WUSCHEL-RELATED HOMEOBOX*) genes form a plant-specific subclade of the eukaryotic homeobox TF superfamily whose members display the specialised functions that are related to either the promotion of cell division and/or the prevention of the premature cell differentiation. Accordingly, WOXs repress or activate their targets depending on the cell type and developmental stage (reviewed in van der Graaff et al. 2009). Fifteen members of *WOX* (*WUS* and *WOX1-14*) family were identified in the Arabidopsis genome, but only a subset of these has yet been characterised in detail. The activity of *WOX* genes that is specific to the tissue and the developmental process was reported. Consequently, in order to maintain stem cells in Arabidopsis, *WOX5* has to be expressed in RAM (Sarkar et al. 2007) and *WOX4* in the cambial meristem (Hirakawa et al. 2010). *WOX2, WOX8* and *WOX9* transcripts accumulate in the early stages of ZE in *Arabidopsis* and *P. abies* to control the polarity of cell divisions (Ueda et al. 2011; Zhu et al. 2014b). *WOX9* regulates cell divisions in SAM and acts upstream of *WUS* (Wu et al. 2005).

Consistent with the *WOX* activity in ZE (Hacker et al. 2004), the expression of the *WOX* family members was also indicated to be associated with somatic embryo development. In an embryogenic culture of *P. abies*, *WOX2*, *WOX8* and *WOX9* were transcribed in the early stage of the somatic embryo and later in the development; the expression of *PaWOX2* was visible in the basal part of the developing embryo while the *PaWOX8/9* transcripts marked the future RAM and the sites of the initiation of the cotyledon (Palovaara et al. 2010). In accordance with this finding, a reduced expression of *WOX8* and *WOX9* was found to result in the aberrant development of somatic embryos because of the deregulation of the cell divisions that were related to the downregulation of the *PaE2F* and *PaCYCBL* genes that control the cell cycle progress (Zhu et al. 2014b).

5.6.1 *WUS and WOX5 in Control of the Apical–Basal Axis of the Embryo*

The *WUS* gene that encodes the WUSCHEL protein was identified as a positive regulator of the stem cells in the SAM formation through the control of the meristematic cell number (Mayer et al. 1998). Parallel to the activation of floral patterning, the encoded TF was also indicated to repress the stem cell regulation and this bifunctional mode of activity placed the WUS TF among the developmental

regulators with unique functions (Ikeda et al. 2009). The role of *WUS* in the promotion of the vegetative-to-embryogenic transition was uncovered in a culture of an Arabidopsis mutant that produced somatic embryos on root explants that were cultured on a hormone-free medium (Zuo et al. 2002a). Moreover, the *WUS* overexpression was indicated to compensate the requirement of auxin treatment in SE induction in *Capsicum chinense* and *Coffea canephora* (Solís-Ramos et al. 2009; Arroyo-Herrera et al. 2008) and to enhance the embryogenic potential in an embryogenic culture of *G. hirsutum* (Zheng et al. 2014).

WUS together with *WOX5* were found to play a key role in the origin of the apical–basal pattern of the shoot–root axis in the zygotic embryos of Arabidopsis and the establishment of SAM and RAM, respectively (Jürgens 2001; Friml et al. 2003). Both genes were also recently demonstrated to specify the establishment of apical–basal polarity during formation of somatic embryos in Arabidopsis; however, some remarkable differences were noticed in comparison to ZE. In contrast to the distinct spatiotemporal separation of the *WUS* and *WOX5* expression that underlies the formation of the opposite embryo poles in early ZE, *WUS* and *WOX5* were simultaneously activated in nearly overlapped callus cells in the embryogenic culture of Arabidopsis, thus implying that the stem cell niches of the SAM and the RAM are developmentally related during SE initiation (Su et al. 2015).

Expression of *WUS* in SAM regeneration in vitro is positively affected by auxin or cytokinin depending on the mode of the morphogenic pathway that is induced. Auxin was found to stimulate *WUS* activity during SE induction (Su et al. 2009) and cytokinin was reported to enhance the gene expression in the regenerating shoots of root-derived cultures (Gordon et al. 2009). Some evidence implies that the mechanism of the WUS-mediated hormonal regulation of SE initiation differs from shoot and root regeneration that is induced separately; however, the genetic interactions that determine this difference need further investigations (Su et al. 2015).

In controlling SAM, WUS directly represses the transcription of the *ARABIDOPSIS RESPONSE REGULATOR* genes (*ARR5*, *ARR6*, *ARR7* and *ARR15*), which act in the negative feedback loop of cytokinin signalling (Leibfried et al. 2005). The differential expression of *ARRs* that is observed during SE of Arabidopsis (Gliwicka et al. 2013) and *M. truncatula* (Imin et al. 2008) provides the possibility for the role of cytokinin signalling in this process; however, the components of the WUS-controlled initiation of embryonic SAM during SE remain unknown. Cytokinin signals and *WUS* were postulated to reinforce each other through multiple feedback loops (Gordon et al. 2009) and a high specificity of these interactions might be expected. The regulatory relation between auxin and *WUS* might also be assumed and in accordance, auxin treatment was found to be essential for the correct regulation of *WUS* expression during somatic embryo induction in Arabidopsis (Su et al. 2009). In addition, *WUS* appears not to interact with the auxin metabolism during SE because the content of IAA was not modulated in response to *WUS* overexpression in the embryogenic callus of cotton (Bouchabké-Coussa et al. 2013). The understanding of the interactions between the endogenous

hormones and *WUS* expression might contribute to the application of this TF in the genetic improvement of plants with a poor capacity for in vitro regeneration, as was demonstrated in *C. chinense* (Solís-Ramos et al. 2009).

5.7 Conclusions

The central role of the transcriptional regulation in the control of the embryogenic transition of somatic cells has been recently documented. However, in contrast to the spectacular progress on the identification of TFs that are decisive for the reprogramming of differentiated cells into totipotent stem cells that has been made in animals, much less is known about these master regulators in plant cells. In the last 10 years intensive analysis of the global transcriptomes of plant cells that are undergoing embryogenic transition and the use of Arabidopsis (a model in plant genomics) in studies on the genetic control of SE have substantially contributed to the identification of the TF regulators of SE. As a result, dozens of TF genes that are differentially expressed in embryogenic cultures have been identified that provide a base in searches for other genetic elements of their decisive roles in SE induction. So far, only a small subset of the potential SE regulators has been verified experimentally. The emerging picture of a TF-controlled process of SE induction shows a complex network of genetic interactions in which the transcriptional regulation of hormone and stress responses appears to play a fundamental role (Fig. 5.2). It is also apparent that the majority of the already identified SE-involved TF genes are also critical for the development of zygotic embryos. Thus, the similarities in the regulatory mechanisms that underlie SE and ZE that were expected from early 1990s have recently become evident at the molecular level.

In addition to ZE-associated regulators, TFs that have less obvious functions in the control of SE induction have been recognised. Among them, TF genes that enhance cell tolerance to stress imposed under in vitro conditions including the activators of storage material accumulation such as LEA proteins were found to be essential for embryogenic transition.

In spite of the apparent progress that has been made, it seems that most of the TF genes that have a decisive role in the reprogramming of somatic cells into embryonic ones still remain uncovered. Special efforts should be focused on the identification of the targets of the SE-involved TF genes that operate at the very early stage of embryogenic transition, which is the most intriguing moment in the reprogramming of somatic cells. In order to recognise the early targets of SE-related TFs, new efficient approaches can be applied including the protein-binding microarray coupled with the analysis of co-regulated genes that was recently recommended for exploring the regulatory networks in plants (Franco-Zorrilla et al. 2014). However, given the fact that many variables determine the cellular and developmental context of TF–DNA interactions (Slattery et al. 2013, 2014), the candidate TF targets that are identified using in vitro approaches should be verified as also acting in vivo during SE induction. To meet this requirement, the versatile

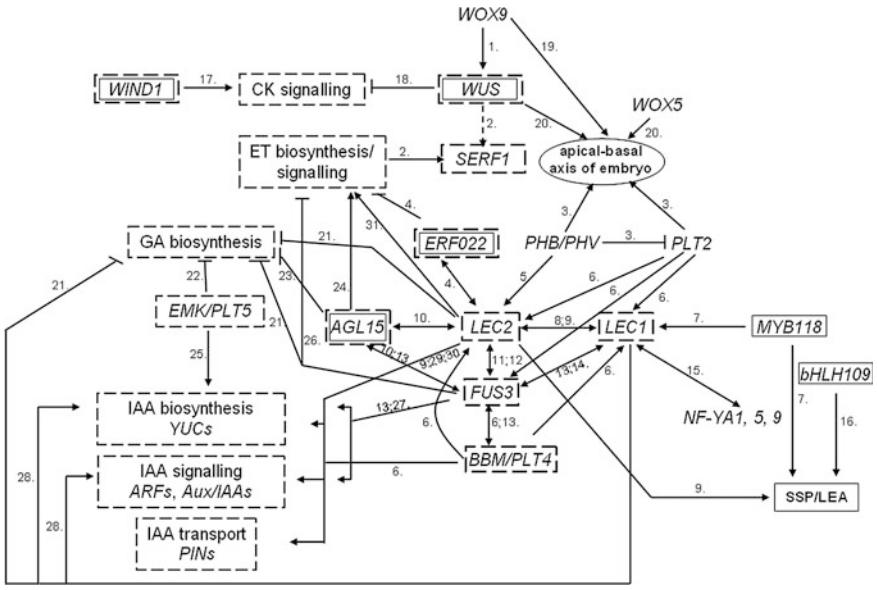

Fig. 5.2 Interaction between SE-related TFs. *Arrows* indicate the activation of the genes expression. The blunt end shows inhibition of gene expression. The *dotted line* indicates the suggested interactions. *Full-line frame* indicates stress-related genes. *Dotted frame* indicates hormone-related genes. *1* Wu et al. (2005); *2* Mantiri et al. (2008b); *3* Smith and Long (2010); *4* Nowak et al. (2015); *5* Tang et al. (2012); *6* Horstman (2015); *7* Zhang et al. (2009); *8* To et al. (2006); *9* Stone et al. (2008); *10* Thakare et al. (2008); *11* Gazzarrini et al. (2004); *12* Yamamoto et al. (2010); *13* Kagaya et al. (2005); *14* Mu et al. (2013); *15* Nowak and Gaj (2016); *16* Iwase et al. (2011); *17* Leibfried et al. (2005); *18* Palovaara et al. (2010); *19* Su et al. (2015); *20* Curaba et al. (2004); *21* Sundaram et al. (2013); *22* Wang et al. (2004); *23* Zheng et al. (2013); *24* Pinon et al. (2013); *25* Lumba et al. (2012); *26* Wang and Perry (2013); *27* Junker et al. (2012); *28* Wójcikowska et al. (2013); *29* Guo et al. (2013); *30* Braybrook et al. (2006). SSP—seed storage protein

molecular tools that are available for functional genomics in Arabidopsis might be helpful. In addition to the dissection of SE-specific TFs and their targets, the recognition of TF regulators, especially the chromatin remodelling factors and miRNAs, is a prerequisite for the full understanding of how already differentiated cells become competent to respond to the embryogenic signal that triggers the developmental switch.

Besides its cognitive value, the efforts that are aimed at the revealing the TF-controlled regulatory network that governs embryonic transition in plants may enable further progress in the genetic improvement of plants (Zuo et al. 2002b). Such perspectives for the use of the TFs that control SE induction in increasing the regeneration potential of some crop species have already been demonstrated for *BBM*, *WUS* and *LEC2* (Arroyo-Herrera et al. 2008; Deng et al. 2009; Solís-Ramos et al. 2009; Belide et al. 2013; Zheng et al. 2014).

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