Chapter 3
The Many Ways of Somatic Embryo Initiation

Attila Fehér, Dóra Bernula and Katalin Gémes

Abstract It is widely believed that all cells of a plant are totipotent and can regenerate the whole organism. This view is supported by uncountable experimental observations demonstrating the regrowth of whole plants from various explants, even from single cells. However, recent investigations have demonstrated that plant regeneration may proceed via transdifferentiation of meristems or root meristem-like callus tissues due to adult stem cells present all over the plants. These pathways do not start from single totipotent cells. There is a strong argument for plant cell totipotency, however, and that is somatic embryogenesis. During this process, differentiated somatic cells change their fate to develop into an embryo. Animal embryos can develop only from the totipotent zygote and its direct descendants (this cell state can also be artificially produced by injecting a somatic cell nucleus into an egg cell cytoplasm during cloning). Plant cells have to be induced to start somatic embryogenesis and not all of them are competent to respond properly to the induction. In conclusion, plant cells cannot be considered as totipotent per se, but some of them can regain totipotency under appropriate conditions. In addition, accumulating evidence supports the view that even somatic embryo development can follow various initial steps not necessarily requiring cellular totipotency. Although, there are experimental observations to support the progression of somatic embryogenesis through a zygote-like state in certain experimental systems, in other instances the reorganization of several cells into an embryo was described. The direct release/induction of the embryo development program in vegetative plant cells may represent a third pathway of somatic embryo development. In this chapter, a brief literature review is provided to support the above view on plant cell totipotency as well as on the various ways to start somatic embryogenesis.

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3.1 Introduction

An intriguing feature of plant embryogenesis is that embryo initiation is not restricted to the fertilized egg cell, the zygote (zygotic embryogenesis) (Radoeva and Weijers 2014). Embryos may form in nature from unfertilized diploid egg cells (gametophytic apomixis) (Koltunow 2012), from maternal cells surrounding the embryo sac (sporophytic apomixis) (Koltunow 2012), from cells of the suspensor (suspensor-derived embryogenesis (Liu et al. 2015), or somatic cells at leaf margins (pseudovivipary) (Garcês et al. 2007). In addition, embryo development can be induced from microspores/pollen grains (microspore embryogenesis) (Soriano et al. 2013) as well as from various somatic cells/tissues under appropriate in vitro conditions (somatic embryogenesis) (Fehér 2015). It has been also observed in many cases that influencing the expression of various regulatory genes controlling cell division and differentiation can also lead to ectopic embryo development (Radoeva and Weijers 2014). In other cases, mutations in chromatin remodelling factors resulted in ectopic embryo or embryogenic callus formation (Fehér 2015). Although there is a clear genetic background defining the capabilities of plant species/genotypes for non-zygotic embryogenesis (Rose et al. 2010), our knowledge about the underlying molecular processes is rather scarce. Even, we cannot answer the central question: how much these pathways converge on the same molecular mechanisms. It is obvious that as soon as the embryos are formed their development follows default pathways independent of their origin. However, the initial conditions that can trigger embryo formation in planta or in vitro are strikingly different (Radoeva and Weijers 2014; Fehér 2015). In this chapter, we compare the general features of various embryogenic systems with the aim of answering the question: how many ways may exist to initiate embryo development in somatic plant cells? At first, however, we discuss the link between cellular totipotency and embryo development.

3.2 The Totipotency of Plant Cells

The default embryo development pathway starts with the fertilization of the egg cell and the formation of the zygote that exhibits developmental totipotency. Totipotency means that the zygote can autonomously develop into all cells of the mature plant. The zygote, at least in certain plant species including Arabidopsis, divides asymmetrically resulting in two cells, one of which forms the embryo and the other the suspensor. It needs to be mentioned that the cells of the suspensor are also embryogenic and may form embryos spontaneously (in certain species) or if the embryo is non-functional or removed. The developmental potential of the suspensor has been shown to be controlled by the embryo via auxin distribution (Liu et al. 2015). The embryogenic capability of suspensor cells is maintained until the globular embryo stage.

The question is, whether any other cells of the plant can be considered being totipotent? One can frequently meet with the statement in the popular as well as scientific literature that "all plant cells are totipotent". This overstatement can be considered as one of the central dogmas of plant biology. In theory, the single cell that can autonomously form an embryo (e.g. the zygote) can only be considered being totipotent, since it is only the embryo that has the capability to develop directly into a whole organism (Verdeil et al. 2007). In contrast, only pluripotency can be ascribed to single cells regenerating plants in two steps, regenerating the shoot followed by adventitious root development, under two separate conditions. Therefore, if all plant cells are totipotent, each should have the capability to form an embryo autonomously.

In plants, embryo development can be achieved starting from various cell types including differentiated somatic cells. Therefore, the view that all or at least many plant cells are totipotent seems to be well supported by observations. However, cellular totipotency is difficult to consider in systems where plant regeneration starts from several cells instead of single ones. This is true for shoot regeneration followed by adventitious root development (Su and Zhang 2014) as well as for indirect embryo formation through the reorganization of cell masses (Su et al. 2015). Even if embryogenesis starts from a single non-zygotic cell, there is a need for harsh changes in the in planta or in vitro conditions to deviate the cell from its default developmental pathway. This means that these cells are not inherently embryogenic but become embryogenic in response to external or internal cues. Therefore, plant cells cannot be considered as totipotent per se, but some of them can regain totipotency (the capability to form an embryo) under appropriate conditions (for reviews see Laux 2003; Verdeil et al. 2007; Fehér 2015).

In theory, plant cells can regain totipotency (the capability to initiate embryo development) in two main ways: regaining embryogenic cell identity via induction or losing vegetative/somatic cell identity via reversion (Fig. 3.1). The possibility that induction or reversion progress through a zygote-like or directly through the embryonic state needs consideration.

3.3 The Direct Ways of Somatic Embryo Development

3.3.1 Embryogenesis Through a Totipotent Zygote-Like State

An interesting question of developmental biology is whether we can also consider the germ cells or only the zygote to be totipotent (Cinalli et al. 2008; Feng et al. 2013). In animals, totipotency is maintained during the first few divisions of the zygote. The germ lines separate from the soma very early during embryo development and there is a view that these cells inherit the developmental potency of the zygote/young embryo (Seydoux and Braun 2006). This potency is kept during egg

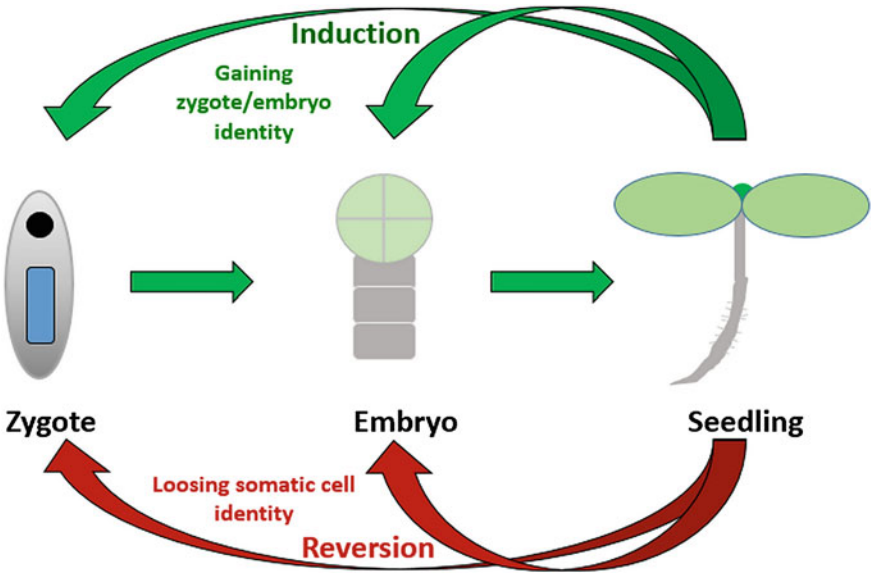

Fig. 3.1 Theoretical ways of somatic embryo formation

cell differentiation but suppressed by epigenetic mechanisms until fertilization takes place (Seydoux and Braun 2006; Cinalli et al. 2008). In contrast, the plant germ lines develop from well-defined differentiated somatic cells of the adult organism during flower formation (Yang et al. 2010; Twell 2011). Nevertheless, diploid egg cells formed during gametophytic apomixis can directly develop into zygotes and embryos indicating the inherent totipotency of plant egg cells. This totipotency is normally suppressed until fertilization by similar epigenetic mechanisms as in animals (Wuest et al. 2010; Feng et al. 2013). Initiation of female germ line development in vegetative tissues means that plants have at least one inherent developmental pathway to convert somatic cells towards totipotent cells (Feng et al. 2013). This pathway starts with the differentiation of the archeospore followed by megasporogenesis and embryo sac development. At present, it seems that embryo sac development is the step when cellular totipotency is re-established. Our knowledge about the molecular background of egg cell totipotency is rather limited. It is clear, however, that egg cell fate is determined by the auxin gradient within the embryo sac (Pagnussat et al. 2009). Manipulation of auxin content and distribution in the embryo sac alters cell fates (Pagnussat et al. 2009). Moreover, the analysis of various mutants demonstrated that cells mispositioned within the embryo sac change their fate depending on the auxin gradient (Sundaresan and Alandete-Saez 2010). Normally, the egg cell forms close to the micropylar end of the embryo sac where there is a relatively high local auxin concentration. High concentration of exogenous and/or endogenous auxin is required to initiate in vitro embryo development from the somatic cells of various explants (Fehér et al. 2003). Therefore,

it is tempting to speculate that somatic embryo development commences with an "egg cell/zygote-like totipotent state". The expression of egg cell/zygote markers in non-zygotic embryogenic cells could strengthen this hypothesis.

The Arabidopsis egg cell and the zygote express the WUSCHEL-like homeotic transcription factors WOX2 and WOX8, which following the asymmetric division of the zygote segregates into the apical and basal cells, respectively (Haecker et al. 2004). The polarity of the zygote is specified by the pollen-derived SHORT SUSPENSOR protein which activates the YODA/MAPK3,6 kinase cascade as well as by the WRKY2 and the RWP-RK-type GROUNDED(GRD)/RKD4 transcription factors (Ueda and Laux 2012). The WOX 2, 8 and 9 transcription factors determining basal and apical cell fates in the dividing Arabidopsis zygote have been implicated in somatic embryogenesis by gene expression data (Palovaara and Hakman 2008; Palovaara et al. 2010; Gambino et al. 2011). However, the exact temporal and spatial expression profile of these genes is yet unknown during this process, especially in the earliest phases.

The indication towards the possible role of these factors in non-zygotic embryogenesis comes from the experiment where the Arabidopsis *RKD4* transcription factor gene was overexpressed in Arabidopsis roots using a dexamethasone-inducible promoter (Waki et al. 2011). Ectopic *RKD4* expression caused overproliferation of root cells. However, if *RKD4* expression was ceased due to dexamethasone removal, somatic embryos appeared on the root surface. This indicates that the transient *RKD4* expression could trigger embryogenesis even in somatic cells. One may hypothesize that *RKD4* expression resulted in an egg cell/zygote-like cell state that favoured the embryogenic pathway. Indeed, ectopic expression of *AtRKD4* in the seedlings resulted in the transcription of genes associated with early embryo development. In a similar study, overexpression of the egg cell-specific *AtRKD1* or *2* transcription factors genes induced the expression of egg cell markers in somatic cells, which were induced to proliferate (Kőszegi et al. 2011). These cells, however, which expressed the *AtRKD1* or *2* transcription factors genes under the control of a constitutive promoter, did not develop into embryos. The expression and role of RKD-type transcriptional regulators during non-zygotic embryogenesis awaits further experimental validation. Detecting the transient expression of these factors may serve as a tool to identify the initial cells starting a zygote-like embryogenic program. This may, however, require a very high sensitivity of detection. The *RKD4* expression could only be detected in Arabidopsis due to a two-component system where the RKD4 promoter was linked to a transcriptional activator regulating the expression of a GFP reporter construct (Waki et al. 2011). In this way, the expression of the *RKD4* gene could be detected in the fertilized zygote and the early embryo (Waki et al. 2011).

Although the expression of zygotic molecular markers during the acquisition of the embryogenic cell fate by somatic cells is unclear, these cells often undergo asymmetric cell division resembling that of the zygote (Rose et al. 2010). Besides the morphological asymmetry, however, not much is known at the molecular level about the two daughter cells. Asymmetric segregation of an arabinogalactan protein epitope specifying cell fate has been described in carrot cell cultures long ago

(Souter and Lindsey 2000). This epitope is recognized by the JIM8 antibody and marks in vitro cultured embryogenic cells in carrot as well as zygotic embryos in Brassica (Pennell et al. 1991). In Brassica, this epitope did not label the zygote only the embryos from the 8-cell stage till protoderm formation as well as the suspensor. This expression pattern is consistent with the expression pattern in the carrot cell culture, where following the asymmetric division of a JIM8-positive cell, the JIM8-positive daughter cell (suspensor-like function?) support the development of the JIM8-negative one (embryogenic fate).

In certain somatic embryogenesis systems starting with an asymmetric cell division, the development of suspensor-like structures from the larger vacuolated cell was reported further supporting the resemblance with zygotic embryogenesis (Smertenko and Bozhkov 2014). These structures, however, often degenerate. The asymmetric divisions of single embryogenic cells can take place even in liquid media indicating that the division asymmetry is defined by intrinsic mechanisms. Only the analysis of asymmetrically dividing single cells devoted to the embryogenic pathway could answer the question on how much the first steps of somatic and zygotic embryogenesis converge. This kind of approaches are now feasible due to recent advances in the sequencing of single cell transcriptomes.

3.4 Release/Induction of the Embryo Maturation Program

Zygotic embryo development is governed among others by a group of transcription factors also implicated in seed maturation (LEAFY COTYLEDON1 (LEC1), LEAFY COTYLEDON2 (LEC2), FUSCA (FUS), ABSCISIC ACID INSENSITIVE3 (ABI3), AGAMOUS-LIKE15 (AGL15)) (Radoeva and Weijers 2014). Mutant phenotypes of these factors are mostly associated with late stages of embryo and seed development including cotyledon differentiation, embryo quiescence, and desiccation. Nevertheless, the overexpression of these genes can result in ectopic embryo development in vegetative tissues, such as in leaves, or at least can promote somatic embryo formation in response to appropriate signals. Considering this feature as well as the fact that they are also expressed during early stages of zygotic and somatic embryogenesis, they are often considered not only as seed maturation but also as embryo identity factors. These transcription factors have to be suppressed during germination to allow seedling development (Holdsworth et al. 2008). This suppression is established at the chromatin level, among others by the chromatin remodelling ATPase, PICKLE (PKL) (Rider et al. 2003; Henderson et al. 2004). Interestingly, the *pickle* mutation, which results in the ectopic expression of some of the above embryo identity factors (e.g. LEC1), may also result in ectopic embryo development (Henderson et al. 2004). Several other chromatin regulators have been shown to repress the embryonic cell fate in vegetative tissues including the POLYCOMB REPRESSOR COMPLEX 1 and 2 or the HISTONE DEACETYLASE 6 and 19 enzymes affecting histone posttranslational modifications (methylation and

acetylation, respectively) (Tanaka et al. 2008; Tang et al. 2012; Fehér 2015; Ikeuchi et al. 2015). Mutations in these genes have also been shown to result in ectopic expression of genes implicated in embryo development and the formation of embryogenic calli, embryo-like structures, or embryos. This supports the view that embryo development is a default pathway that is, however, efficiently suppressed in vegetative tissues at the chromatin level. Factors inducing (or rather releasing) somatic embryogenesis via this pathway therefore, should also act at the chromatin level. These factors might include exogenous plant hormones or stress factors altering the overall gene expression pattern (Fehér 2015).

Not only the released, but the induced expression of the above mentioned and other seed maturation/embryo development factors can result in somatic embryogenesis, including LEC1 (Lotan et al. 1998). *LEC1* gene expression could be detected in zygotic embryogenesis as early as the eight-celled stage. The earliest roles of LEC1 were hypothesized to be at the globular stage of zygotic embryogenesis when the *lec1* mutants exhibit aberrant cell divisions in the suspensor (Harada 2001). Although these observations indicate the role of LEC1 during early embryogenesis, it is still unclear how the overexpression of *LEC1* gene triggers the formation of embryo-like structures on leaves. LEC1 seems to induce embryonic gene expression programs only in seedlings and not in mature plants and therefore, it may need other co-factors to act (Lotan et al. 1998).

The action of the overexpressed *LEAFY COTYLEDON 2* (*LEC2*) gene is better revealed (Stone et al. 2001). This transcription factor also induces somatic embryo formation if ectopically expressed in seedlings. In addition to positively regulating the expression of embryo and seed maturation genes, LEC2 was shown to activate auxin synthesis through the expression of *YUCCA* genes (Stone et al. 2008). It was hypothesized that in *LEC2* overexpressing transgenic plants auxin synthesis is triggered in the genetic environment similar to that of maturating embryos resulting in the ectopic embryo development (Stone et al. 2008). This hypothesis is in agreement with the wide use of immature Arabidopsis embryos as explants for efficient auxin (2,4-D)-induced somatic embryogenesis (Gaj 2001; Ikeda-Iwai 2002). In general, it can be stated that the manifestation of the embryo maturation program (or at least part of it) in vegetative plant cells/tissues favours the ectopic initiation of embryo (or embryogenic callus) development. This favourable genetic environment can be achieved either via the released (e.g. chromatin remodelling mutants) or the induced expression of at least one of the several transcription factors related to embryo development (Ikeda et al. 2006; Radoeva and Weijers 2014; Fehér 2015). The inducer might be exogenous auxin or other factors triggering local auxin synthesis/accumulation (Fehér 2015). Seed and zygotic embryo maturation is dependent on the proper ratio of the plant hormones abscisic acid and gibberellic acid (Holdsworth et al. 2008). Not surprisingly, both hormones have been strongly implicated in somatic embryogenesis (Fehér 2015) further supporting the view that the conditions favouring zygotic and somatic embryo development are the same.

3.5 Indirect Embryogenesis from Embryogenic Cell Clusters

3.5.1 Callus Formation: Dedifferentiation or Transdifferentiation?

In many experimental systems, somatic embryos are not directly formed from somatic cells but there is in between an intervening callus phase. Callus is generally considered to be an unorganized mass of dividing dedifferentiated cells, which are capable of switching cell fate in response to hormonal signals. However, recent investigations suggest that there are various types of calli exhibiting different degrees of differentiation (Ikeuchi et al. 2013). For example, calli formed on Arabidopsis roots cultured in a high auxin/low cytokinin medium (callus-inducing medium, CIM) have a characteristic gene expression pattern reminiscent of partly organized root tip meristems (Sugimoto et al. 2010). This type of callus originates from pericycle or pericycle-like cells surrounding the veins in roots or aerial organs, respectively (Atta et al. 2009). Its development follows the initial steps of lateral root formation (Atta et al. 2009). Therefore, this callus tissue cannot be considered as a dedifferentiated but rather a misdifferentiated root meristem. Subsequent organogenesis from this type of callus, in response to cytokinin or auxin, respectively, can be regarded as transdifferentiation of the root meristem-like tissue into shoot or root meristem (Sugimoto et al. 2011). Embryogenesis has not been linked yet to root meristem-like callus, although initiation of embryos from cells surrounding the veins (procambial cells) was frequently observed (Guzzo et al. 1994; Rose et al. 2010; de Almeida et al. 2012; Fehér 2015). Whether in these cases embryogenesis shares the initial steps of lateral root formation still needs to be experimentally addressed.

Elaboration of a somatic embryogenic system from Arabidopsis roots would represent an excellent experimental system to answer this question due to the availability of mutants and cellular markers associated with lateral root initiation and callus formation.

In carrot, somatic embryo formation could be tracked back to single cells or small cell clusters of provascular origin in the fresh liquid culture of hypocotyl explants (Schmidt et al. 1997). In established cultures, proembryogenic cell masses (PEMs) form as a transitional stage towards embryogenesis in the presence of auxin (2,4-D). Whether PEMs can be regarded as misdifferentiated root meristems or embryos, is an interesting question to be answered.

There are, however, cases where callus and indirect embryo development seems to be preceded by dedifferentiation. Callus development in response to wounding follows a pathway independent of the pericycle or pericycle-like stem cells (Ikeuchi et al. 2013). This type of callus does not express root tip markers but is dependent on the WIND1 (WOUND-INDUCED DEDIFFERENTIATION1) transcription factor (Iwase et al. 2011a, b). Callus development from leaf protoplasts suffering a kind of wounding during the isolation process involves a transient dedifferentiated

stage (Grafi 2004; Grafi et al. 2011a, b). Wound-induced callus might also exhibit the capability for indirect embryo formation. Moreover, experimental observations demonstrate that embryogenic callus formation frequently initiates in epidermal or other highly differentiated cell types instead of procambial or perivascular cells (Nishiwaki et al. 2000; Yamamoto et al. 2005; Wang et al. 2011).

3.6 Embryo Initiation: Single Cell Origin or Reorganization of Cell Clusters?

In many indirect somatic embryogenesis systems, embryogenic calli or PEMs are formed in the presence of high concentration of auxin (especially 2,4-D) and removal of auxin triggers embryo formation on their surfaces (de Vries et al. 1988; Rose et al. 2010). In these cases, embryos may form through the reorganization of cell clusters instead of developing from single totipotent cells. Recent data obtained in the case of indirect Arabidopsis somatic embryogenesis support this view.

In this system, several molecular steps associated with initiation of the somatic embryogenesis pathway have been revealed (Su et al. 2009, 2015; for review see Fehér 2015). Arabidopsis calli (PEMs) formed in the presence of 2,4-D were used as explants. Using fluorescent markers of gene expression and confocal laser scanning microscopy (Su et al. 2009; Bai et al. 2013; Su et al. 2015), resulted in the following model (for a comprehensive figure see Fehér 2015). When the embryogenic calli are moved to auxin-free medium, endogenous auxin synthesis is induced via the expression of *YUCCA* (*YUC*) genes (Bai et al. 2013). The subsequently induced expression of *PINFORMED1* (*PIN1*) gene and the orientation of auxin transport proteins results in local auxin accumulations (Su et al. 2009). This is required to induce the expression of the *WUSCHEL* (*WUS*) meristem identity regulator gene in regions with auxin minima (Su et al. 2009). Interestingly, during auxiliary meristem formation in Arabidopsis, *WUS* expression also appears in cells with low auxin levels (Wang et al. 2014a, b), indicating the possibility that the regulation of the two pathways is similar. Redistribution of PIN1 proteins and auxin, as well as the expression of *WUS* ultimately, lead to the establishment of a shoot meristem organizing centre. The expression of *WUS-RELATED HOMEOBOX 5* (*WOX5*), a master regulator gene of root meristem organization, appears in parallel and almost overlapping with that of *WUS* in response to auxin removal (Su et al. 2015). However, later its expression is correlated with cytokinin rich regions and root meristem emergence. The apical-basal axis of the embryo is established before the formation of somatic embryos would be visible (Su et al. 2009, 2015). These observations indicate that indirect somatic embryogenesis proceeds via the reorganization of hormone synthesis, distribution, and gene expression within groups of callus cells. Formation of shoot and root meristems is followed by the organization of the cells into an embryo-like structure. These somatic embryos are often much larger than the zygotic ones and have no properly formed protoderm, which might be the

consequence of their multicellular origin (Su et al. 2009). The whole process can rather be considered as transdifferentiation of a partly differentiated callus tissue than redifferentiation of single cells within a dedifferentiated cell mass. The observations are also in line with the view that the various types of calli are not merely homogenous masses of fully dedifferentiated cells (Sugimoto et al. 2011). Moreover, this model argues that the regression to a fully dedifferentiated (totipotent) state is not an absolute prerequisite for embryo regeneration from vegetative tissues.

3.7 Transdifferentiation of the Shoot Meristem

A similar mechanism takes place during the formation of embryo-like propagules on the leaf margins of viviparous *Kalanchoe* species. In these species, the SHOOT MERISTEMLESS (STM) transcription factor, which serves to maintain shoot meristem function in cooperation with WUS, is required for the initiation of plantlet regeneration at the leaf margins (Garcês et al. 2007). This is, however, followed by the recruitment of an embryogenic pathway indicated by the expression of *FUS3* and *LEC1*. Interestingly, the *LEC1* expression is not indispensable for plantlet formation, as several species of the genus carry a non-functional *LEC1* allele preventing seed production and making the vegetative plantlet formation the default reproductive pathway. The embryo-like structures of *Kalanchoe* have no root poles but the plantlets form adventitious roots. This may be the consequence of their shoot meristem origin. In certain *Kalanchoe* species, vegetative propagation requires stress induction that is in line with the important role of stress in somatic embryogenesis (Fehér 2015). Arabidopsis shoot meristems have also been shown to be efficiently transformed into somatic embryos in response to stress (Ikeda-Iwai et al. 2003). The ectopic expression of the *STM* gene has been shown to enhance somatic embryogenesis in *Brassica* (Elhiti et al. 2010). Moreover, mutations enlarging shoot meristems have been shown to enhance somatic embryogenesis (Mordhorst et al. 1998). All these observations indicate that the shoot meristem to embryo conversion is generally feasible.

Transient *WUS* overexpression is sufficient to trigger embryo development in various vegetative tissues (Zuo et al. 2002). *WUS*-triggered embryogenesis was demonstrated to start with an asymmetric cell division indicating direct embryo formation. *WUS* itself was actually shown to repress *LEC1* expression suggesting that *WUS* does not directly act through the *LEC1* pathway, which is activated in this system only when the embryos appear on the explants (Zuo et al. 2002). *WUS* is the central regulator of shoot meristem identity, and its expression is detected in the Arabidopsis embryo from the dermatogen stage onward. Therefore, it is unclear how *WUS* overexpression triggers embryo development in a single “zygote-like” cell. One can suppose that *WUS* overexpression initially induces genes required for shoot meristem identity, but the inappropriate signals from the surrounding tissues result in embryo development that might be considered as a kind of transdifferentiation.

3.8 Conclusions

In plants, the gametophytes producing germ cells develop from well-defined somatic cells of the adult organism in response to developmental as well as environmental signals. The formed germ cells are considered to convey developmental totipotency for the zygote following their fusion. It is hypothesized that totipotency is already present in the egg cell but suppressed by various epigenetic mechanisms until fertilization takes place. In agreement, the existence of defined developmental and environmental conditions resulting in the establishment of totipotency in somatic plant cells cannot be questioned. This does not mean, however, that every plant cells is totipotent, as generally thought. Only those single cells, which have the capability to develop directly into an embryo can be considered to possess developmental totipotency. Embryo formation, however, not necessarily progresses through this zygote-like totipotent state. Accumulating evidence supports the view that the development of embryos from somatic cells can follow several pathways as far as the initial steps are considered. At present, at least there main models can be suggested: (1) direct embryogenesis from single cells through a zygote-like stage; (2) direct embryogenesis dependent on seed/embryo identity factors (LEC1 and other related embryo identity transcription factors); and (3) indirect embryogenesis dependent on WUS and WOX5 (as well as related auxin and cytokinin transport, synthesis and signalling pathways). The existence of the two latter pathways is in agreement with the observation that the direct formation of Arabidopsis somatic embryos is *LEC1* dependent while the *lec1* mutants can follow the indirect (WUS-centred) pathway for embryo formation (Gaj et al. 2005). Indirect somatic embryo development has been reported for *stm* and *wus* mutants (Mordhorst et al. 2002). However, the function of these proteins may be redundant considering somatic embryo induction (see above). It cannot be excluded either that in these mutants the *LEC1*-dependent pathway was followed.

Comparison of the various systems using modern experimental techniques that make possible to follow the development of single cells at the genome and epigenome scale might give the final answer on how much the above embryo development pathways are indeed separated or interrelated.

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