Cellular Markers for Somatic Embryogenesis

Ewa U. Kurczynska, Izabela Potocka, Izabela Dobrowolska, Katarzyna Kulinska-Lukaszek, Katarzyna Sala and Justyna Wrobel

Laboratory of Cell Biology, Faculty of Biology and Environmental Protection, University of Silesia, Katowice, Poland

1. Introduction

Somatic embryogenesis (SE) is a process in which somatic cells under special conditions develop into embryos and - in the end - into a plant. That is why SE is a good model system for studying the genetic, molecular, physiological, biochemical, histological and cellular mechanisms underlying not only somatic but also zygotic embryogenesis and the totipotency of plant cells. SE begins with a transition of somatic cells to an embryogenic state and it can be induced under certain in vitro conditions. The mechanisms which determine SE induction - the transition of cells from the vegetative to the embryogenic state and the conditions underlying such changes - are the main questions of developmental biology (for a review see: de Jong et al., 1993; von Arnold et al., 2002; Fehér et al., 2003; Namasivayam, 2007; Yang & Zhang, 2010).

A description of the events taking place during SE requires the application of different scientific methods such as genetic, molecular or biochemical analysis and also histological studies of explant cells. Moreover, the morphological, histological and cytological analysis of SE is also an object of studies leading to an understanding of the basis of the totipotency, differentiation, dedifferentiation, redifferentiation and changes in cell fate (Quiroz-Figueroa et al., 2006). It could help us to understand the developmental processes taking place during plant growth and development, including pattern formation.

In this review we describe the cellular markers which can be used to identify different groups of cells within the explant during the process of SE. The aim of this review is to summarise information concerning the morphology and histology of explant cells, such as changes in the apoplast and symplast of explants, which can be used as markers to identify a cell/cells which changed their fate from the somatic to the embryogenic state.

2. Definitions

The first information about somatic embryo development in in vitro conditions was presented by Steward and co-workers (1958). From that moment on, this kind of plant propagation forced many scientists to study the mechanisms involved in changes from the
somatic to the embryogenic state and to improve the efficiency of this process as a method for plant propagation. Since during SE different processes leading to changes in cell fate are taking place, some important definitions concerning this phenomenon are reminded below.

Somatic embryogenesis is divided into direct and indirect embryogenesis (DSE and ISE respectively; Sharp et al., 1980; Evans & Sharp, 1981). In DSE, somatic embryos develop directly from the somatic cells of explants, and in ISE they develop from callus cells. Somatic embryogenesis is also divided depending upon the type of explants. If somatic embryos develop from primary explants it is called primary somatic embryogenesis; if they develop from primary somatic embryos, this is called secondary somatic embryogenesis.

In normal plant development, cells differentiate from an unspecialised to a mature state with the determined function. The term ‘cell differentiation’ can be interpreted as spatiotemporal and it focuses on the diverging path of differentiation among the constituent cells in a population (Romberger et al., 2004).

During SE, some explant cells change the direction of differentiation. For example, the epidermal cell is the “source” of the somatic embryo, and the parenchyma cell becomes a callus cell and afterwards develops into a somatic embryo. The processes by which cells can change their state of development are dedifferentiation, transdifferentiation and redifferentiation. It is well-documented that most of plant cells retain the possibility to dedifferentiate and as a consequence to change their fate (Grafi, 2004). Such changes are possible because plant cells are totipotent (or at least most of them are), where totipotency is the property of the cell which retains the potential for developing into a complete adult organism (Verdeil et al., 2007). For the most recent analysis of the definitions mentioned above, the article written by Sugimoto and co-workers (2011) is recommended.

According to Nagata (2010) and Grafi (2004), dedifferentiation is the process where differentiated non-dividing cells become meristematic. This concept explains many observations which had shown that cells divisions precede changes in the direction of their differentiation. During dedifferentiation, cells return to the undifferentiated, meristematic state. Transdifferentiation involves processes which lead cells or tissues from one differentiated state of development into a new one, and probably - first of all - such cells dedifferentiate and then redifferentiate along another developmental path (Thomas et al., 2003; Gunawardena et al., 2004). Redifferentiation is the ability of non-differentiated, meristematic cells to differentiate into a new direction, e.g., into new plant organs.

It is worth reminding ourselves of another definition concerning SE. According to Verdeil and co-workers (2007), the embryogenic callus is an undifferentiated, unorganised tissue enriched in embryogenic cells, and the embryogenic cell is a cell that requires no further external stimulus to produce a somatic embryo.

3. General description of SE

During SE, changes in explant tissues cause the development of the somatic embryo. Many studies have shown that somatic embryos are going through the same developmental stages as their zygotic counterparts, which in dicotyledonous plants are called the globular, heart, torpedo and cotyledonary stages (Fig. 1; sometimes such stages were named differently, as with, e.g., Quiroz-Figueroa et al., 2006).
Different parts of plant organs or zygotic embryos are used as an explant for the induction of SE. The literature describing this aspect of SE is huge and it is not possible to even mention here most of them. In some species, zygotic embryos are the best source of somatic ones and the explant organs involved in SE are cotyledons or shoot apical meristem (e.g. Canhoto & Cruz, 1996; Gaj, 2001; Kurczyńska et al., 2007; Raghavan, 2004; Rocha et al., 2011). Cultures of leaves, stems and roots parts are also efficient in SE induction (Mathews et al., 1993; Quiroz-Figueroa et al., 2002). In some cases, the production of protoplasts from different plant tissues or suspension cultures is the best for SE (Pennel et al., 1992; Quiroz-Figueroa et al., 2002).

Somatic embryos have a single-cell or multicellular origin. Analyses performed by Canhoto & Cruz (1996) on *Feijoa sellowiana* cotyledons of zygotic embryos, as an explant, showed that somatic embryos developed from a single protodermal cell or from a group of cells including sub-protodermis. Similar results were obtained during the histological analysis of somatic embryogenesis of *Arabidopsis thaliana*, where the single-cell and multicellular origins of somatic embryos were also detected (Kurczyńska et al., 2007). Cork oak somatic embryos are of a multicellular origin or a single-cell origin depending on the explant cells which participated in the embryo’s formation (Puigderrajols et al., 2001). The single- and multicellular origins of somatic embryos was also described (among others) in *Borago officinalis* (Quinn et al., 1989), *Camellia japonica* (Barciela & Vieitez, 1993), *Elaeis guinensis* (Schwendiman et al., 1990) and...
The unicellular origins of somatic embryos was described (among others) in the leaf explant of *Coffea arabica* (Quiroz-Figueroa et al., 2002), coconut (Verdeil et al., 2001) and *Dactylis glomerata* (Trigiano et al., 1989). In some species, only the multicellular origins of somatic embryos were described, as, for example, in *Carya illinoinensis* (Rodriguez & Wetzstein, 1998) and *Passiflora cincinnata* (Rocha et al., 2011).

It is well-documented that dividing explant cells (e.g., in callus cultures) can follow different developmental pathways, such as organogenesis, SE or unorganised growth (Fehér et al., 2003). Distinguishing between somatic embryo and organ-like structural development within explants can sometimes be difficult. The most distinctive features in the histology of somatic embryos are the anatomically closed radicular end and the lack of a vascular connection with the maternal tissues (Fig. 2 A, B). Moreover, analysis of the distribution of starch in the radicular pole of the embryo showed that starch was present in both zygotic embryos and their somatic counterparts (Fig. 2 C). Using such a criterion it is much easier to distinguish somatic embryo formation from organogenesis, which can take place within the same explant.

**Fig. 2.** Schematic differences in the histology of the basal region of the embryo (A) and buds (B; this resembles organogenesis) and starch distribution in the radicular pole of *Arabidopsis* somatic embryo (the red lines on A and B represent the vascular tissue; C – brownish colour after staining with Lugol solution marks starch; bar = 150 μm).

In the case of *Arabidopsis thaliana* (a system described by Gaj, 2001), somatic embryos develop via a DSE from explant cells located on the adaxial side in the cotyledon node (Fig. 3; Kurczyńska et al., 2007).

**Fig. 3.** Schematic representation of a longitudinal section through *Arabidopsis* explants. The location of the embryogenic and non-embryogenic regions is indicated.
From many observations and histological analysis, it appears that in this system only those cells located on the adaxial side of cotyledons undergo transition from a somatic to embryogenic state in the manner of DSE. Sometimes, if zygotic embryos are cultured in a different way, somatic embryos which developed from the callus were also detected (Fig. 4).

Fig. 4. Structure detected within the callus during SE in an Arabidopsis explants, which resembles a very early stage (a few cells) of a somatic embryo developed via an ISE (bar = 10 µm).

4. Changes in cell fate during SE

In the process of somatic embryogenesis, some somatic cells start to divide, becoming totipotent, and then enter the new pathway which is SE (Fehér et al., 2002). The most important question concerns the mechanisms underlying the changes (the transition) of the differentiated state of the plant cell into a totipotent and finally an embryogenic state (Fehér et. al., 2002). It was documented that during DSE somatic cells acquire their embryogenic competence through dedifferentiation (Harada, 1999; Fehér et al., 2003; Steinmacher et al., 2011). Such big changes in cell fate depend on the possibility of acquiring the ability to divide (Nagata, 2010). It is accepted that dedifferentiation is preceded by cell divisions (Fehér et al., 2002; Nagata et al., 1994; Wang et al., 2011) and it is postulated that existing developmental information must be changed so as to allow cells to respond to new signals (Fehér et al., 2002).

The transition from the somatic to the embryogenic state requires the induction of embryogenic competence (Verdeil et al., 2001). How should one recognise this stage of SE? The answer to this question is still far away, as it is very difficult to recognise the very early stages of somatic embryo development, starting from the changes in competence and transition from the somatic to the embryogenic state. Some studies were undertaken to answer this question and the results and the conclusions drawn from them are described below.

4.1 Cell division

From studies on the explants of different species it appears that the direction of cell division can be a marker of cells undergoing changes in cell fate. In Arabidopsis explants, during DSE, the protodermal cell is involved in somatic embryo formation and divides.
periclinally (Kulinska-Lukaszek et al., in press; Kurczyńska et al., 2007). Such a direction of cell division in the protodermal cell is unusual. In normal conditions, epidermal cells divide anticlinally (Considine & Knox, 1981) and periclinal division means that the phenotype of the protodermal cells was changed. This kind of division can be also called asymmetric (asymmetric does not necessarily mean that cells are of a different size after a division) because two daughter cells after periclinal division have a different neighbourhood; one of them still is in contact with the external environment while the other one is not. Other examples where unusual and asymmetric division was detected during SE were described in the case of the development of the secondary somatic embryos of *Trifolium repens* (Meheswaran & Williams, 1985), *Juglans regia* and *Medicago sativa* (Polito et al., 1989; Uzelac et al., 2007) and in the case of *Helianthus annuus* x *H. tuberosus* (Chiappetta et al., 2009).

### 4.2 Meristematic and embryogenic cells within explants

From many studies, it appears that the development of somatic embryos begins from the explant areas which are described as meristematic. Such a characteristic is typical not only for DSE but also for ISE.

The question now arises whether meristematic cells are histologically, morphologically and ultrastructurally equal to embryogenic ones? Next, how can we recognise meristematic and embryogenic explant cells?

Histological and ultrastructural analysis during the SE of pineapple guava showed that meristematic cells are rich in cytoplasm and containing many ribosomes, some amyloplasts and numerous mitochondria (Canhoto & Cruz, 1996; Canhoto et al., 1996). In this system, meristematic cells were similar on the ultrastructural level to embryogenic (proembryo) cells, with the only exception that the meristematic cells were more vacuolated. In the case of coconut, the meristematic cells were also characterised by dense cytoplasm, many ribosomes, reduced vacuole and a voluminous central nucleus with one or two nucleoli (Fig. 5 A; Verdeil et al., 2001). Cells with the same characteristics were described for *Carya* (Rodriguez & Wetzstein, 1998).

According to many studies, the most widely-described characteristic of the embryogenic cells involved in somatic embryo development are as follows: small cells with an isodiametric shape with dense cytoplasm, a nucleus located in the cell centre with a highly visible nucleolus and with small starch grains and vacuoles (Fig. 5 B; C; Canhoto & Cruz, 1996; Namasivayam et al., 2006; Verdeil et al., 2001). Pasternak and co-workers (2002) have also shown that embryogenic cells can be distinguished from non-embryogenic cells in the case of *Medicago* by the character of these cells. The embryogenic ones are characterised by their small size, with rich cytoplasm and filled with starch. The similar character of embryogenic cells was described in *Passiflora cincinnata* (Rocha et al., 2011) and cork oak (Puigderrajols et al., 2001). Cells with the same characteristics were described for the embryogenic parts of the explants of *Carya* (Rodriguez & Wetzstein, 1998). Nomura and Komamine (1985, 1995) have shown that isolated, small, cytoplasm-rich carrot cells have the ability to develop into somatic embryos. In carrot cultures, several phenotypes of cells capable for SE (embryogenic) were described (Toonen et al., 1994) but the efficiency of SE was highest in cells with a small size, a rich cytoplasm and which are spherical. The
comparison of the embryogenic and non-embryogenic parts of explants is much easier as the non-embryogenic parts of explants are highly vacuolated (Fig. 5 D).

Fig. 5. Semi-thin sections through the *Arabidopsis* explant showing the examples of meristematic (A), meristematic and embryogenic (B), embryogenic (C) and non-embryogenic cells (D; the arrows point to embryogenic cells; arrowheads – to meristematic, note several nucleoli; V – vacuoles; sections stained with toluidine blue; bar = 10 μm; author – Izabela Potocka).

From the features of meristematic and embryogenic cells presented above, it appears that these differences are not distinct. According to Verdeil and co-workers (2007), some other features can be used for better distinguishing between meristematic and embryogenic cells, being the shape and the structure of the nucleus. In meristematic (in that case, the authors described the meristematic cells of shoot meristem) cells, the nucleus is spherical, with several nucleoli and heterochromatin (electron-dense areas under TEM) uniformly distributed within the nucleus. In the case of embryogenic cells, the nucleus is irregular in shape and contains one large nucleolus (Verdeil et al., 2007).

Some observations point to changes in the cell cytoskeleton which in embryogenic cells is organised in a different manner in comparison to non-embryogenic cells (Dijak & Simmonds, 1988; Dudits et al., 1991).

**In conclusion:** during the analysis of the cell morphology of explants during SE, one must remember that not all meristematic cells become an embryogenic cell, and not all embryogenic cells develop into somatic embryos. The direction of cell division within an explant can be a marker of cells which changed their direction of differentiation. The main features of embryogenic cells are their small size, low elongation rate, their small vacuoles, cells reach with cytoplasm, the high cytoplasm-nucleus ratio, changes in the nucleus and the nuclear envelope and their starch content.

**5. Apoplast and symplast during SE**

Between the somatic and embryogenic states of development, crucial processes called the transition and induction of embryogenic competence take place. This step is the most
important, but at the same time it is less understood (Verdeil et al., 2001). During this step, competent cells are those which are in a transitional state and which still require some stimuli to become embryogenic cells (Namasivayam, 2007). It is not clear how the embryogenic cells originate within the explants and what mechanisms control this process. Changes in cell fate and the direction of differentiation rely on the erasing of the genetic developmental program and switching on of a new one. How this is realised by explant cells is unclear. Some studies indicate that changes in the developmental program rely on physical isolation of a cell or a group of cells from the surroundings. This process may proceed by the isolation of the symplast and/or apoplast. The analysis of these plant compartments has shown that there are some features of the transition from the somatic to the embryogenic state on the cellular and histological level which allows the recognition of this developmental stage.

5.1 Changes in apoplast as a markers for SE

A unique feature of plants is the presence of a system of cell walls which is called ‘apoplast’. For many years, apoplast has not been perceived as an important part of plant organisms. At present, it is no longer a dead part of the plant body but a temporally and spatially changing extracellular matrix. It is well-known that many processes depend not only on changes in the chemical and structural composition of the cell wall, but that the cell wall is a place where signal transduction takes place (Fry et al., 1993). If so, also process of SE was investigated from that point of view.

Studies with the secondary embryogenesis of Brassica napus have shown some features which should be convenient for the recognition of the transitional stage from the somatic to the embryogenic state (Namasivayam et al., 2006). It was shown that the explant epidermal cells involved in somatic embryogenesis were irregular in shape and size and covered by a layer of additional material deposited on their surface, while such material was not found in the non-embryogenic tissue (Namasivayam et al., 2006). What is interesting is that this material disappeared in the adult somatic embryos, suggesting that such a feature of embryogenic tissue could be a cellular marker for cells which changes their way of development. The staining of this material with Azur II/methylene blue suggested the presence of a mucilage/polysaccharide component (Namasivayam et al., 2006). A similar substance at the surface of the pre-embryogenic tissues was present in Coffea arabica (Sondahl et al., 1979), Cichorium (Chapman et al., 2000a, 2000b; Dubois et al. 1991, 1992), Camellia japonica (Pedroso & Pais, 1992, 1995), Drosera (Bobák et al., 2003; Šamaj et al., 1995), Zea mays (Šamaj et al., 1995), Papaver (Ovečka et al., 1997; Šamaj et al., 1994), Pinus (Jasik et al., 1995), Citrus (Chapman et al., 2000a) and coconut (Verdeil et al., 2001). The detected material was present only up to the globular stage of embryo development. Because of the time of its appearance and the location, it is postulated that this material is a cellular marker for the acquisition of embryogenic competency (Namasivayam et al., 2006). In some cases, this structure was called a ‘supraembryonic network’ (Chapman et al., 2000a, 2000b) or an ‘extracellular matrix’ (Namasivayam et al., 2006).

Another feature of apoplast during SE are the changes in the thickness of the cell wall (Fig. 6). Information about the necessity of the presence of the thick cell wall around developing somatic embryos showed that in some examples such an isolation is necessary (Dubois et al., 1991; Schwendiman et al., 1990; Verdeil et al., 2001). The thickening of the cell walls in the
explants’ tissues was described for *Gentiana punctata* (Mikula et al., 2004) and *Feijoa sellowiana*, where thick cell walls were detected around the proembryos (Canhoto & Cruz, 1996).

![Differences in wall thickness between cells within the explant through the example of Arabidopsis](image)

**Fig. 6.** Differences in wall thickness between cells within the explant through the example of *Arabidopsis* (PAS+tobuidine blue staining; bar = 20 µm; author - Czekała).

It seems that the thicker cell walls surrounding the cell with a morphology which is typical for the embryogenic state is the result of the origin of these cells. Namely, if an embryo develops from the one cell and successive cell walls are formed within this mother cell, it is obvious that the cell wall at the surface of the proembryo is thicker, as is the older wall in such a complex. According to Williams and Meheswaran (1986), such isolation is necessary only if the embryogenic cells are surrounded by non-embryogenic ones.

### 5.1.1 Lipid transfer proteins

The lipid transfer proteins (LTPs) are proteins which can be divided into two classes, depending on the molecular weight. In *in vitro* conditions, it was shown that these proteins are able to transfer phospholipids between cellular membranes (Kader, 1997). The role of LTPs in the process of somatic embryogenesis was shown for the first time in the case of carrot embryos (Sterk et al., 1991). It is postulated that LTPs are involved in cutin biosynthesis and that they can be used as a cellular marker for the development of protodermis in somatic embryos (for a review, see Zimmerman, 1993). LTPs were also found in the extracellular proteins secreted by grapevine somatic embryos (Coutos-Thevenot et al., 1993). In *Arabidopsis* culture, LTPs were also observed outside the meristematic explant cells, which may indicate that LTPs can be used as a cellular marker during the transition from the somatic to the embryogenic state (Fig. 7).

Analysis of the presence of LTPs during somatic embryogenesis has rarely been performed, but studies on gene expression were more abundant and have shown that taking this expression pattern it is possible to distinguish between the embryogenic and non-embryogenic parts of a *Dactylis glomerata* suspension culture (Tchorbadjieva et al., 2005). Similar results indicating the role of LTPs in SE were performed on *Camellia* leaf cultures (Pedroso & Pais, 1995) and cotton (Zeng et al., 2006).
5.1.2 Arabinogalactan proteins (AGPs)

Arabinogalactan proteins are the group of extracellular and membrane-bound proteins which are very diverse in their composition and which are involved in many morphogenetic processes in plants, such as growth and development, cell expansion, cell proliferation and zygotic and somatic embryogenesis (Kreuger & van Holst, 1993; Qin & Zhao, 2006; for a review, see Seifert & Roberts, 2007). Many antibodies against different AGP epitopes have been introduced in order to study the role of this class of proteins in plant development. The role of AGP is postulated both during the early stages of embryogenesis and in the different developmental stages of the embryo (Stacey et al., 1990). It is also known that AGP secreted into the culture medium can promote the production of somatic embryos (Egertsdotter & von Arnold, 1995; Hengel et al., 2001; Kreuger & van Holst, 1993).

Developmental changes during somatic embryogenesis were described in detail in the case of *Daucus carota* and showed that cells “decorated” by the JIM8 antibody developed into somatic embryos, which suggests that this AGP epitope can serve as a cellular/wall marker for the very early transitional cell stage into an embryogenic pathway (Pennell et al., 1992).

The AGP epitope which was recognised by the JIM8 antibody was able to force the somatic cell of *Daucus carota* to produce somatic embryos, which points to the role of AGP in somatic embryogenesis (McCabe et al., 1997). Within the explant cells of *Arabidopsis*, only some of them during the culture period are characterised by the presence in their wall of AGP epitopes recognised by the JIM8 antibody (Fig. 8).

It was shown that the JIM4 monoclonal antibody can be an early marker for the development of somatic embryos (Stacey et al., 1990). Analysis with the use of the JIM13 antibody showed that in PEM (proembryogenic masses), in the case of *Picea abies* culture, this kind of AGP epitope was present in PEM cell walls and was not found in young somatic embryos, suggesting that this AGP epitope can be a good cellular marker for distinguishing between PEM and somatic embryos (Filonova et al., 2000).
5.1.3 Pectic epitopes

Pectins are the main component of the middle lamella and the primary cell wall. Pectins are acidic polysaccharides with a heterogeneous nature. The most important function of pectins is the attachment of cells.

During immunohistological studies of *Cichorium* SE with the use of the JIM5 antibody, the pectic epitopes recognised by this antibody were present in the supraembryonic network which covered the embryogenic parts of explant. It was postulated that unesterified pectic epitopes can be used as an early marker of SE (Chapman et al., 2000b).

Detected differences between the embryogenic and non-embryogenic calluses of *Daucus carota* in the amount of neutral sugars of pectin in comparison to the acidic parts of pectin are postulated as a marker for embryogenic cells (Kikuchi et al., 1995).

High levels of esterified pectins were detected during the embryogenesis of *Capsicum annuum* (Bárány et al., 2010), indicating that such a composition of cell walls is not only marker of cell proliferation but also an early marker of microspore reprogramming for embryogenesis.

In the *Arabidopsis* explants, the distribution of pectin epitopes recognised by the JIM5 and JIM7 antibodies was almost the same, but what is interesting in those parts of the explant which do not participate in embryogenesis is that neither pectin epitope was detected in the cells’ walls (Fig. 9).

5.1.4 Callose

Callose is a (1→3)-β-D-linked homopolymer of glucose (Gibeaut & Carpita, 1994) present in different plant cells and what is most interesting is synthesized in response to wounding or other stress treatments (Fortes et al., 2002). However, the role of callose is not well-understood and as was pointed out by Fortes and co-workers (2002) - in some tissues callose can prevent the absorption of water and in others it can enhance this process, which can also be important during SE.
The deposition of callose in the vicinity of plasmodesmata disturbs symplasmic communication (this will be described in detail below) between cells and - in this manner - influences the exchange of signals through plasmodesmata (Fig. 10 A). When callose is deposited in the cell wall it can interrupt the exchange of signals through the apoplast (Fig. 10 B).

Studies with Cichorium and Camellia japonica showed that the deposition of callose is a prerequisite for somatic embryogenesis (Dubois et al., 1990; Pedroso & Pais, 1992). The same results were described for Trifolium (Meheswaran & Williams, 1985) and coconut (Verdeil et al., 2001).

Ultrastructural and histological studies on Cichorium during SE have shown that the first sign of SE is the deposition of callose in the cell wall (Verdus et al., 1993). Analysis performed on Eleutherococcus senticosus explants showed that after plasmolysis the amount of callose increased in comparison with untreated explants and - moreover - it was shown that callose is deposited between the plasma membrane and the cell wall (You et al., 2006).
5.1.5 Lipid substances

The deposition of lipid substances in the form of lamellae within the cell walls is postulated as being an important factor in the isolation of cells undergoing changes in their fate (Pedroso & Pais, 1992, 1995). It is postulated that apoplast isolation through the deposition of lipid substances is necessary for the abortion of the exchange of molecules through the cell wall. That is why this marker can be used in the detection of cells during the transition from the somatic to the embryogenic state. Unfortunately, there is not much information on the presence of lipid lamellae during the acquisition of embryogenic competence of explant cells.

Histological analysis of the series of a section of the Arabidopsis explant showed that within the callus cells some of them are isolated from the others by the lipid lamellae within the cell walls (Fig. 11). If this feature is characteristic of cells in their transition state then it requires further study.

In conclusion

The markers for the early stages of SE during the transition from the somatic to the embryogenic stage of cell development are present within the cell walls. These markers refer to the chemical composition of the extracellular matrix of a cell undergoing the process of transition, which involves changes in AGP and LTP, pectic epitopes, and callose and lipid substances deposited within the cell wall.

5.2 Changes in symplasm during SE

During SE, not only do changes in apoplast take place but changes also take place within the symplasm. Among the different mechanisms which control the process of plant development, including zygotic and non-zygotic embryogenesis (somatic embryogenesis and androgenesis), symplasmic communication/isolation is also postulated (Gisel et al., 1999; Kurczyńska et. al., 2007, Wrobel et al., 2011). This process is an important mechanism for the exchange of information between cells within a plant body. Such exchange of information is also a part of pattern formation within the plant organism, as it is known that
the process of cell differentiation relies on the cell’s position (for a review, see Scheres, 2001). The exchange of information is important and it allows cells to realise the proper developmental program.

Symplasmic communication relies on a unique feature of plant organisms - the presence of plasmodesmata (PD) which links the cytoplasm of neighbouring cells and which creates the system called ‘symplasm’ (Romberger et al., 2004). It should be noticed that during plant growth and development, the connection through PD between cells changes and depends on the stage of development. As a result, plant organisms can be divided into symplasmic domains and subdomains (Zambryski & Crawford, 2000). Symplasmic domains present in the plant body can be permanent (for example stomata; Fig. 12 A). Symplasmic subdomains can be also temporal, which means that they changed spatially and temporally and may be composed of several cells or just one cell (Fig. 12). Analysis of the symplasmic tracer distribution within the protodermal cells of Arabidopsis explants showed that fluorochrome was present only in some cells (Fig. 12 B, C). What is interesting is that after the division of mother cell, only one of the daughter cells was filled with a fluorochrome, which suggests that communication between these cells is restricted (Fig. 12 B).

The main characteristic of PD is the upper limit of the molecules’ size that can freely diffuse through PD, which is called the ‘Size Exclusion Limit’ (SEL). It was shown that SEL changed during the development because the PD diameter can be changed temporally, spatially and physiologically (Zambryski & Crawford, 2000). PD also can disappear during the development or may be created de novo. Thus, the limitation in symplasmic communication is a result of PD disappearance, lowering of their number or else the downregulation of SEL.

Fig. 12. The distribution of the symplasmic tracer (HPTS -8-hydroxypyrene-1,3,6-trisulfonic acid) within the protodermal cells of Arabidopsis explants. A – stomata as an example of the permanent symplasmic domain. B-C examples of the temporal symplasmic domains composed of a few cells (C) or in a single cell (D; as to B, note the unequal distribution of fluorochromes in the daughter cells after a division - arrow; bar = 10 μm).
As is known, molecules which can be exchanged between neighbouring cells through PD are not only ions, hormones, minerals, amino acids and sugars, but also proteins, transcriptional factors and different types of RNA (Kempers & van Bell, 1997; Lucas et al., 1993; Roberts & Oparka, 2003). This indicates that PD can regulate cell-to-cell movement and in this way participates in the regulation and coordination of plant development. It is known that PD plays an important role during the zygotic embryogenesis of Arabidopsis thaliana (Kim et al., 2002). Studies of the role of symplasmic communication during zygotic embryogenesis were based on the analysis of the movement of fluorochromes, dextrans conjugated to fluorescein and GFP (Green Fluorescent Protein) between embryo cells in different stages of their development. It appeared that the Arabidopsis embryo is one symplasmic domain up to the mid-torpedo stage (Kim et al., 2002). From that moment of development, the embryo is no longer a single symplast and the movement of symplasmic transport tracers of different molecular weights is restricted to different symplasmic domains and subdomains which correlate with the development of primary tissues and organs. This means that the downregulation of PD as the embryo develops is important for proper embryogenesis (Zambryski & Crawford, 2000). The studies mentioned above also indicate that disturbance in the normal permeability of PD leads to disorder in the development of Arabidopsis. The changes in PD permeability also took place when embryo changed its development from radial to bilateral symmetry (Kim & Zambryski, 2005). Detailed analysis of the GFP movement between cells also revealed the existence of subdomains which correspond to the establishment of the apical-basal axis of the Arabidopsis embryo (Kim et al., 2005b). These results clearly show that the regulation of embryogenesis is based (among others) on changes in symplasmic transport between embryo cells and they reveal the temporal and spatial correlation between the stages of embryo development and the formation of symplasmic domains and subdomains (Kim et al., 2002; Kim et al., 2005a; Kim et al., 2005b; Kim & Zambryski 2005; Stadler et al., 2005).

It is worth noting that there are some similarities between PD in plant organisms and the gap junctions in animal organisms. Namely, gap junctions play a control role during animal development (Warner, 1992).

The role of the disruption of symplasmic connection between cells which undergo different fate of differentiation has been postulated for many years. It is suggested that such a disruption allows those cells which are no longer connected by PD to differentiate in independent ways. Such physiological isolation is needed for reprogramming the cells. The question is: is the closing or decreasing of symplasmic communication a prerequisite for changing in direction of cell differentiation or is it the result of other changes which lead to the downregulation of symplasmic communication? The answer is not obvious. Some reports point to the first possibility while the other may suggest that it is a secondary cell reaction.

Symplasmic communication within explant cells during the initiation and development of somatic embryos was not intensively studied. Analysis of the distribution of CFDA (fluorescent tracer 5-(and-6) Carboxyfluorescein Diacetate) during the DSE in Arabidopsis explants showed the presence of the fluorochrome only in the protodermis and subprotodermis of the explants, indicating that the downregulation of plasmodesmata connection within an explant took place (Kurczyńska et al., 2007).
Studies on the explants of *Panax ginseng* have shown that the disruption of plasmodesmata generated more somatic embryos than in normal conditions, indicating that cell-to-cell communication must be decreased for obtaining more efficient somatic embryogenesis (Choi & Soh, 1997). Similar results were obtained in *Morus alba* (Agarwal et al., 2004).

In the case of coconut, the cells forming the meristematic layer were connected by plasmodesmata, indicating that symplasmic communication between the cells in this layer is present (Verdeil et al., 2001). As somatic embryogenesis proceeds, the decreasing in symplasmic communication between proembryo and meristematic cells occurred, but plasmodesmata within the proembryo and embryo were present (Verdeil et al., 2001). This is an example that cells belonging to the same developmental stage - which is at the beginning of somatic embryo formation - are connected by plasmodesmata but are isolated from their neighbours.

Studies on the zygotic embryos of *Eleutherococcus senticosus* as explants showed that the disruption of plasmodesmata between explants cells promotes the formation of somatic embryos even on the medium without auxin (You et al., 2006). The interpretation of these results is as follows: the interruption of symplasmic communication stimulates the reprogramming of cells into cells competent for the embryogenic pathway (You et al., 2006).

In *Ranunculus*, analysis of the formation of somatic embryos showed that at the early stages of embryoid connection development by plasmodesmata between the embryoid and surrounding tissues were present, but in the latter stage the connection was disturbed (Konar et al., 1972). The isolation of competent cells during the formation of proembryos by disrupting plasmodesmata was also postulated by Yeung (1995). In *Gentiana punctata*, the disappearance of plasmodesmata during somatic embryogenesis was also detected (Mikuła et al., 2004).

Timmers and co-workers (1996), during the analysis of the level of calcium ions in the cells of *Daucus carota* culture, have also shown that an increasing level of these ions can cause the closure of the plasmodesmata between embryogenic cells and the proembryogenic mass.

The analysis of the presence of plasmodesmata in the callus cells of *Cichorium* shows the disappearance of connection by plasmodesmata during somatic embryogenesis, indicating that cells which will undergo new a physiological state are isolated from their neighbouring cells (Sidikou-Seyni et al., 1992). Similar results were described in the case of grasses, where the plasmodesmata connection existed only between cells belonging to the same group of cells creating aggregates (Karlsson & Vasil, 1986).

However, not all of the results described so far are in agreement with those presented above. In the case of *Pineapple guava* symplasmic, isolation was not detected during the formation of somatic embryos (Canhoto et al., 1996). Plasmodesmata were present between the cells of the embryo, but also between the embryo and the surrounding cells. This suggests that symplasmic isolation is not a prerequisite for somatic embryo formation (Canhoto et al., 1996). In other tissue culture systems, the same conclusion was drawn (Jasik et al., 1995; Thorpe, 1980; Williams & Meheswaran, 1986).

Symplasmic communication within somatic embryos is also not well-described. It was shown for barley androgenic embryos that the symplasmic barrier exists between protodermis and the underlying tissues up to the late globular stage, in the isolation of
meristematic cells of the embryo in the transitional and coleoptilar stage, and between the embryo proper and the scutellum and the coleorhizae at the mature stage of the embryo (Wrobel et al., 2011). In the case of *Arabidopsis*, symplasmic isolation was correlated with the morphogenesis of somatic embryos (Fig. 13; Wrobel, 2010). In the case of *Cephalotaxus harringtonia*, numerous plasmodesmata connecting the embryo cells were noticed (Rohr et al., 1997). Similar results were described when the secondary somatic embryos of *Eucalyptus globulus* were investigated (Pinto et al., 2008).

![Fig. 13. The distribution of the symplasmic tracer (CMNB - caged fluorescein (fluorescein bis-(5-carboxymethoxy-2-nitrobenzyl ether, dipotassium salt) within the *Arabidopsis* somatic embryo, showing a border in symplasmic communication between the root meristem and other parts of the somatic embryo, which indicates that the symplasmic subdomains correspond with the main morphological parts of the embryo (fluorescence microscope; h – hypocotyl, c – cotyledon, r – root; bar = 150 μm; author – Wrobel, PhD thesis).](image-url)

6. Conclusions

Knowledge of the cellular markers of somatic embryogenesis from the very early stages of changes in the direction of cell differentiation is important not only from a biotechnological point of view but also in helping in the understanding of the mechanisms underlying the changes in the direction of cell differentiation in general and the transition from the somatic to the embryogenic stage in particular.

It seems that promising cellular markers of cell fate changes exist at the ultrastructural and molecular level (Kiyosue et al., 1992; Pennell et al., 1992; Schmidt et al., 1997; Yeung, 1995).
The analysis of the cell wall’s components and symplasmic communication during the changes in the direction of cell differentiation requires further study. Probably, both symplasm and apoplast are involved in the control of the synchronisation of cell division, histodifferentiation and primary organ development.

7. Acknowledgement

This work was supported in part by grant N303092 32/3176 from the Ministry of Science and Higher Education of Poland. We would like to apologise that not every wonderful paper describing somatic embryogenesis has been mentioned in the text, but the only cause of this was a lack of space.

8. References

Agarwal, S., Kanwar, K. & Sharma, D.R. (2004). Factors affecting secondary somatic embryogenesis and embryo maturation in Morus alba L. Scientia Horticulturae, Vol. 102, No. 3, (November 2004), pp. 359–368, ISSN 0304-4238

Bárány, I., Fadón, B., Risueño, M.C. & Testillano, P.S. (2010). Cell wall components and pectin esterification levels as markers of proliferation and differentiation events during pollen development and pollen embryogenesis in Capsicum annuum L. Journal of Experimental Botany Vol. 61, No. 4, (January 2010), pp. 1159-1175, ISSN 0022-0957

Barciela, J. & Vieitez, A.M. (1993). Anatomical sequence and morphometric analysis during somatic embryogenesis on cultured cotyledon explants of Camellia japonica L. Annals of Botany, Vol. 71, No. 5, (May 1993), pp. 395-404, ISSN 0305-7364

Bobák, M., Šamaj, J., Hlinková, E., Hlavačka, A. & Ovečka, M. (2003) Extracellular matrix in early stages of direct somatic embryogenesis in leaves of Drosera spatulata. Biologia Plantarum, Vol. 47, No. 2, (September 2003), pp. 161-166, ISSN 0006-3134

Canhoto, J.M. & Cruz, G.S. (1996). Histodifferentiation of somatic embryos in cotyledons of pineapple guava (Feijoa sellowiana Berg). Protoplasma, Vol. 191, Nos. 1-2, (March 1996), pp. 34-45, ISSN 0033-183X

Canhoto, J.M., Mesquita, J.F & Cruz, G.S. (1996) . Ultrastructural changes of pineapple guava (Myrtaceae) during somatic embryogenesis. Annals of Botany, Vol. 78, No. 4, (October 1996), pp. 513-521, ISSN 0305-7364

Chapman, A., Blervaqc, A.-S., Tissier, J.-P., Delbreil, B., Vasseur, J. & Hilbert, J.-L. (2000a). Cell wall differentiation during early somatic embryogenesis in plants. I. Scanning and transmission electron microscopy study on embryos originating from direct, indirect, and adventitious pathways. Canadian Journal of Botany, Vol. 78, No. 6, (June 2000), pp. 816-823, ISSN 0008-4026

Chapman, A., Blervaqc, A.-S., Hendriks, T., Slomianne, C., Vasseur, J., Hilbert, J.-L. (2000b). Cell wall differentiation during early somatic embryogenesis in plants. II. Ultrastructural study and pectin immunolocalization on chicory embryos. Canadian Journal of Botany, Vol. 78, No. 6, (June 2000), pp. 824-831, ISSN 0008-4026

Chiappetta, A., Fambrini, M., Petraru1o, M., Rapparini, F., Michelotti, V., Bruno, L., Greco, M., Baraldi, R., Salvini, M., Pugliesi, C. & Bitonti, M.B. (2009). Ectopic expression of LEAFY COTYLEDON1-LIKE gene and localized auxin accumulation mark
embryogenic competence in epiphyllous plants of *Helianthus annuus* × *H. tuberosus*. *Annals of Botany*, Vol. 103, No. 5, (March 2009), pp. 735-747, ISSN 0305-7364

Choi, Y.-E. & Soh, W.-Y. (1997). Enhanced somatic single embryo formation by plasmolyzing pretreatment from cultured ginseng cotyledons. *Plant Science*, Vol. 130, No. 2, (December 1997), pp. 197-206, ISSN 0168-9452

Considine, J.A. & Knox, R.B. (1981). Tissue origins, cell lineages and patterns of cell division in the developing dermal system of the fruit of *Vitis vinifera* L. *Planta*, Vol. 151, No. 5, (May 1981), pp. 403-412, ISSN 0032-0935

Coutos-Thevenot, P., Jouenne, T., Maes, O., Guerbette, F., Grosbois, M., Le Caer, J.P., Boulay, M., Deloire, A., Kader, J.C. & Guern, J. (1993). Four 9-kDa proteins excreted by somatic embryos of grapevine are isoforms of lipid-transfer proteins. *European Journal of Biochemistry*, Vol. 217, No. 3, (November 1993), pp. 885-889, ISSN 0014-2956

de Jong, A.J., Schmidt, E.D.L. & de Vries, S.C. (1993). Early events in higher-plant embryogenesis. *Plant Molecular Biology*, Vol. 22, No. 2, (May 1993), pp. 367-377, ISSN 0167-4412

Dijak, M. & Simmonds, D.H. (1988). Microtubule organization during early direct embryogenesis from mesophyll protoplasts of *Medicago sativa* L. *Plant Science*, Vol. 58, No. 2, pp. 183-191, ISSN 0168-9452

Dubois, T., Guedira, M., Dubois, J. & Vasseur, J. (1990). Direct somatic embryogenesis in roots of *Cichorium*: is callose an early marker? *Annals of Botany*, Vol. 65, No. 5, (May 1990), pp. 539-545, ISSN 0305-7364

Dubois, T., Guedira, M., Dubois, J. & Vasseur, J. (1991). Direct somatic embryogenesis in leaves of *Cichorium*. A histological and SEM study of early stages. *Protoplasma*, Vol. 162, No. 2-3, (June 1991), pp. 120-127, ISSN 0033-183X

Dubois, T., Dubois, J., Guedira M., Diop, A. & Vasseur, J. (1992). SEM characterization of an extracellular matrix around somatic proembryos in roots of *Cichorium*. *Annals of Botany*, Vol. 70, No. 2, (August 1992), pp. 119-124, ISSN 0305-7364

Dudits, D., Bögre, L. & Györgyey, J. (1991). Molecular and cellular approaches to the analysis of plant embryo development from somatic cells in vitro. *Journal of Cell Science*, Vol. 99, No. 3 (July 1991), pp. 475-484, ISSN 0021-9533

Egertsdotter, U. & von Arnold, S. (1995). Importance of arabinogalactan proteins for the development of somatic embryos of Norway spruce (*Picea abies*). *Physiologia Plantarum*, Vol. 93, No. 2, (February, 1995), pp. 334-345, ISSN: 0031-9317

Evans, D.A. & Sharp, W.R. (1981). Plant regeneration from cell cultures [Methodology, genetic regulation]. *Horticultural reviews*, Vol. 3, (January, 1981), pp. 214-314, ISSN: 0163-7851

Fehér A., Pasternak T., Otvos K., Miskolczi P., Dudits D. (2002). Induction of embryogenic competence in somatic plant cells: a review. *Biologia*, Vol. 57, No.1, pp. 5-12, ISSN 1335-6372

Fehér, A., Pasternak, T. P., Dudits D. (2003). Transition of somatic plant cells to an embryogenic state. *Plant Cell, Tissue and Organ Culture*, Vol. 74, No. 3, (September, 2003), pp.201-228, ISSN: 0167-6857

Filonova, L.H., Bozhkov, P.V. & von Arnold, S. (2000). Developmental pathway of somatic embryogenesis in *Picea abies* as revealed by time-lapse tracking. *Journal of Experimental Botany*, Vol. 51, No. 343, (February, 2000), pp. 249-264, ISSN: 0022-0957

---

www.intechopen.com
Fortes, A.M., Testillano, P.S., Risueno, M.D. & Pais, M.S. (2002). Studies on callose and cutin during the expression of competence and determination for organogenic nodule formation from internodes of *Humulus lupulus* var. Nugget. *Physiologia Plantarum*, Vol. 116, No. 1, (September, 2002), pp. 113-120, ISSN: 0031-9317

Fry, S.C., Aldington, S., Hetherington, P.R. & Aitken, J. (1993). Oligosaccharides as signals and substrates in the plant cell wall. *Plant Physiology*, Vol. 103, No. 1 (September 1993), pp. 1-5, ISSN 0032-0889

Gaj, M.D. (2001). Direct somatic embryogenesis as a rapid and efficient system for *in vitro* regeneration of *Arabidopsis thaliana*. *Plant cell, tissue and organ culture*, Vol. 64, No. 1, (January, 2001), pp. 60-73, ISSN 1040-4651

Gibebaut, D.M. & Carpita, N.C. (1994). Biosynthesis of plant cell wall polysaccharides. *The FASEB Journal*, Vol. 8, No. 12, (September, 1994), pp. 904-915, ISSN: 0892-6638

Gisel, A., Barella, S., Hempel, F.D. & Zambryski, P.C. (1999) Temporal and spatial regulation of symplasmic trafficking during development in *Arabidopsis thaliana* apices. *Development*, Vol. 126, No. 9, (May, 1999), pp. 1879-1889, ISSN: 0950-1991

Grafi, G. (2004). How cell dedifferentiate: a lesson from plants. *Developmental Biology*, Vol. 268, No. 1, (April 2004), pp. 1-6, ISSN: 0012-1606

Gunawardena, A.H.L.A.N., Greenwood, J.S. & Dengler, N.G. (2004). Programmed cell death remodels lace plant leaf shape during development. *Plant Cell*, Vol. 16, No. 1, (January 2004), pp. 60-73, ISSN 1040-4651

Harada, J.J. (1999). Signaling in plant embryogenesis. *Current Opinion in Plant Biology*, Vol. 2, No. 1, (February, 1999), pp. 23-27, ISSN: 1369-5266

Hengel Van, A.J., Tadesse, Z., Immerzeel, P., Schols, H., Kammen Van, A. & de Vries, S.C. (2001). N-acetylglucosamine and glucosamine-containing arabinogalactan proteins control somatic embryogenesis. *Plant Physiology*, Vol. 125, No. 4, (April, 2001), pp. 1880-1890, ISSN: 0032-0889

Jasik, J., Salajova, T. & Salaj, J. (1995). Developmental anatomy and ultrastructure of early somatic embryos in European black pine (*Pinus nigra* Arn.). *Protoplasma*, Vol. 185, Nos. 3-4 (September 1995), pp. 205-211, ISSN 0033-183X

Kader, J.-C. (1997). Lipid-transfer proteins: a puzzling family of plant proteins. *Trends in Plant Science*, Vol. 2, No. 2 (February 1997), pp. 66-70, ISSN 1360-1385

Karlsson, S.B. & Vasil, I.K. (1986). Morphology and ultrastructure of embryogenic cell suspension cultures of *Panicum maximum* (Guinea Grass) and *Pennisetum purpureum* (Napier Grass). *American Journal of Botany*, Vol. 73, No. 6 (June 1986), pp. 894-901, ISSN 00029122

Kempers, R. & van Bell, A.J.E. (1997). Symplasmic connections between sieve element and companion cell in the stem phloem of *Vicia faba* L. have a molecular exclusion limit of at least 10 kDa. *Planta*, Vol. 201, No. 2, (June 1997), pp. 195-201, ISSN 0032-0935

Kim, I., Hempel, F.D., Sha, K., Pfluger, J. & Zambryski, P.C. (2002). Identification of a developmental transition in plasmodesmatal function during embryogenesis in *Arabidopsis thaliana*. *Development*, Vol. 129, No. 5, (March 2002), pp. 1261-1272, ISSN 0950-1991

Kim, I., Cho, E., Crawford, K., Hempel, F.D & Zambryski, P.C. (2005a). Cell-to-cell movement of GFP during embryogenesis and early seedling development in *Arabidopsis*. *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 102, No. 6, (February 2005), pp. 2227-2231, ISSN 0027-8424
Kim, I., Kobayashi, K., Cho, E. & Zambryski, P.C. (2005b). Subdomains for transport via plasmodesmata corresponding to the apical-basal axis established during Arabidopsis embryogenesis. Proceedings of the National Academy of Sciences of the United States of America, Vol. 102, No. 33, (August 2005), pp. 11945-11950, ISSN 0027-8424

Kim, I. & Zambryski, P.C. (2005). Cell-to-cell communication via plasmodesmata during Arabidopsis embryogenesis. Current Opinion in Plant Biology, Vol. 8, No. 6, (December 2005), pp. 593-599, ISSN 1369-5266

Kiyosue, T., Yamaguchi-Shinozaki, K., Shinozaki, K., Higashi, K., Satoh, S., Kamada, H., & Harada, H. (1992). Isolation and characterization of a cDNA that encodes ECP31, an embryogenic-cell protein from carrot. Plant Molecular Biology, Vol. 19, No. 2 (May 1992), pp. 239-249, ISSN 0167-4412

Konar, R.N., Thomas, E., & Street, H.E. (1972). Origin and structure of embryoids arising from epidermal cells of the stem of Ranunculus sceleratus L. Journal of Cell Science, Vol. 11 No. 1 (July 1972), pp. 77-93, ISSN 0021-9533

Kreuger, M. & van Holst, G.J. (1993). Arabinogalactan proteins are essential in somatic embryogenesis of Daucus carota L. Planta, Vol. 189, No. 2 (February 1993), pp. 243-248, ISSN 0032-0935

Kulinska-Lukaszek, K., Tobojka, M., Adamiok, A. Kurczynska, E.U. (in press). Spatio-temporal expression of the BBM gene in explants and embryos during somatic embryogenesis of Arabidopsis. Biologia Plantarum, in press, ISSN: 0006-3134

Kurczyńska, E.U., Gaj, M.D., Ujczak, A. & Mazur, E. (2007). Histological analysis of direct somatic embryogenesis in Arabidopsis thaliana (L.) Heynh. Planta, Vol. 226, No. 3 (August 2007), pp. 619-628, ISSN 0032-0935

Lucas, W.J., Ding, B. & van der Shoot, C. (1993). Plasmodesmata and the supracellular nature of plants. New Phytologist, Vol. 125, No. 3, (November 1993), pp. 435-476, ISSN 0002-646X

Kikuchi, A., Satoh, S., Nakamura, N. & Fujii, T. (1995). Differences in pectic polysaccharides between carrot embryogenic and nonembryogenic calli. Plant Cell Reports Vol. 14, No. 5, (February 1995), pp. 279-284, ISSN 0721-7714

Maheswaran, G. & Williams, E.G. (1985). Origin and development of somatic embryoids formed directly on immature embryos of Trifolium repens L. vitro. Annals of Botany, Vol. 56, No. 5, (November 1985), pp. 619-630, ISSN 0305-7364

Mathews, H., Schöpke, C., Carcamo, R., Chavarriago, P., Fauquet, C. & Beachy, R.N. (1993). Improvement of somatic embryogenesis and plant recovery in cassava. Plant Cell Reports, Vol. 12, No. 6 (April 1993), pp. 328-333, ISSN 0721-7714

McCabe, P.F., Valentine, T.A., Forsberg, L.S., & Pennell, R.I. (1997). Soluble signals from cells identified at the cell wall establish a developmental pathway in carrot. The Plant Cell Vol. 9, No. 12, (December 1997), pp. 2225-2241, ISSN 1040-4651

Mikula, A., Tykarska, T., Zielinska, M., Kuraś, M. & Rybczyński, J.J. (2004). Ultrastructural changes in zygotic embryos of Gentiana punctata L. during callus formation and somatic embryogenesis. Acta Biologica Cracoviensia, Series Botanica, Vol. 46, pp. 109-120, ISSN 0001-5296

Nagata, T., Ishida, S., Hasezawa, S. & Takahashi, Y. (1994). Genes involved in the dedifferentiation of plant cells. International Journal of Developmental Biology, Vol. 38, No. 2 (June 1994), pp. 321-327, ISSN 0214-6282
Embryogenesis

Nagata, T. (2010). A journey with plant cell division: reflection at my halfway stop. In: Progress in Botany, Lüttge, U., Beylschlag, W., Büdel, B., Francis, D. (Eds.), Vol. 71, pp. 5-21, Springer-Verlag, ISBN 978-3-642-02166-4, Berlin, Heidelberg

Namasivayam, P., Skepper, J. & Hanke, D. (2006). Identification of a potential structural marker for embryogenic competency in the Brassica napus ssp. Oleifera embryogenic tissue. Plant Cell Reports, Vol. 25, No. 9 (September 2006), pp. 887-895, ISSN 0721-7714

Namasivayam, P. (2007). Acquisition of embryogenic competence during somatic embryogenesis. Plant Cell, Tissue and Organ Culture, Vol. 90, No. 1 (July 2007), pp. 1-8, ISSN 0167-6857

Nomura, K. & Komamine, A. (1985). Identification and isolation of single cells that produce somatic embryos at a high frequency in a carrot cell suspension culture. Plant Physiology, Vol. 79, No. 4 (December 1985), pp. 988-991, ISSN 0032-0889

Nomura, K. & Komamine, A. (1995). Physiological and biological aspects of somatic embryogenesis. In: In vitro embryogenesis in plants, Thorpe, T.A. (Ed.), pp. 249-266, Kluwer Academic Publishers, ISBN 0-7923-3149-4, Dordrecht, The Netherlands

Ovečka, M., Bobák, M., Blehová, A. & Krištín, J. (1997). Papaversomniferum regeneration by somatic embryogenesis and shoot organogenesis. Biologia Plantarum, Vol. 40, No. 3 (November 1997), pp. 321-328, ISSN 0006-3134

Pasternak, T.P., Prinsen, E., Ayaydın, F., Miskolczi, P., Potters, G., Asard, H., Van Onckelen, H.A., Dudits, D. & Fehér, A. (2002). The role of auxin, pH, and stress in the activation of embryogenic cell division in leaf protoplast-derived cells of alfalfa. Plant Physiology, Vol. 129, No.4 (August 2002), pp. 1807-1819, ISSN 0032-0889

Pedroso, M.C. & Pais, M.S. (1992). A scanning electron microscope and x-ray microanalysis study during induction of morphogenesis in Camellia japonica L. Plant Science, Vol. 87, No. 1, pp. 99-108, ISSN 0168-9452

Pedroso, M.C. & Pais, M.S. (1995). Factors controlling somatic embryogenesis. Cell wall changes as an in vivo marker of embryogenic competence. Plant, Cell, Tissue and Organ Culture, Vol. 43, No. 2 (November 1995), pp. 147-154, ISSN 0167-6857

Pence, V.C., Hasegawa, P.M. & Janick, J. (1980). Initiation and development of asexual embryos of Theobroma cacao L. in vitro. ZeitschriftfuerPflanzenphysiologie, Vol. 98, No. 1 (June 1980), pp. 1-14, ISSN 0044-328X

Pennell, R.I., Janniche, L., Scofield, G.N., Booij, H., de Vries, S.C. & Roberts, K. (1992). Identification of a transitional cell state in the developmental pathway to carrot somatic embryogenesis. The Journal of Cell Biology, Vol. 119, No. 5 (December 1992), pp. 1371-1380, ISSN 0021-9525

Pinto, G., Silva, S., Park, I.-S., Neves, L., Araújo C. & Santos, C. (2008). Factors influencing somatic embryogenesis induction in Eucalyptus globulus Labill.: basal medium and anti-browning agents. Plant, Cell, Tissue and Organ Culture, Vol. 95, No. 1 (October 2008), pp. 79-88, ISSN 0167-6857

Polito, V.S., McGranahan, G., Pinney, K. & Leslie, C. (1989). Origin of somatic embryos from repetitively embryogenic cultures of walnut (juglans regia L.): implications for Agrobacterium-mediated transformation. Plant Cell Reports, No. 8 (December 1989), pp. 219-221, ISSN 0721-7714

Puigderrajols, P., Mir, G. & Molinas, M. (2001). Ultrastructure of early secondary embryogenesis by multicellular and unicellular pathways in cork oak (Quercus
Qin, Y. & Zhao, J. (2006). Localization of arabinogalactan proteins in egg cells, zygotes, and two-celled proembryos and effects of β-D-glucosyl Yariv reagent on egg cell fertilization and zygote division in Nicotiana tabacum L. *Journal of Experimental Botany*, Vol. 57, No. 9, (June 2006), pp. 2061-2074, ISSN 0022-0957

Quinn, J., Simon, J.E. & Janick, J. (1989). Histology of zygotic and somatic embryogenesis in borage. *Journal of the American Society for Horticultural Science*, Vol. 114, No. 3, (May 1989), pp. 516-520, ISSN 0003-1062

Quiroz-Figueroa, F.R., Fuentes-Cerda, C.F.J., Rojas-Herrera, R. & Loyola-Vargas, V.M. (2002). Histological studies on the developmental stages and differentiation of two different somatic embryogenesis systems of Coffea arabica. *Plant Cell Reports*, Vol. 20, No. 12, (June 2002), pp. 1141-1149, ISSN 0721-7714

Quiroz-Figueroa, F.R., Rojas-Herrera, R., Galaz-Avalos, R.M. & Loyola-Vargas, V.M. (2006). Embryo production through somatic embryogenesis can be used to study cell differentiation in plants. *Plant Cell, Tissue and Organ Culture*, Vol. 86, No. 3, (September 2006), pp. 285-301, ISSN 0167-6857

Raghavan, V. (2004). Somatic embryogenesis, In: *Journey of a single cell to plant*, Murch, S.J. & Saxena, P.K., pp. 203-226, Oxford & IBH Publishing, ISBN 1578083524, New Delhi, India

Roberts, A.G. & Oparka, K.J. (2003). Plasmodesmata and the control of symplasmic transport. *Plant, Cell & Environment*, Vol. 26, No. 1, (January 2003), pp. 103-124, ISSN 0140-7791

Rocha, D.I., Vieira, L.M., Tanaka, F.A.O., da Silva, L.C. & Otoni, W.C. (2011). Somatic embryogenesis of a wild passion fruit species Passiflora Cincinnata Masters: histocytological and histochemical evidences. *Protoplasma*, (17 September 2011), doi: 10.1007/s00709-011-0318-x, ISSN 1615-6102

Rodriguez, A.P.M. & Wetzstein H.Y. (1998). A morphological and histological comparison of the initiation and development of pecan (Carya illinoinensis) somatic embryogenic cultures induced with naphthaleneacetic acid or 2,4-dichlorophenoxyacetic acid. *Protoplasma*, Vol. 204, No. 1-2 (March 1998), pp. 71-83, ISSN 0033-183X

Rohr, R., Piola, F. & Pasquier P. (1997). Somatic embryogenesis in Cephalotaxus harringtonia embryo-megagametophyte co-culture. *Journal of Forest Research*, Vol. 2, No. 2 (May 1997), pp. 69-73, ISSN 1341-6979

Romberger, J.A., Hejnowicz, Z. & Hill, J.F. (2004). *Plant structure: function and development. A treatise on anatomy and vegetative development*, with special reference to woody plants (Reprint of First Edition), The Blackburn Press, ISBN 1-930665-95-4, New Jersey, U.S.A.

Šamaj, J., Bobák, M., Ovečka, M., Kríštin, J. & Blehová A. (1994). Extracellular matrix in early stages of plant regeneration *in vitro*. *Cell Biology International*, Vol. 18, No. 5 (May 1994), p. 545, ISSN 1065-6995

Šamaj, J., Bobák, M., Kríštin, J. & Auctová-Šamajová, O. (1995). Developmental SEM observations on an extracellular matrix in embryogenic calli of Drosera rotundifolia and Zea mays. *Protoplasma*, Vol. 186, No. 1-2 (March 1995), pp. 45-49, ISSN 0033-183X
Scheres, B. (2001). Plant cell identity. The role of position and lineage. *Plant Physiology*, Vol. 125, No. 1 (January 2001), pp. 112-114, ISSN 0032-0889

Schmidt, E.D.L., Guzzo, F., Toonen, M.A.J. & de Vries, S. (1997). A leucine-rich repeat containing receptor-like kinase marks somatic plant cells competent to form embryos. *Development*, Vol. 124, No. 10 (May 1997), pp. 2049-2062, ISSN 0950-1991

Schwendiman, J., Pannetier, C. & Michaux-Ferriere, N. (1990). Histology of embryogenic formations during *in vitro* culture of oil palm *Elaeis guineensis* Jacq. *Oléagineux*, Vol. 45, No. 10, pp. 409-418, ISSN 0030-2082

Seifert, G.J. & Roberts, K. (2007). The biology of arabinogalactan proteins. *Annual Review of Plant Biology*, Vol. 58 (January 2007), pp. 137-161, ISSN 1543-5008

Sharp, W.R., Sondahl, M.R., Caldas, L.S. & Maraffa, S.B. (1980). The physiology of *in vitro* asexual embryogenesis. *Horticultural Reviews*, Vol. 2, pp. 268-310, ISSN 0163-7851

Sidikou-Seyni, R., Rambaud, C., Dubois, J. & Vasseur, J. (1992). Somatic embryogenesis and plant regeneration from protoplasts of *Cichorium intybus* L. x *Cichorium endivia* L. *Plant Cell, Tissue and Organ Culture*, Vol. 28, No. 1 (January 1992), pp. 83-91, ISSN 0167-6857

Sondahl, M.R., Salisbury, J.L. & Sharp, W.R. (1979). SEM characterization of embryogenic tissue and globular embryos during high-frequency somatic embryogenesis in coffee callus cells. *Zeitschrift für Pflanzenphysiologie*, Vol. 94, pp. 94, ISSN 0044-328X

Stacey, N.J., Roberts, K. & Knox, J.P. (1990). Patterns of expression of the JIM4 arabinogalactan-protein epitope in cell cultures and during somatic embryogenesis in *Daucus carota* L. *Planta*, Vol. 180, No. 2 (January 1990), pp. 285-292, ISSN 0032-0935

Stadler, R., Lauterbach, C. & Sauer, N. (2005). Cell-to-cell movement of green fluorescent protein reveals post-phloem transport in the outer integument and identifies symplasmic domains in Arabidopsis seeds and embryos. *Plant Physiology*, Vol. 139, No. 2, (October 2005), pp. 701-712, ISSN 0032-0889

Steinmacher, D.A., Guerra, M.P., Saare-Surminski, K. & Lieberei, R. (2011). A temporary immersion system improves *in vitro* regeneration of peach palm through secondary somatic embryogenesis. *Annals of Botany*, (25 February 2011), doi: 10.1093/aob/mcr033, ISSN 1095-8290

Sterk, P., Booij, H., Schellekens, G.A., van Kammen, A. & de Vries, S.C. (1991). Cell-specific expression of the carrot EP2 lipid transfer protein gene. *The Plant Cell*, Vol. 3, No. 9 (September 1991), pp. 907-921, ISSN 1040-4651

Steward, F.C., Mapes, M.O. & Mears, K. (1958). Growth and organized development of cultured cells. I. Growth and division of freely suspended cells. *American Journal of Botany*, Vol. 45, No. 9 (November 1958), pp. 693-703, ISSN 0002-9122

Sugimoto, K., Gordon, S.P. & Meyerowitz, E.M. (2011). Regeneration in plants and animals: dedifferentiation, transdifferentiation, or just differentiation? Trends in Cell Biology, Vol. 21, No. 4(April 2011), pp. 212-218, ISSN 0962-8924

Tchobadjieva, M., Kalmukova, R., Pantchev, I. & Kyurchiev, S. (2005). Monoclonal antibody against a cell wall marker protein for embryogenic potential of *Dactylis glomerata* L. suspension cultures. *Planta*, Vol. 222, No. 5 (November 2005), pp. 811-819, ISSN 0032-0935

www.intechopen.com
Thomas, H., Ougham, H.J., Wagstaff, C & Stead, A.D. (2003). Defining senescence and death. *Journal of Experimental Botany*, Vol. 54, No. 385 (April 2003), pp. 1127-1132, ISSN 0022-0957

Thorpe, T.A. (1980). Organogenesis in vitro: structural, physiological and biochemical aspects. In: *Perspectives in plant cell and tissue culture. International Review of Cytology – Supplement 11A*, Vasil, I.K. (Ed.), pp. 71-111, ISSN 0074-770X New York Academics Press

Timmers, A.C.J., Reiss, H.-D., Bohsung, J., Traxel, K. & Schel, J.H.N. (1996). Localization of calcium during somatic embryogenesis of carrot (*Daucus carota* L.). *Protoplasma*, Vol. 190, No. 1-2 (March 1996), pp. 107-118, ISSN 0033-183X

Toonen, M.A.J., Hendriks, T., Schmidt, E.D.L., Verhoeven, H.A., van Kammen, A. & de Vries S.C. (1996). Description of somatic-embryo-forming single cells in carrot suspension cultures employing video cell tracking. *Planta*, Vol. 194, No. 4 (December 1994), pp. 565-572, ISSN 0032-0935

Trigiano, R.N., Gray, D.J., Conger, B.V. & McDaniel, J.K. (1989). Origin of direct somatic embryos from cultured leaf segments of *Dactylis glomerata*. *Botanical Gazette*, Vol. 150, No. 1, (March 1989), pp. 72-77, ISSN 0006-8071

Uzelac, B., Ninković, S., Smigocki, A. & Budimir S. (2007). Origin and development of secondary somatic embryos in transformed embryogenic cultures of *Medicago sativa*. *Biologia Plantarum*, Vol. 51, No. 1, pp. 1-6, ISSN 0006-3134

Verdeil, J.L., Hocher, V., Huet, C., Grosdemange, F., Escoute, J, Ferriere, N. & Nicole, M. (2001). Ultrastructural Changes in Coconut Calli Associated with the Acquisition of Embryogenic Competence. *Annals of Botany*, Vol. 88, No. 1, (July 2001), pp. 9-18, ISSN 0305-7364

Verdeil, J.L., Alemano, L., Niemenak, N. & Trambarger, T.J. (2007). Pluripotent versus totipotent plant stem cells: dependence versus autonomy? *TRENDS in Plant Science*, Vol. 12, No. 6 (May 2007), pp. 245-252, ISSN 1360-1385

Verdus, M.C., Dubois, T., Dubois, J. & Vasseur, J. (1993). Ultrastructural Changes in Leaves of *Cicorium* during Somatic Embryogenesis. *Annals of Botany*, Vol. 72, No. 4, (April 1993), pp. 375-383, ISSN 0305-7364

von Arnold, S., Sabala, L., Bozhkov, P., Dyachok, J. & Filonova, L. (2002). Developmental pathways of somatic embryogenesis. *Plant Cell, Tissue and Organ Culture*, Vol. 69, No. 3, (June 2002), pp. 233-249, ISSN 0167-6857

Wang, X.D., Nolan, K.E., Irwanto, R.R., Sheahan, M.B. & Rose, R.J. (2011). Ontogeny of embryogenic callus in *Medicago truncatula*: the fate of the pluripotent and totipotent stem cells. *Annals of Botany*, Vol. 107, No. 4, (April 2011), pp. 599-609

Williams, E.G. & Maheswaran, G. (1986). Somatic embryogenesis: factors influencing coordinated behavior of cells as an embryogenic group. *Annals of Botany*, Vol. 57, No. 4, (October 1985), pp. 443-462, ISSN 0305-7364

Warner, A. (1992). Gap junctions in development-a perspective. *Seminars in Cell Biology*, Vol. 3, No. 1, (February 1992), pp. 81-91, ISSN 1043-4682

Wrobel, J. (2010). Symplasmic communication during somatic embryogenesis of chosen species. PhD thesis, University of Silesia, Katowice, Poland

Wrobel, J., Barlow, P.W., Gorka, K., Nabialkowska, D. & Kurczynska, E.U. (2011). Histology and symplasmic tracer distribution during development of barley androgenic embryos. *Planta*, Vol. 233, No. 5, (May 2011), pp. 873-881, ISSN 0032-0935
Yang, X. & Zhang, X. (2010). Regulation of Somatic Embryogenesis in Higher Plants. Critical Reviews in Plant Science, Vol. 29, No. 1-3, (January 2010), pp. 36-57, ISSN 0735-2689

Yeung, E.C. (1995). Structural and developmental patterns in somatic embryogenesis. In vitro embryogenesis in plants. In: Kluwer Academic Publishers, Thorpe, T.A. (Ed.), pp. 205-247, Netherlands

You, X.L., Yi, J.S. & Choi, Y.E. (2006). Cellular change and callose accumulation in zygotic embryos of Eleutherococcus senticocous caused by plasmolyzing pretreatment result in high frequency of single-cell-derived somatic embryogenesis. Protoplasma, Vol. 227, No. 5, (May 2006), pp. 105-112, ISSN 0033-183x

Zambryski, P. & Crawford, K. (2000). Plasmodesmata: gatekeepers for cell-to-cell transport of developmental signals in plants. Annual Review of Cell and Developmental Biology, Vol. 16, (November 2000), pp. 393-421, ISSN 1081-0706

Zeng, F., Zhang, X., Zhu, L., Tu, L., Guo X., & Nie Y. (2006). Isolation and characterization of genes associated to cotton somatic embryogenesis by suppression subtractive hybridization and macroarray. Plant Molecular Biology Vol. 60, No. 2, (January 2006), pp. 167-183, ISSN 0167-4412

Zimmerman, J.L. (1993). Somatic Embryogenesis: A Model for Early Development in Higher Plants. The Plant Cell, Vol. 5, No. 10, (October 1993), pp. 1411-1423, ISSN 1040-4651
The book "Embryogenesis" is a compilation of cutting edge views of current trends in modern developmental biology, focusing on gametogenesis, fertilization, early and/or late embryogenesis in animals, plants, and some other small organisms. Each of 27 chapters contributed from the authorships of world-wide 20 countries provides an introduction as well as an in-depth review to classical as well as contemporary problems that challenge to understand how living organisms are born, grow, and reproduce at the levels from molecule and cell to individual.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Ewa U. Kurczynska, Izabela Potocka, Izabela Dobrowolska, Katarzyna Kulinska-Lukaszek, Katarzyna Sala and Justyna Wrobel (2012). Cellular Markers for Somatic Embryogenesis, Embryogenesis, Dr. Ken-Ichi Sato (Ed.), ISBN: 978-953-51-0466-7, InTech, Available from: http://www.intechopen.com/books/embryogenesis/cellular-markers-for-somatic-embryogenesis
