Chapter 7
The Current Status of Proteomic Studies in Somatic Embryogenesis

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Abstract Somatic embryogenesis includes the genetic reprogramming of somatic cells to acquire the embryogenic potency necessary to generate an embryo, which can develop into a whole plant. Acquisition of embryogenic capacity requires rigorous biochemical coordination that includes several metabolic and signal transduction pathways. Recent genomic and epigenetic studies in somatic embryogenesis have shown interconnection among signals associated with growth regulators, stress factors, and modulation of the genome structure. A broad range of key proteins, posttranslational modifications, protein turnover, and protein–protein interactions are common factors associated with the establishment of the necessary biochemical status of cells during the acquisition of the embryogenic potential. Recent proteomic studies have begun describing the molecular basis of somatic embryogenesis. However, the diversity of the embryogenic response among plant species makes it difficult to define key protein factors associated with embryogenic cultures or specific stages during the transdifferentiation of somatic embryos. In this chapter, we review the most prominent proteomic studies carried out in the past

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decade and discuss the contributions of proteomics studies to elucidating the molecular basis of somatic embryogenesis.

7.1 Introduction

The capacity of a somatic cell to acquire embryogenic competence and generate a new plant by somatic embryogenesis (SE) has been studied for several decades. Strategies that have been used to induce SE across plant species include: different types of explant, modification of the culture media (addition, subtraction, and combination of culture media components), plant growth regulators (different types, concentration, and combinations), culture conditions (different wavelength of light, dark culturing, and ventilation), and the application of culture media coming from embryogenic cultures (conditioned media). These studies have provided empirical information, which has identified key physiological cues that lead to embryogenic potential, although specific culture condition requirements vary with species. In addition, microscopy studies in several plant species defined key morphological characteristics associated with embryogenic cultures (Popielarska-Konieczna et al. 2008; Xu et al. 2011; Kikuchi et al. 1995; Sterk et al. 1991; Steinmacher et al. 2012; Pennell et al. 1992). However, the molecular bases of induction, embryo development, maturation, and germination are not well-known. Several studies have shed light on the important roles of growth regulators, protein receptors, and transcription factors (Martin 2004; Zeynali et al. 2010; Cheung and Wu 2011; Wolf and Hofte 2014; Karami et al. 2009). Recently, with the surge of "omics" technologies we have been able to generate a more comprehensive panorama of the genome-wide expression profiles during SE (Silva et al. 2014; Noah et al. 2013; Gomez-Garay et al. 2013; Salvo et al. 2014; Hoenemann et al. 2010; Rode et al. 2011, 2012). These comprehensive studies allow us to identify specific modifications of genome architecture through epigenetic regulation during SE (Feher 2015; Nic-Can et al. 2013; Imin et al. 2005). Furthermore, proteomic studies of SE in several species have created protein profiles of cultures in different conditions during the induction of embryogenic potential (Varhanikova et al. 2014; Correia et al. 2012; Marsoni et al. 2008; Sharifi et al. 2012; Almeida et al. 2012; Guzman-Garcia et al. 2013). These studies have been followed up with the focused identification of key proteins associated with each stage of the transdifferentiation of somatic embryos (Rode et al. 2012; Bian et al. 2010; Vale Ede et al. 2014). Moreover, the morphological and physiological similarities between zygotic and somatic embryogenesis have suggested similar biochemical status for cells undergoing both of these processes, and protein profiles for zygotic and somatic embryogenesis have been compared in a few plants species (Sghaier-Hammami et al. 2009; Winkelmann et al. 2006; Noah et al. 2013). Altogether, the proteins identified in several conditions and stages of SE reflect the biochemical status of cells. These biochemical statuses suggest that cells need to overcome stress conditions during genome reprogramming and the development of SE (Smertenko and Bozhkov 2014; Feher 2015; Zavattieri et al. 2009). However, the technical difficulties of establishing somatic embryogenesis,

such as the differential response among plant species, the asynchronous transdifferentiation of the somatic embryos and the participation of several types of cells, increase the complexity of proteomics studies. Moreover, the key proteins associated with cellular division, expansion, and differentiation are expressed in very narrow windows of time in each stage of SE. Regulatory proteins such as receptors and transcription factors are mostly present in low abundance in a specific subcellular compartment (Gupta et al. 2015; Qi and Katagiri 2009; Chen et al. 2001; Van Leene et al. 2007; Smaczniak et al. 2012).

In this chapter, we review the important proteomics studies associated with somatic embryogenesis during induction, embryo transdifferentiation, and maturation. We mention the major contribution of proteomic technology to understanding the basis of the molecular foundation of SE and we discuss the current proteomics tools suitable for overcoming the hurdles of analyzing the proteome of SE.

7.2 Induction Stage: The Starting Point

The starting point of somatic embryogenesis in plant systems is the induction of embryogenic competency. This allows somatic cells to be reprogrammed and develop into mature embryos capable of germination. Due to of the broad diversity of embryogenic response in the plant kingdom, several culture conditions have been proposed. Many include the addition of growth regulators (auxin and/or cytokinin) during the induction stage (Imin et al. 2005; Nolan et al. 2003; Schmidt et al. 1997). Several types of explants have been used for the establishment of SE including leaflets, stems, cambium, immature zygotic embryos, cell suspensions, and callus (Imin et al. 2005; Varhanikova et al. 2014; Correia et al. 2012; Sharifi et al. 2012; Guzmán-García et al. 2013). SE can be induced directly from the explant (direct embryogenesis) or indirectly through the formation of somatic embryos from callus or suspension cultures (George et al. 2008). Pioneering microscopy observations distinguished the generation of embryogenic and non-embryogenic tissues from the same explants. Both of these tissues can be maintained in culture for long periods of time, maintaining their particular identities (Varhanikova et al. 2014; Correia et al. 2012; Marsoni et al. 2008; Sharifi et al. 2012; Nomura and Komamine 1985; Pennell et al. 1992). In general embryogenic cultures comprise compact globular masses with clusters of meristematic cells (small, highly cytoplasmic, and mostly containing starch) while in non-embryogenic cultures it is common to observe fast growing large parenchymal cells (vacuolated, amorphous, and translucent in appearance). However, the morphological and physiological features of embryogenic and non-embryogenic tissues are very specific to the plant species studied. Although, embryogenic cultures in most species develop into somatic embryos while non-embryogenic cultures remain undifferentiated (Fig. 7.1). These pairs of distinct cultures have been analyzed using proteomics tools with the goal of identifying proteins markers associated with embryogenic potency (Varhanikova et al. 2014; Correia et al. 2012; Marsoni et al. 2008; Sharifi et al. 2012; Almeida

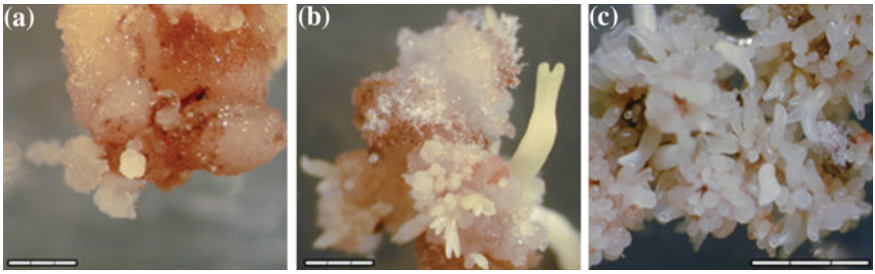

Fig. 7.1 Different responses can be obtained from zygotic embryos of *Magnolia dealbata,* cultured on WP media supplemented with 2.26 μM 2,4-D. **a** Non-embryogenic callus. **b** Using a zygotic embryo it is possible to induce non-embryogenic and embryogenic calluses. The growth of somatic embryos in some cases is observed in specific areas of the explant. **c** Somatic embryos at different stages of development after induction of direct secondary somatic embryogenesis from embryogenic callus. Scale bar: 3 mm

et al. 2012; Guzmán-García et al. 2013). However, these studies have not used sufficiently robust proteomics pipelines and therefore have not covered a comprehensive proportion of the proteome during initiation of the acquisition of the embryogenic potency (Rode et al. 2011, 2012). In most of the proteomics studies of somatic embryogenesis, proteins have been analyzed with 2D-SDS-PAGE and mass spectrometry tools (Rode et al. 2011, 2012; Varhanikova et al. 2014; Correia et al. 2012; Marsoni et al. 2008; Sharifi et al. 2012; Almeida et al. 2012; Guzmán-García et al. 2013; Vale Ede et al. 2014).

In this chapter, we compile the most relevant proteomic information by considering prominent proteomic studies in embryogenic and non-embryogenic cultures from different plant species (Varhanikova et al. 2014; Correia et al. 2012; Marsoni et al. 2008; Sharifi et al. 2012; Almeida et al. 2012; Guzmán-García et al. 2013). We group the identified proteins from each plant species by functional category. Our analysis indicates that the two most prominent differences between embryogenic and non-embryogenic cultures are proteins associated with oxidation–reduction processes and proteins associated with protein folding and binding domains (Fig. 7.2). In fact, several proteomic studies have shown over-accumulation of reactive oxygen species (ROS), reactive nitrogen species (RNS) (Zavattieri et al. 2009; Ötvös et al. 2005), and ROS-scavenging enzymes including extracellular peroxidases, superoxide dismutase (SOD), catalases, thioredoxins, and isoflavone reductases occur in embryogenic cultures (Sharifi et al. 2012; Marsoni et al. 2008; Nomura and Komamine 1985). Interestingly, an outburst of constant oxidative stress can lead to irreversible post-translational modifications in proteins inducing oligomerization, fragmentation, destabilization, aggregation, and degradation of unfolded proteins (Meriin et al. 2010; Nystrom 2005; Lindermayr et al. 2005; Spadaro et al. 2010). Furthermore, these oxidative conditions overload the protein quality control (PQC) system, which can cause a second major wave of stress conditions able to destabilize the proteostasis system of the cell (Tu and Weissman 2004; Dahl et al. 2015). This reduction in protein integrity is reflected in proteomic studies, where several proteins associated with a

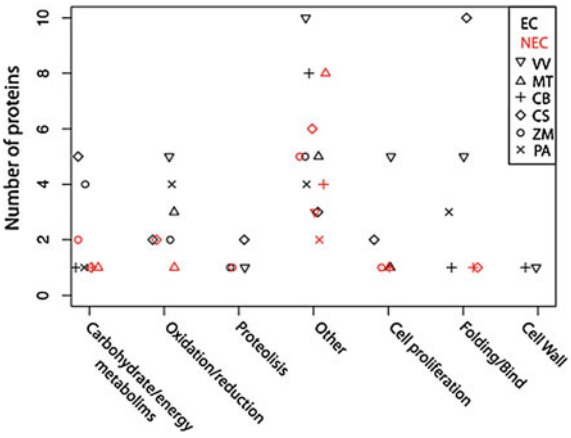

Fig. 7.2 Comparison of proteomic data from embryogenic and non-embryogenic cultures. We considered the most recent and representative proteomic data. Proteins identified in each study were grouped in function families both in embryogenic (*EC*) and non-embryogenic cultures (*NEC*). Species depicted are *VV Vitis vinifera*; *MT Medicago truncatula*; *CB Cyphomandra betacea*; *CS Crocus sativus*; *ZM Zea mays*; *PA Persea americana*

folding and binging activity such as heat shock proteins 60 (HSP60) and 70 kDa (HSP70), were identified more frequently in embryogenic than non-embryogenic cultures (Fig. 7.2). Therefore, it seems likely that fine scale regulation of gene expression and the over-accumulation of ROS/RNS scavenging enzymes and folding associated proteins at the induction stage of SE are key factors for overcoming stress conditions and continuing the acquisition of embryogenic potency in embryogenic cultures. This interpretation is consistent with the pattern of identification of proteins associated with cellular proliferation, which mirrors the induction and development of somatic embryos in embryogenic cultures (Fig. 7.1). It is noteworthy that this oxidation–reduction regulation may be part of global master regulation and contribute to crosstalk among several regulatory networks (Feher 2015).

7.3 Transdifferentiation of Somatic Embryos Resembles Zygotic Embryogenesis

One of the main goals of the application of somatic embryogenesis is the large-scale production of homogeneous plants with defined characteristics. This task has been difficult to accomplish in all species in which it has been tried. The first challenge, described above, is establishing optimal conditions for the induction of SE. After the induction of SE, involving a global shift in gene expression and the rigorous coordination of several metabolic pathways, each embryo undergoes transdifferentiation. In some cases, this process includes multiple stages with unique morphological, molecular, and physiological conditions. Transdifferentiation of somatic embryos can be very different across species depending on the genomic background. In some species, it is possible to observe the globular, heart, torpedo, and cotyledon stage for dicotyledons, and globular, elongated, scutelar, coleoptilar

stages for monocotyledons (von Arnold et al. 2002; Zimmerman 1993). However, the asynchronous nature of the embryo transdifferentiation gives rise to a mix of embryos at different morphogenetic stages (Gray and Purohit 1991). Moreover, somatic embryos in several plant species show abnormal development during transdifferentiation. In most of the cases, aberrant embryos cannot be completely transdifferentiated, halting at some point in SE. There is even variation in somatic embryogenesis between species of the same plant genus. For instance, although generation of *Coffea canephora* through SE is straightforward, the induction of SE in *C. arabica* is a very difficult task (Nic-Can et al. 2015; Tonietto et al. 2012). Recent studies have suggested that molecular factors secreted by *C. arabica* cultures including the phenolic compounds, caffeine and chromogenic acid, act as repressors of somatic embryogenesis (Nic-Can et al. 2015). Caffeine and chromogenic acid are known to affect DNA methylation (Nic-Can et al. 2015) and it is possible that they affect the proteome and secretome of the cultures of *C. arabica* as well. Altogether, the molecular basis of the physiological, morphological and biochemical differences during asynchronous transdifferentiation is not well understood and difficult to control. Since somatic embryogenesis (SE) resembles zygotic embryogenesis (ZE) in several aspects, molecular, and microscopy studies have analyzed the two processes simultaneously (Rode et al. 2011; Takac et al. 2011; Sghaier-Hammami et al. 2009; Rodríguez-Sanz et al. 2014; Dobrowolska et al. 2012). Several studies have suggested that zygotic embryos may provide important clues about the physiological and biochemical preconditions necessary for proper transdifferentiation, maturation, and germination of somatic embryos. Unfortunately, few massive proteomic studies have broadly analyzed the proteome at each stage of SE and ZE (Rode et al. 2011, 2012; Balbuena et al. 2009).

In the absence of a single comprehensive study, we resort to a meta-analysis of public data across studies. However, this approach is non ideal because of the high variability of transdifferentiation in somatic embryos which makes it very difficult to compare proteomics data even within plant species. In addition, proteomic studies have used a broad range of extraction procedures, mass spectrometers, and databases. Future comparison of data would be facilitated by the establishment of universals procedures, proteomics pipelines, and more compatible bioinformatic platforms among laboratories around the world studying SE. We examined the most representative and recent proteomic studies, where either somatic or zygotic embryogenesis have been analyzed at least in three different stages of development, by classifying cellular stages and functions in broad groups and comparing counts of uniquely identified proteins (Fig. 7.3). We can clearly observe a wide variety of proteins identified in the proliferation stage for SE, as opposed to ZE. However, at the globular and torpedo stages, the proteins identified in both somatic and zygotic embryogenesis belong to similar functional categories (Fig. 7.3). Similar proteins were identified in proteomic studies at the torpedo stage of SE and ZE in *Theobroma cacao*. In both cases, proteins associated with stress and folding/sorting/degradation were more prominent in SE, while proteins associated with carbohydrate metabolism were more prominent in ZE (Noah et al. 2013).

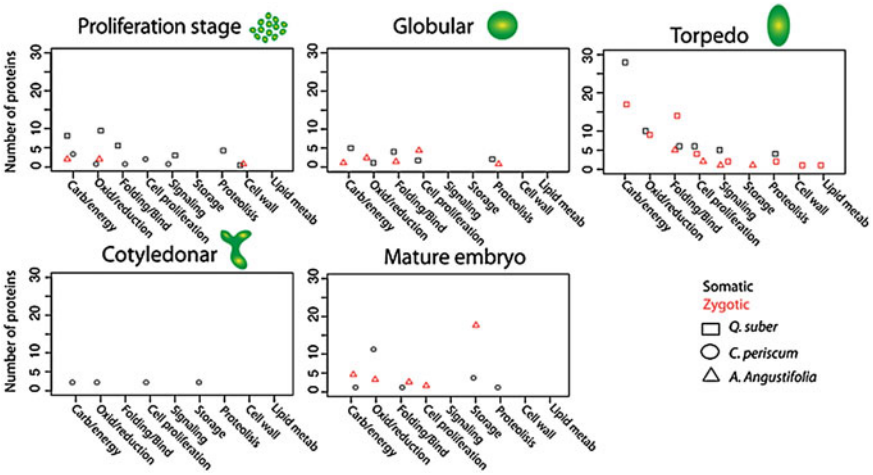

Fig. 7.3 Comparison of proteomics studies carried out during the transdifferentiation of somatic and zygotic embryos. Proteins identified in each study were grouped in functional families for both somatic (*SE*) and zygotic embryogenesis (*ZE*)

7.4 Maturation of Somatic Embryos

Maturation of the somatic embryos is a critical step, which is defined by the conversion of a mature somatic embryo to a plantlet with a functional shoot and root system (Stasolla and Yeung 2003). Maturation is characterized by active cellular expansion and differentiation and accumulation of storage proteins such as globulins and small enolases (Rode et al. 2011, 2012; Vale Ede et al. 2014). Although the function of these small enolases is unknown, they have a lower than theoretically predicted molecular weight (Rode et al. 2011) and, therefore, may be the result of proteolysis of larger inactive enolases, which are then repurposed as storage proteins (Rode et al. 2011). During recent decades great effort has been devoted to understanding and improving the maturation of somatic embryos and preventing precocious germination, because these processes define the efficiency of plant regeneration through SE (Stasolla and Yeung 2003; Gutmann et al. 1996; Merkle et al. 1990; Bapat et al. 1988). There are several factors which alter maturation of somatic embryos including mineral salts, carbohydrates, gelling agent, coconut water, polyethylene glycol, amino acids, cytokinins, and abscisic acid (Rode et al. 2011, 2012; Vale Ede et al. 2014; Sghaier-Hammami et al. 2010). These treatments have been mainly associated with the over-accumulation of storage proteins and proteins involved in carbohydrate/energy metabolism (Rode et al. 2011, 2012; Vale Ede et al. 2014). The accumulation of storage molecules is a common feature during the maturation of zygotic embryos; this accumulation can serve as a marker to compare quality and fidelity of somatic embryogenesis. This has most notably been accomplished using small enolases in *Cyclamen persicum* (Thorpe 1995; Rode et al.

2011). Additionally, findings across proteomic studies indicate that the main difference between matured somatic and zygotic embryos is the availability of storage compounds (carbohydrates, lipids, and proteins). This is clear in *Phoenix dactylifera* (date palm), where comparative proteomic studies between SE and ZE of showed an over-accumulation of storage proteins in zygotic embryos (Sghaier-Hammami et al. 2009). Furthermore, proteomic studies during ZE of *Agave angustifolia* found large accumulations of storage proteins at the maturation stage (Fig. 7.3) (Balbuena et al. 2009; Thorpe 1995). Clearly, in ZE, storage proteins and other storage molecules (small enolases, oligosaccharides, and lipids) are key factors that provide energy to the seedling until the establishment of photosynthesis (Winkelmann et al. 2006; Rode et al. 2011). Therefore, increasing the availability of storage molecules in somatic embryos could improve conditions and promote the generation of high quality in vitro plantlets.

In order to move beyond identifying necessary protein categories (e.g., storage) and toward identifying the specific protein requirements for efficient SE, a standardized approach across labs is necessary. A universal proteomics approach with powerful technologies applied to each stage of somatic embryogenesis in a variety of plant species will lead to a better understanding of the molecular nature of each embryo stage during SE. Identifying and characterizing key proteins, such as the small enolases, can provide invaluable molecular markers necessary to the establishment of efficient and effective protocols for somatic embryogenesis and massive propagation.

7.5 New Technologies for Proteomics Studies in Somatic Embryogenesis

The goal of proteomic studies is the global analysis of the proteome of a cell, tissue, or organ at a specific time under defined conditions. Recently, with the establishment and application of new pipelines extensive progress has been made in proteomics studies, resulting in the in-depth characterization of bacteria and yeast proteomes (Zielinska et al. 2012; Nothaft and Szymanski 2010). Moreover, studies in a multicellular model organism such as *Mus musculus*, *Homo sapiens*, *Arabidopsis thaliana* and *Solanum lycopersicum* have demonstrated the application of new proteomic tools in a broad range of organisms (Lopez-Casado et al. 2012; Zielinska et al. 2012; Kim et al. 2014). This may be possible even when whole genome sequence is not available. However, these techniques have not been applied systematically or in sufficient detail to somatic embryogenesis. Studies are needed to create detailed profiles of each stage of embryo transdifferentiation (Rode et al. 2012). Moreover, considering the intercommunication among several tissue layers, specific cell types, and subcellular compartments during cellular differentiation, division, and expansion, profiles of individual tissues are necessary as well.

The major hurdle for proteomic studies in SE is asynchronous transdifferentiation and the limited amount of biological material available in each stage. Specifically, the bottleneck for proteomics studies of SE is obtaining sufficiently large samples of protein (Gupta et al. 2015; Zawadzka et al. 2014). Even when samples are available in large quantity, in most cases more than 50 % of the proteins present are at low concentration, falling below the limit of detention for even the most powerful mass spectrometry instrumentation available. As mentioned above, most of the proteomics studies in somatic embryogenesis have been conducted with 2D-SDS-PAGE and mass spectrometry tools. However, considering the limitations of 2D-SDS–PAGE (poor reproducibility, the narrow dynamic range of identification, limitations in detecting membrane and low abundance proteins) (Rabilloud and Lelong 2011), proteomic studies in SE should be conducted using alternative proteomic tools. In fact, several approaches have been used to isolate and analyze low abundance proteins including, precipitations, depletion of abundant proteins, and affinity chromatographic tools (Hage and Matsuda 2015; Polaskova et al. 2010; Jiang et al. 2004). We suggest, that affinity chromatography, combinatorial peptide ligands library (CPLL), and powerful mass spectrometers will be key to digging deeper into the proteome of somatic embryogenesis in several plant species.

Affinity chromatography has been the favorite approach for low abundant protein enrichment, including for studies emphasizing identification of proteins with posttranslational modifications. This versatile technique utilizes a broad range of ligands covalently linked to different types of matrixes (e.g., Sepharose, agarose, and others) (Zielinska et al. 2012; Ruiz-May et al. 2014). A wide variety of ligands is now commercially available including: antibodies used to enrich specific antigens, substrates to trap enzymes, ligands to isolate receptors, and lectins to enrich and characterize low abundance glycoproteins (Wilchek and Chaiken 2000; Steen et al. 2006; Hage and Matsuda 2015). Pioneering work in carrot somatic embryogenesis indicated glycosylated secreted proteins as the main factor associated with the activation of the somatic embryogenesis (Lo Schiavo et al. 1990; Cordewener et al. 1991; Sterk et al. 1991; van Engelen et al. 1991). However, follow-up studies are needed. Combining affinity chromatography with powerful mass spectrometers could provide important clues about low abundant glycoproteins with key roles during the induction of somatic embryogenesis and the transdifferentiation of the SE and ZE. Recently, several pipelines including affinity chromatography with lectins with multiple affinities to broad types of sugar structures in glycoproteins have been reported as effective approaches for the enrichment of a comprehensive population of glycopeptides and glycoproteins (Ruiz-May et al. 2014; Zielinska et al. 2012).

Another important consideration for future work in understanding signal transduction associated with embryogenic potency is the proteomic analysis of redox-based posttranslational modifications (PTM). This is especially relevant because of the association between the induction of somatic embryogenesis and the oxidative status of embryogenic cultures. For instance, protein carbonylation is an irreversible PTM that marks proteins under oxidative stress conditions (Lounifi

et al. 2013; Madian and Regnier 2010; Moller et al. 2011). Protein carbonylation can be direct, through the oxidation of amino acid residues (proline, lysine, arginine, and threonine), or indirect, through the formation of adducts with lipid peroxidation products or glycation products (Madian and Regnier 2010). In addition, it has become increasingly clear that protein *S*-nitrosylation is an important PTM in plant biological processes (Spadaro et al. 2010; Corpas et al. 2008). Recent studies have shown that cysteine residues are the major site of action for ROS/RNS species leading to the formation of *S*-nitrosylation and *S*-glutathionylation, and sulphenic acid, sulphinic acid, and disulphide formation (Spadaro et al. 2010). Both carbonylation and *S*-nitrosylation affect the structure and function of several proteins (Moller et al. 2011; Tada et al. 2008; Lindermayr and Durner 2009; Davies 2005). Therefore, great effort has been focused on establishing proteomic pipelines for the isolation, enrichment, and characterization of carbonylated and *S*-nitrosylated proteins (Lindermayr et al. 2005; Lindermayr and Durner 2009). The implementation of new technologies of affinity chromatography and powerful mass spectrometers will underpin the new era of proteomics studies in somatic embryogenesis.

The CPLL is another fascinating approach that diminishes the dynamic range of protein extracts, thereby, allowing analysis of very diluted proteins, which are often invisible due to highly abundant proteins. This technology consists of several million hexapeptides, which are covalently linked to porous beads, capable of binding to a great number of proteins (both high and low abundance) in all cases tested thus far (Boschetti and Giorgio Righetti 2008; Boschetti et al. 2009; Antonioli et al. 2007; Fortis et al. 2006; Guerrier et al. 2007; Sennels et al. 2007; Castagna et al. 2005). This approach drastically reduces the presence of highly abundant proteins, because the most abundant proteins saturate the ligands for which they have affinity faster than low abundance proteins. Consequently, continuous overloading of the ligand libraries will allow the enrichment of very dilute proteins while the unbound highly abundant proteins are discarded in the flow through. Therefore, using CPLL coupled with mass spectrometry during the transdifferentiation of somatic or zygotic embryos will allow the identification of key protein factors present at very low concentration either in a specific layer of tissue or extracellular culture media. Furthermore, CPLL may serve to increase the effectiveness of other technologies. For instance, in studies of the tomato pericarp *N*-glycoproteome, a high dynamic range of identified *N*-glycoproteins was found even after enrichment with lectin affinity chromatography (Ruiz-May et al. 2014). Combining an affinity chromatography approach with CPLL may provide an alternative pipeline to reduce the high dynamic range of proteins with posttranslational modifications.

The above-mentioned chromatography tools, combined with peptide fractionation and cutting edge mass spectrometry technology will provide the means to a better understanding of somatic embryogenesis. For several decades manufacturers of mass spectrometers and scientists around the globe have been working to overcome the challenges posed by the size and heterogeneity of peptides and proteins, and solubility of protein complexes (Marcoux and Cianferani 2015;

Tsiatsiani and Heck 2015; Hu et al. 2005; Hardman and Makarov 2003; Eliuk and Makarov 2015). Recently, several excellent reviews of improvements in mass spectrometry and bioinformatics tools have been published (Larance and Lamond 2015; Marcoux and Cianferani 2015; Tsiatsiani and Heck 2015; Yates et al. 2009; Vowinckel et al. 2014). The new advances in proteomics pipelines include: the improvement of peptide fractionation with nano-HPLC/nano-UPLC systems, the combination of mass analyzers, alternative fragmentation methods (CID, ECD, HCD, and ETD), database search algorithms, and new implementation of data independent analysis (SWATH), which have allowed the identification and characterization of several thousands of glycoproteins and phosphoproteins (Hu et al. 2005; Liu et al. 2014; Vowinckel et al. 2014; Yates et al. 2009; Choudhary et al. 2015). Furthermore, today it is possible to identify and quantify proteins with less than 100 copies per cell within short period of time (Picotti et al. 2009). In addition, bottom-up, middle-down and top-down proteomics have emerged as integrative tools for the characterization of posttranslational modifications and the structural analysis of complex isoforms (Rosati et al. 2012; Moradian et al. 2014). The coming years will bring exciting discoveries in somatic embryogenesis with the application of cutting-edge proteomics approaches.

7.6 Conclusions

SE is a fascinating biological process that consists of a series of complex molecular mechanisms precisely located spatially and temporally both within particular cells and within specific tissue layers in those cells. The major players associated with the activation and regulation of embryogenic response is still unknown. Several cellular aspects of SE are still poorly studied including the molecular and structural modifications associated with the cell wall and cytoskeleton of various cell types during early stage SE, embryo transdifferentiation, maturation, and germination (Šamaj et al. 2006). There is clear interconnection among the cytoskeleton, plasma membrane, and cell wall which appears to play a key role during cellular expansion and division (Šamaj et al. 2006; Thomas and Staiger 2014). In fact, microscopy and immunocytochemistry data have strongly suggested differences between embryogenic and non-embryogenic cultures in the methyl-esterification of pectic epitopes in the cell wall (Sala et al. 2013). However, the characterization of the cell wall and membrane proteome during SE is still needed in a broad range of plant species. In addition, embryogenic cells differ from non-embryogenic cells in several other aspects including subcellular compartments and the ultrastructure of organelles (Šamaj et al. 2006). However, massive proteomic characterization of subcellular compartments such as nucleus, mitochondria, chloroplast, and extracellular space (culture media) has not yet been carried out. The next frontier in proteomics studies in plants systems will be the simultaneous characterization of multiple PTM, the crosstalk between PTM, and massive profiling of protein–protein interaction in single cells and specific tissues. Proteomics studies in SE are still in their infancy.

Nevertheless, with the increasing number of new and accessible proteomic technologies, the application of proteomics tool to SE is easier than ever before.

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