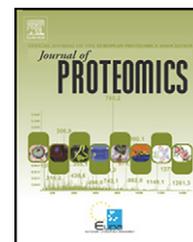


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## Review

# Differential proteomics of plant development

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### ABSTRACT

In this mini-review, recent advances in plant developmental proteomics are summarized. The growing interest in plant proteomics continually produces large numbers of developmental studies on plant cell division, elongation, differentiation, and formation of various organs. The brief overview of changes in proteome profiles emphasizes the participation of stress-related proteins in all developmental processes, which substantially changes the view on functional classification of these proteins. Next, it is noteworthy that proteomics helped to recognize some metabolic and housekeeping proteins as important signaling inducers of developmental pathways. Further, cell division and elongation are dependent on proteins involved in membrane trafficking and cytoskeleton dynamics. These protein groups are less prevalently represented in studies concerning cell differentiation and organ formation, which do not target primarily cell division. The synthesis of new proteins, generally observed during developmental processes, is followed by active protein folding. In this respect, disulfide isomerase was found to be commonly up-regulated during several developmental processes. The future progress in plant proteomics requires new and/or complementary approaches including cell fractionation, specific chemical treatments, molecular cloning and subcellular localization of proteins combined with more sensitive methods for protein detection and identification.

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## 1. Introduction

Generally, proteomics copes with the analysis of proteins in organisms, organs, cell populations and subcellular compartments, all under diverse developmental and environmental conditions. Early plant developmental processes often occur as a consequence of protein expression change in one particular cell, e. g. during the embryogenesis and the developmental switch of stem cells. Therefore, the choice of a proteomic approach for studying developmental processes should be carefully considered, especially when being aware of constrained sensitivity of proteomics methods for the detection of low-abundant proteins. Nevertheless, the number of plant developmental studies using various proteomics approaches is steadily growing [1–3]. Considerable experimental effort was devoted to the proteomic investigation of hormonal pathways regulating plant development such as brassinosteroid signaling [4], auxin signaling [5], or cytokinin regulation [6,7]. In this mini-review, we aim to summarize the most recent achievements in developmental plant proteomics during the last five years. In addition, we spot light how proteomics contributes and can contribute to current knowledge about particular developmental processes.

## 2. Cell proliferation and elongation

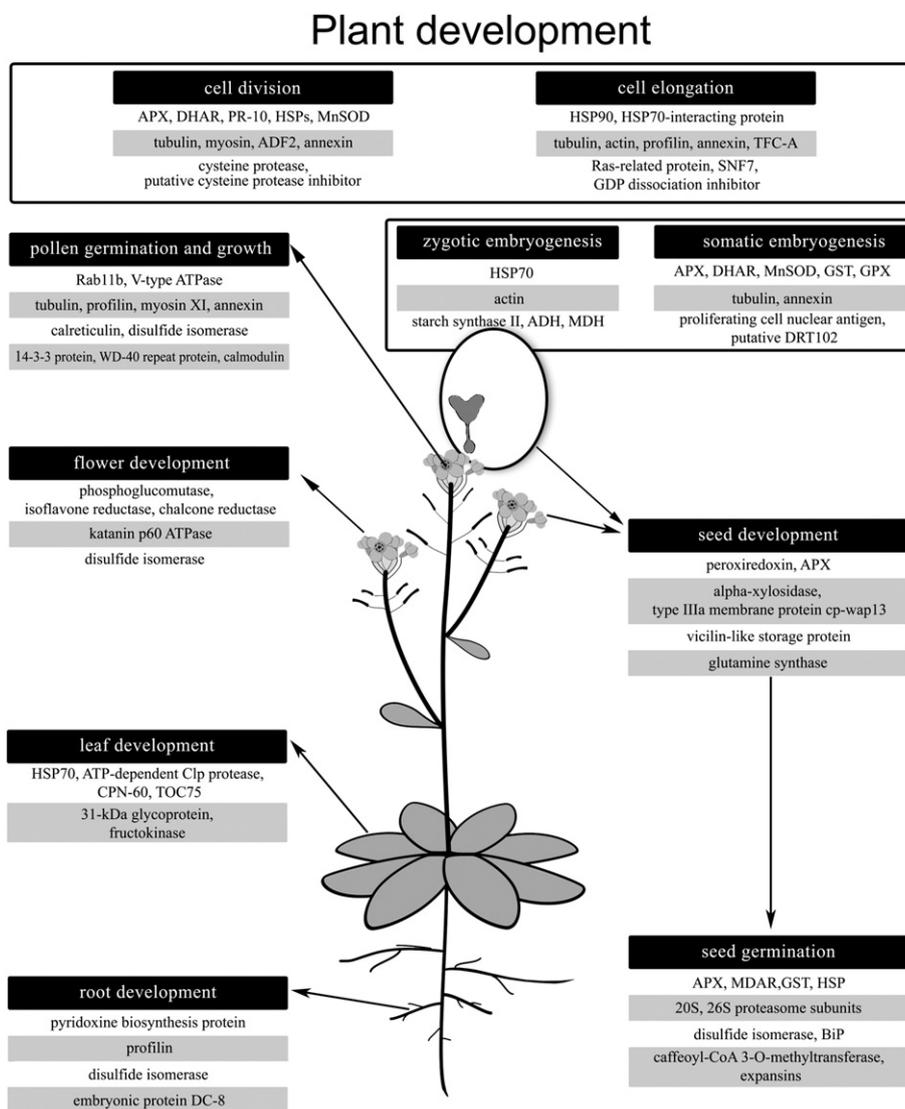
Cell division is a crucial process for growth and development of plants. It is regulated via hormonal, mainly auxin signaling and is accompanied by cytoskeleton rearrangement [8,9]. At the molecular level, plant cell cycle is controlled by an evolutionarily conserved mechanism involving cyclin-dependent kinase/cyclin complexes. Because of its complexity, the organization of a proteomic study to investigate the cell cycle has to be carefully thought out. The observed changes in crude extract protein expression can elucidate primarily the cell division-accompanying processes such as metabolism, energy housekeeping or the control of protein synthesis, due to limited abundance of the cell cycle regulating proteins. Stress-related proteins preferentially accumulate in *Medicago truncatula* dividing tissues, such as root meristem [10] and proliferating protoplasts [11]. In both cases, mainly pathogenesis-related proteins, such as PR-10 and heat shock proteins exhibited higher abundance in dividing tissues. Further, elevations of beta-glucosidase homolog 1 and annexin expression were found in *Arabidopsis* plants altered in transcription factor NTM (for NAC with trans-membrane motif 1) and exhibiting reduced cell division rate [12]. Differential regulation of annexins is also linked to other plant developmental processes including pollen germination, cotton fiber elongation and somatic embryogenesis, as reported in recent proteomic studies [13–16] and highlighted in Fig. 1.

It is important to note that reactive oxygen species (ROS) and ROS-related proteins are crucial components of redox signalling. These ROS originate in the photosynthesis, photorespiration/respiration or apoplastic redox state, and they together with ROS-induced proteins contribute to the regulation of plant development, especially under unfavourable conditions [17,18]. Additionally, experimental evidence suggests that ROS play roles also in the regulation of plant cell cycle [19–21]. Recent proteomic studies revealed that proteins induced after the oxidative stress, such as ascorbate peroxidase [10], dehydroascorbate reductase, glutathione transferase [12] and mitochondrial manganese superoxide dismutase [5] were also connected to higher cell division activity (Fig. 1). These findings confirm and enrich the data supporting a functional role of some ROS-related proteins in the cell division regulation.

During mitosis and cytokinesis, arrays of actin filaments and microtubules closely co-exist and play indispensable roles during formation of the pre-prophase band and the phragmoplast [22]. The increase in cell division activity was consistently accompanied by higher accumulation of actin binding proteins such as actin-depolymerizing factor 2 [10], as well as by up-regulation of myosin heavy chain protein ([5]; Fig. 1). The later serves as molecular motor capable of producing motive force along actin filaments indicating intensive vesicle movement during cell division as it was proposed by Dhonukshe et al. [23]. In addition to the mobilization of actin filaments, the higher abundance of alpha-tubulin in the root meristem of *M. truncatula* [10] as compared to non-meristematic tissue, likely contributes to the well-known involvement of microtubules in cell division including cell plate formation [24]. Furthermore, cell division is accompanied with high energy flux, as demonstrated by up-regulation of energy production-related 76 kDa mitochondrial complex I subunit [5]. Other authors [11] reported down-regulation of proteins involved in energy metabolism in dividing protoplast culture, which can be related to the exogenous supply of sucrose to culture media. New synthesis of proteins is often reported in association with cell division as well [5,11]. When the extracellular proteome of rice (*Oryza sativa*) suspension cell culture was analyzed [25], distinct secreted proteins were more abundant in cells grown for 6 days, in comparison to those grown for 3 days. This suggests changes in secretion dynamics during the cell division followed by cell expansion. Notably, putative cysteine protease inhibitor may directly regulate cell growth by inhibiting extracellular cysteine protease activity ([25]; Fig. 1).

Unfortunately, to our best knowledge, there is no study about subcellular proteome fractions during cell division. Such a study could contribute substantially to the better understanding of cell division regulation.

Similarly to cell division, cell elongation in plants is controlled by environmental cues and plant growth regulators,



**Fig. 1 – Plant model highlighting the most representative proteins important for various developmental processes as identified in proteomics experiments. APX = ascorbate peroxidase, DHAR = dehydroascorbate reductase, PR-10 = pathogenesis-related protein 10, MnSOD = manganese superoxide dismutase, HSP = heat shock protein, ADF2 = actin depolymerisation factor 2, TFC-A = tubulin forming cofactor A, SNF7 = protein found in sucrose non-fermenting mutant, ADH = alcohol dehydrogenase, MDH = malate dehydrogenase, GST = glutathione transferase, GPX = glutathione peroxidase, MDAR = monodehydroascorbate reductase, CPN-60 = chaperonin 60.**

such as brassinosteroids, auxins and cytokinins. However, from the physiological point of view, elongation represents a separate process, mainly depending on the loosening and synthesis of the cell wall. To investigate the cell growth and expansion/elongation, cotton (*Gossypium hirsutum*) fiber and pollen tube experimental model systems are used prevalently [14,26–28].

The cell expansion is determined mostly by vigorous actin and microtubule cytoskeleton dynamics [29,30]. In fact, this is reflected also in proteomic studies (Fig. 1). For example, two independent comparative studies showed down-regulation of alpha-tubulin, beta-tubulin, tubulin forming cofactor-A and profilin in mutant cotton fibers with inhibited elongation [14,26]. Similar results showing up-regulation of five actin and two beta-tubulin isoforms were obtained during fiber elongation

[27]. Notably, also five annexin isoforms, which could interact with cytoskeleton were involved in fiber elongation. Coincidentally, vesicular trafficking regulatory proteins such as vacuolar-sorting protein SNF7, a  $\gamma$ -soluble NSF-attachment protein and a coatomer-like protein were suppressed in mutant cotton fibers [14].

During pollen tube growth, small Ras-related protein and its negative regulator, GDP dissociation inhibitor, contribute to the regulation of vesicular trafficking [27]. In addition, cell elongation requires proper protein folding as was found for both cotton fiber elongation [14] and pollen tube growth [31]. Among the proteins involved in metabolism, nucleotide sugar metabolism was activated most significantly during elongation of cotton fiber cells. Further investigation revealed that this is mediated via ethylene production, and a similar

mechanism also participates in *Arabidopsis* root hair elongation [26,27]. The enhanced translational activity, as well as redox homeostasis maintenance was connected to fiber elongation [14,27]. These proteomics data show that cell elongation comprises wide range of pathways at the signaling, structural and metabolic level (Fig. 1).

Pollen tubes, unlike cotton fiber cells, possess several unique features, which make this system an excellent model to investigate polar tip growth, cell fate determination, cell differentiation, and cell-to-cell communication [32]. Therefore, pollen and pollen tube growth have been widely studied also by proteomic approaches (reviewed in [13]). More recent proteomic studies broadened the knowledge of pollen development via the comparison of mature and germinating pollen [31,33] including changes in membrane proteins during pollen grain germination and pollen tube growth [28]. Further, time-course examinations of *Picea meyeri* [34] and *Pinus bungeana* [35] pollen tube proteomes revealed perturbations in calcium homeostasis and signaling after treatments with chemical inhibitors.

At the cellular level, tip growth of pollen tube is achieved through polar- and cell domain-specific trafficking of secretory and endocytotic vesicles [36]. Thus Rab11b in pollen tubes regulates vesicular trafficking within the clear zone (free of bigger organelles), and defines the *trans*-Golgi compartment, which is essential for polarized secretion and endocytosis [32]. Valuable contribution to the elucidation of pollen tube polar growth was achieved by using high throughput proteomic approach on *Lilium longiflorum* pollen grain membrane proteins, fractionated by the sucrose density gradient method [28]. Remarkably, the expression levels of proteins involved in membrane/protein trafficking (Rab 11b GTPase, V-type ATPase and the H<sup>+</sup> pyrophosphatase) raised simultaneously with proteins involved in signal transduction, stress response, protein biosynthesis and folding, during the germination of pollen grains (Fig. 1). In contrast, proteins involved in cytoskeleton, carbohydrate, energy metabolism and transport of ions were up-regulated earlier, when the pollen just started to germinate [28]. Furthermore, Ca<sup>2+</sup>-CAM signaling substantially determines the polarized growth of *P. meyeri* pollen tube as proposed in a proteomics study which employed chemical inhibitor trifluoperazine [34]. Signaling proteins and proteins involved in organelle functions and energy production were identified as primary responders, followed by proteins related to cytoskeletal organization, secretory pathways, and polysaccharide synthesis.

Cell wall biogenesis and remodeling (loosening and extensibility) are primary factors that regulate cell division and elongation. Accordingly, proteins involved in cell wall biosynthesis were preferentially accumulated in germinating pollen, compared to mature pollen [31,33]. In addition to well-known proteins participating in cell wall remodeling (such as expansins, beta-glucosidase and reversibly glycosylated polypeptide), the pectin methylesterase inhibitor, which is involved in regulation of pectin synthesis, was identified in the above studies.

Despite technical limitations of the cell wall protein extraction, cell wall proteomics belongs to dynamically developing research areas. This research area was comprehensively reviewed by Jamet et al. [37]. More recently, large

number of studies investigating changes in the cell wall protein expression under various stress conditions, such as dehydration [38], flooding [39], pathogenesis [40], salt [41], and nutritional phosphate deficiency [42] appeared. In regard to improving methodology for cell wall and membrane protein extraction, utilization of differential detergent fractionation was reported while profiling the maize (*Zea mays*) rachis proteome [43]. Unfortunately, only a limited number of proteomic studies actually examined the cell wall proteome during developmental processes. For example, the cell wall proteome was investigated in half- and fully-grown etiolated *Arabidopsis* hypocotyls [44]. A new extraction approach based on high performance cation exchange chromatography resulted in detection of 51 proteins previously not identified by other cell wall proteomic approaches. Furthermore, the occurrence of cell wall proteins known to be related to cell wall extension after growth arrest, such as expansins, peroxidases, xyloglucan endotransglucosylase-hydrolases, polygalacturonase and pectin methylesterase, indicated that these proteins may have also other functions in mature cell walls [44]. Just as in case of primary cell wall, different experimental settings were exploited also to investigate the secondary cell wall formation. The unique system of *Populus tremula x alba* xylem isolation provided various stages of cell types during secondary xylem development. In this case, use of multidimensional protein identification technology (MudPit) together with sample fractionation into crude, nuclear and pellet fractions allowed identification of proteins mainly associated with nuclear structure and processes, such as DNA replication, recombination and repair [45]. Thus, a CtBP-like transcription factor was implicated to be likely involved in the control of the MER15 gene, a xyloglucan endotransglycosylase required for cell wall remodeling.

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### 3. Cell differentiation

Basically, all developmental processes are controlled via hormonal regulation. In plant tissue cultures, the endogenous level of hormones is artificially modified to specifically direct cell fate. The use of proteomics in tissue culture systems could help to detect proteins which are regulated by hormones. For instance, different relative concentrations of cytokinin and auxin were used for seed-derived callus differentiation in rice [46]. Roots, which formed from calli on different regeneration media, showed different pattern of epidermal cells. This was reflected also by protein profile of the calli, showing differences mainly in carbohydrate, energy/metabolism and stress/defense-related proteins [46]. Interestingly, these protein groups were also activated in *Vanilla planifolia* calli directed for shoot organogenesis [47]. In addition to cell differentiation, it is possible to reprogram differentiated cells to retain the competency of cell division and organ regeneration by using particular external hormone composition. Kinetin and 2,4-dichlorophenoxyacetic acid induced a dedifferentiation of *Arabidopsis* cotyledon cells, was accompanied by protein phosphorylation [48]. This hormonal treatment induced also protein synthesis, changes in the chromatin structure, cytoskeleton reorganization, and prevalent down-regulation of chloroplast proteins [49].

In addition to the organogenesis, the somatic and gametic embryogenesis serve as convenient systems for plant developmental studies under controlled *in vitro* conditions. For example, somatic embryogenesis in maize proceeds through well-defined cellular structures and it follows distinct growth patterns [50,51]. During early embryogenic stages, both induction of cell division and suppression of cell growth are necessary. On the other hand, polarized cell growth is required for advanced embryogenic stages. During the last five years, various proteomic approaches were applied to study somatic embryogenesis of diverse plant species such as cassava (*Manihot esculenta*, [52,53]), oak (*Quercus suber*, [15]), valencia sweet orange (*Citrus sinensis*, [54]), grape wine (*Vitis vinifera*, [55]), cyclamen (*Cyclamen persicum*, [56–58]), cowpea (*Vigna unguiculata*, [59]), date palm (*Phoenix dactylifera*, [60]) and feijoa (*Acca sellowiana*, [61]). These reports included studies on protein expression changes during somatic embryogenesis and comparative studies between embryogenic and non-embryogenic calli as well as between gametic and somatic embryogenesis. Because stress might force cells to switch into an embryogenic state, current proteomic studies put strong emphasis on the role of stress proteins in somatic embryogenesis ([15,54,55]; Fig. 1). Among these, the proteins involved in oxidative stress such as ascorbate peroxidase and manganese superoxide dismutase in grape wine [55,62] as well as glutathione S-transferase, phospholipid hydroperoxide and glutathione peroxidase in valencia sweet orange [54] were shown to be differentially regulated. In addition, ascorbate metabolism was shown to be involved in microspore-derived embryo development [63].

Somatic cells must regain their cell division activity in order to develop into somatic embryos. Therefore, the division associated proteins, such as proliferating cell nuclear antigen in grape wine [55] and putative citrus DRT102 in valencia sweet orange [54] are activated during embryogenesis. Moreover, cytoskeletal proteins, such as cell division associated tubulins are also differentially regulated [52–54]. Somatic embryogenesis also requires strong induction of protein biosynthesis [53]. The dynamic protein turnover during somatic embryogenesis of cyclamen is indicated by the up-regulation of putative 26S proteasome regulatory particle triple-A ATPase subunit 5a [58].

Along with characterization of processes associated with somatic embryogenesis, differential time-course proteomic studies could detect protein markers during distinct embryonic developmental stages. The hypothetical protein similar to L-isoaspartyl-O-methyltransferase in torpedo stage, and an osmotin-like protein in the pre-cotyledonar stage of somatic embryos were suggested as embryonic markers for *A. sellowiana* [61]. Moreover, several protein markers of microspore gametic embryogenesis were established, such as glutathione S-transferases, BNM2 (gene expressed in *Brassica napus* microspore-derived embryos), and lipid transfer proteins [63].

In addition to somatic embryogenesis, the proteomic analysis was also performed on zygotic embryos of coffee (*Coffea arabica*, [64,65]), and date palm [66]. The time-course studies of zygotic embryo development showed significant requirements for energy production, mainly during the early stages [64,66]. Differential expression of alcohol dehydroge-

nase indicates that energy production is followed by the fermentation of pyruvate into ethanol in date palm, since zygotic embryos are developing in an environment with low oxygen supply [66]. Early zygotic embryos showed also changes in the abundance of proteins involved in mRNA splicing, signaling [64] and starch synthesis [66]. The advanced stages of coffee zygotic embryogenesis were accompanied by differential expression of Rubisco, Myb transcription factor, and by changed biosynthetic activity of phosphatidylcholine [64].

Comparison of somatic and zygotic embryos revealed that their proteomes reflected mainly the different environmental conditions, which resulted in differential expression of proteins involved in metabolic pathways and stress response [56]. Another comparative proteomic study conducted on somatic and gametic embryos of *Q. suber* showed preferential up-regulation of actin and protein synthesis-related proteins in gametic embryos [15]. Despite that stress induction was used for both gametic and somatic embryo formation, stress-related proteins such as superoxide dismutase, peroxidase, vacuole-associated annexin VCaB42 and Ypr-10 were up-regulated preferentially in somatic embryos. On the other hand, proteins of the phenylpropanoid and flavonoid pathways involved in lignification and cork synthesis were up-regulated in both gametic and somatic embryos, indicating an intensive secondary cell wall differentiation.

## 4. Developmental processes at organ level

### 4.1. Seed development

Seed development studies highlighted an active oxidative stress metabolism (ascorbate peroxidase as well as peroxidoxin) in early seed development. Higher abundance of enzymes involved in cell wall expansion (alpha-xylosidase and type IIIa membrane protein cp-wap13) in Brazilian pine was observed as well [67]. In the later stages of seed development, storage proteins (e.g. vicilin-like storage protein), as well as proteins involved in respiration (triosephosphate isomerase, fructose-bisphosphate aldolase and isocitrate dehydrogenase) were accumulated. The up-regulation of glutamine synthase during the early-cotyledonary stage indicated active biosynthesis and conversion of glutamine to glutamic acid ([67]; Fig. 1). Another study on *Jatropha curcas* compared the endosperm and embryo proteomes of dry seeds [68]. Surprisingly, both tissues exhibit relatively similar protein expression profiles, indicating some similarities in metabolic pathways between them. However, embryos generally possess proteins mainly involved in anabolic processes, and accumulate stress-related proteins, implying increased embryo requirements for protection against stress.

### 4.2. Seed germination

Extensive effort was also dedicated to the proteomic investigation of seed germination. This led to better knowledge about molecular regulation of seed germination including post-translational control of seed germination via redox signaling

[69]. Recently, novel information was gained when the proteomes of individual seed parts, such as endosperm and embryo, were examined [70]. Abscisic acid (ABA) is a key regulator of seed germination, inhibiting the expression of germination-related genes. The comparison of the endosperm cap proteome of ABA-inhibited vs. non-inhibited germinating cress (*Lepidium sativum*) seeds showed specific, ABA-responsive, early germination processes, such as lipid mobilization, energy production, proteolysis, and increase in abundance of antioxidant enzymes [70]. These data suggested that the cress endosperm cap is not a storage tissue similar to cereal endosperm. Instead, it is a metabolically very active tissue regulating the rate of radicle protrusion. The investigation of changes in the proteome of rice embryo during germination [71] revealed that enzymes detoxifying reactive oxygen species, as well as protein degradation proteins and cytoskeleton-associated proteins play an important role during seed germination (Fig. 1).

Phosphorylation is a major post-translational modification of proteins by which cellular signals are transferred within a cell [72–75]. One of the last studies on protein phosphorylation during maize seed germination revealed 39 protein kinases, 16 phosphatases and 33 phosphoproteins containing 36 phosphorylation sites [76]. At least one-third of these phosphoproteins represented key components involved in biological processes related to the seed germination. Among them, there were proteins regulating DNA repair, gene transcription, RNA splicing and protein translation. Thus, these data suggest that protein phosphorylation plays an important role in seed germination.

Besides seed germination, some other studies focused on seed abortion, which is of great interest, mainly in important fruits like longan (*Dimocarpus longan*). One of the recent proteomic reports highlighted the role of cysteine protease along with oxidative stress proteins and chaperonins during seed abortion [77].

#### 4.3. Root, leaf and flower development

The mechanisms of maize lateral and seminal root formation were extensively studied by comparative proteomics approaches using the *rum1* (rootless with undetectable meristems 1, [78]), and *rtcs* (rootless concerning crown and seminal roots, [79]) maize mutants. The *rum1* mutant line is altered in both seminal and lateral root formation, while the *rtcs* line does not form seminal roots. The comparison of *rtcs* and wild type maize embryos showed differences in energy production. Moreover, disulfide isomerases involved in protein folding, as well as embryonic protein DC-8, generally seem to have a role in various pathways, that are essential for the formation of different root types [79]. In addition, the proteomics study on *rum1* transgenic line revealed that the proteins related to pyridoxine biosynthesis are involved in RUM1 dependent pathway of root formation [78].

Since the formation of lateral roots occurs in pericycle cell layer, pericycle and non-pericycle cells were harvested using laser-capture microdissection, and were subjected to a proteomic comparative study [80]. Metabolism, energy and defense-related proteins were found to be preferentially accumulated in pericycle cells vs. non-pericycle cells. Inter-

estingly, the comparison of this dataset with previous transcriptomic data revealed that the pericycle specification and lateral root formation are regulated via different molecular networks.

Developmental changes in root, stem and leaf proteomes were followed in rice during the first 10 weeks after budding [81]. Interestingly, this study showed that 19 proteins were present in all developmental stages in all tissues. Among them were metabolic proteins as well as oxidative stress-related proteins such as catalase isozyme A, superoxide dismutase ascorbate peroxidase and peroxiredoxin. Further, protein transport regulatory proteins, especially those involved in the transport of nuclear encoded chloroplastic protein into chloroplasts, were presumably involved in leaf development and maturation in soybean [82].

Proteome changes during bud development [83] were elucidated in *Pinus sylvestris* L. var. *mongolica* in order to study mechanisms of bud dormancy induction and release. Stress-induced ascorbate peroxidase, pathogenesis-related proteins and heat shock proteins were involved in bud dormancy induction. On the other hand, proteins involved in protein synthesis, cell wall biogenesis and cytoskeleton were up-regulated during dormancy release. Moreover, changes in metabolism indicated accumulation of carbohydrate and protein reserves during dormancy induction, as well as their mobilization when dormant bud turns to growth phase.

The comparison of flower and bud proteomes suggested that sucrose generation derived by up-regulated phosphoglucosyltransferase and down-regulated glycoprotein could serve as an inducer of flavonoid and anthocyanin-related genes important for petal growth and color development in mature flower. Active organelle transport mediated by katanin p60 ATPase, as well as protein assembly, is also important for flower development (Fig. 1). Interestingly, disulfide isomerase, shown to be involved in root formation [79,80], was also developmentally regulated in flowers [82]. This protein involved in root and flower development plays an important role also in other organs such as potato tubers [84].

#### 4.4. Other plant organs

Beside the roots, leaves and flowers, the proteomic approach was found to be powerful for the investigation of potato (*Solanum tuberosum*) tuber formation [84–86], tuber wound healing [87] and tuber ageing processes [88]. Changes in the proteome during tuber initiation and growth reflect mainly the processes connected to the accumulation of storage reserves and starch synthesis. Thus, storage proteins, protease inhibitors and proteins involved in secondary metabolism were up-regulated during tuber growth. Additionally, some isoforms of patatins, a large family of primary storage proteins, were shown to accumulate in non-swelling stolons, possibly indicating their involvement in tuber initiation [84,85].

A somewhat non-traditional tissue of interest, the corn rachis, was a subject of recent study by Pechanova et al. [89]. The rachis is a vital structure of the maize ear, delivering essential nutrients to the developing kernels. It also plays an important role in pathogenic fungal (*Aspergillus flavus*) proliferation, and subsequent kernel contamination by aflatoxin, a potent carcinogen. Proteomic investigations of rachis tissue

during maturation (25 vs. 50 days after silking) revealed significantly increased expression (2.4- to 14.5-fold) of many stress/defense-related proteins in mature rachis. They included PRm3 (class III chitinase), PR-1, PR-10, beta 1,3 glucanase, endo-1,3 beta glucanase, germin-like protein subfamily 1 member 17, permatin, and Asr protein (Fig. 2). Additionally, profilin, an actin binding protein which regulates actin polymerization [90], was also up-regulated during rachis development and maturation. Profilins are known to be involved in the development of seedlings (both hypocotyls and cotyledons), microspores and pollen, as well as in the cell elongation, polarized growth of root hairs and determination of flowering time in *Arabidopsis* [91–93]. Previous proteomic study revealed that an inhibition of pollen tube tip growth by latrunculin b (an inhibitor of actin polymerization) was well correlated with down-regulation of profilin [94]. Recently, profilin2 was identified by proteomic and cell biology approaches as a new cytoskeletal protein modulating vesicular trafficking in *Arabidopsis* roots [95].

## 5. Experimental proteomic approaches to study plant development

Proteomic studies listed in this review have used diverse comparative approaches for the investigation of plant development. These included the time-course analysis, the comparison of different organs, as well as the comparison of transgenic vs. non-transgenic tissues (summarized in Table 1).

The most limiting factor in performing reproducible differential proteomic analysis in plant developmental studies was reliable quantification of protein abundance. Moreover, to increase the proteome resolution substantially, other issues such as sample preparation and protein identification need also proper attention in proteomic workflow [96].

Despite of the labor intensive and time-consuming procedure of the conventional two-dimensional electrophoresis, it remains the most frequently used separation and quantification method in plant proteomic developmental studies (about 95% of studies listed in Table 1). In this gel-based approach, the protein stained on two-dimensional gels is identified by mass

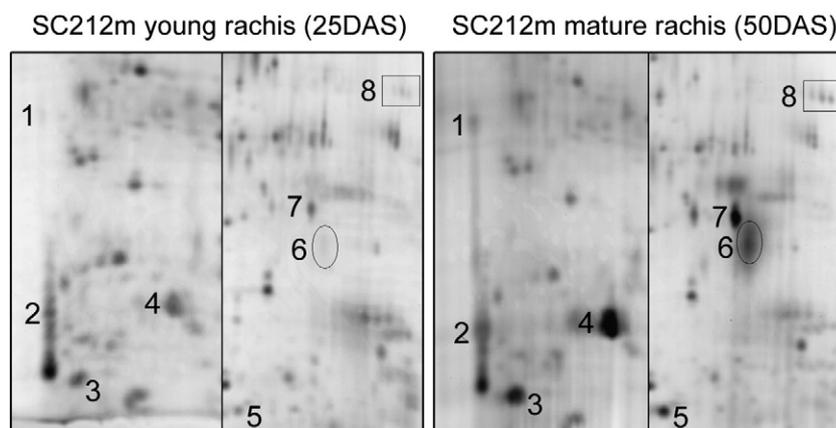
spectrometry and quantified on the base of normalized spot density by using specialized gel analysis software. Differential gel electrophoresis (DIGE) allows the simultaneous separation of two samples and one internal standard on one two-dimensional gel, by the pre-labeling of the samples with fluorescent cyanine dyes Cy2, Cy3 and Cy5 [97]. That results in overall better reproducibility and lower number of gels per experiment.

Perhaps due to higher labeling costs and need for specialized 2D gel imaging and analysis software, it was not widely used in differential proteomic studies in plant development (only about 10% of studies listed in Table 1).

In addition to gel-based, number of sophisticated shotgun gel-free proteomic approaches were also exploited in plant proteomic developmental studies [43,49,76] representing promising advances in the field of differential proteomics. Gel-free approach effectively overcomes some limitations of 2D electrophoresis (e.g. detection sensitivity, co-migration of different proteins in gel, limited detection of hydrophobic proteins, high costs) and enables true high throughput global proteome analysis of complex biological systems. In contrast to separation of proteins on a gel, the gel-free approach utilizes, an “in-solution” digestion of complex protein sample, followed by liquid chromatography and in- or off-line mass spectrometry [98]. Protein quantification is achieved either via stable isotope labeling, mass tags, or label-free methods. All of them possess specific advantages, which were comprehensively discussed in recent publications [99,100].

There are marketed proteomics software tools available to researchers for both qualitative and quantitative gel-free analysis, offered by major mass spectrometer manufacturers (e.g. Proteome Discoverer and SIEVE by THERMO, MassLynx by Waters, ProteinScape by Bruker, Mass Profiler by Agilent) or by third commercial parties (e.g. PEAKS by Bioinformatics Solutions, ProteoIQ by NuSEP/Bioinquire). Even in-house made, freely available quantification software appeared recently, for example, the unique sum-of -Xcorr-based method [101].

New advances in nano-scale separation techniques [102] could also help to reduce the required sample amount for identification, which might promote the analysis of



**Fig. 2 – Accumulation of stress/defense-related proteins during development and maturation of SC212m *Zea mays* L. rachis, 25 and 50 days after silking (DAS). 1. PRm3 (class III chitinase); 2. PR-1 (pathogen-related protein 1); 3. profilin; 4. PR-10 (pathogen-related protein 10); 5. Asr protein; 6. permatin; 7. germin-like protein subfamily 1 member 17; 8. endo-1,3 beta glucanase.**

**Table 1 – The overview of subjects, plant samples and proteomic techniques/methods used in plant developmental studies.**

Reference	Subject	Sample	Extraction	Separation	Identification
[5]	Auxin signaling	Auxin vs. inhibitor treated sample ( <i>Oryza sativa</i> )	Precipitation	2D DIGE	EDMAN sequencing ESI-Q-TOF MS/MS MALDI-TOF MS
[6]	Cytokinin signaling	Mutant vs. vector transfected sample ( <i>Agrostis stolonifera</i> )	Precipitation	2DE	MALDI-TOF/TOF MS/MS
[7]	Cytokinin signaling	Transformed vs. wild type sample ( <i>Arabidopsis thaliana</i> )	Precipitation	2DE	MALDI-MS; LC-MS/MS
[10]	Cell proliferation	Meristematic vs. non-meristematic tissues ( <i>Medicago truncatula</i> )	Precipitation	2DE	MALDI-TOF/TOF MS/MS LC-MS/MS
[11]	Cell proliferation	Time-course study ( <i>Medicago truncatula</i> )	Precipitation	2DE	MALDI-TOF MS MALDI-TOF/TOF MS/MS
[12]	Cell proliferation	Transformed vs. wild type sample ( <i>Arabidopsis thaliana</i> )	Precipitation	2DE	MALDI-TOF/TOF MS
[25]	cell proliferation	Time-course study ( <i>Oryza sativa</i> )	Precipitation	2DE	MALDI-TOF/TOF MS/MS LC-MS/MS
[26]	Cell elongation	Transformed vs. wild type sample ( <i>Gossypium hirsutum</i> , <i>Arabidopsis thaliana</i> )	Precipitation	2DE	MALDI-TOF/TOF MS/MS
[27]	Cell elongation	Time-course study ( <i>Gossypium hirsutum</i> )	Phenol extraction	2DE	MALDI-TOF/TOF MS/MS
[14]	Cell elongation	Transformed vs. wild type sample ( <i>Gossypium hirsutum</i> )	Precipitation	2DE	MALDI-TOF/TOF MS/MS
[28]	Cell elongation	Time-course study ( <i>Lilium longiflorum</i> )	Sucrose gradient-separated microsomal fractions	1DE	LC-MS/MS of chopped 1D gel slices
[44]	Cell elongation	Elongating vs. non-elongating hypocotyl ( <i>Arabidopsis thaliana</i> )	Cell wall	1DE <sup>a</sup>	MALDI-TOF/TOF MS
[31]	Pollen germination	Mature vs. germinating pollen ( <i>Brassica napus</i> )	Precipitation	2D DIGE, 2DE	MALDI-TOF/TOF MS/MS
[33]	Pollen germination	Mature vs. germinating pollen ( <i>Arabidopsis thaliana</i> )	Precipitation	2DE	MALDI-TOF MS
[34]	Pollen tube growth	Control vs. drug-treated sample ( <i>Picea meyeri</i> )	Precipitation	2DE	ESI-Q TOF MS/MS
[35]	Pollen tube growth	Control vs. drug-treated sample, time-course study ( <i>Pinus bungeana</i> )	Precipitation	2DE	ESI-Q TOF MS/MS
[46]	Cell differentiation	Control vs. phytohormon-treated sample ( <i>Oryza sativa</i> )	Precipitation	2DE	MALDI-TOF/TOF MS/MS
[47]	Cell differentiation	Control vs. phytohormon-treated sample ( <i>Vanilla planifolia</i> )	Extraction in lysis buffer	2DE	MALDI-TOF/TOF MS/MS
[48]	Cell dedifferentiation	Phospho/proteomics on time-course study ( <i>Arabidopsis thaliana</i> )	Phenol extraction	2DE	MALDI-TOF/TOF MS/MS
[49]	Cell dedifferentiation	Time-course study ( <i>Arabidopsis thaliana</i> )	Phenol extraction	Gel free	LC-MS/MS
[53]	Somatic embryogenesis	The comparison of different organs ( <i>Manihot esculenta</i> Crantz)	Phenol extraction	1DE	LC-MS/MS
[54]	Somatic embryogenesis	Time-course study ( <i>Citrus sinensis</i> )	Precipitation	2DE	MALDI-TOF/TOF MS/MS
[55]	Somatic embryogenesis	Embryonic vs. non-embryonic calli ( <i>Vitis vinifera</i> )	Phenol extraction	2DE	LC-MS/MS
[15]	Somatic and zygotic embryogenesis	Somatic vs. zygotic embryos ( <i>Quercus suber</i> )	Extraction in lysis buffer; phenol extraction; precipitation	DIGE	MALDI-TOF/TOF MS/MS
[66]	Somatic and zygotic embryogenesis	Somatic vs zygotic embryos ( <i>Phoenix dactylifera</i> )	TCA-acetone-phenol	2DE	MALDI-TOF/TOF MS/MS
[66]	Somatic and zygotic embryogenesis	Somatic vs zygotic embryos ( <i>Phoenix dactylifera</i> )	TCA-acetone-phenol	2DE	MALDI-TOF/TOF MS/MS
[56]	Somatic and zygotic embryogenesis	The comparison of different organs ( <i>Vitis vinifera</i> )	Extraction in Lysis buffer	2DE	LC-MS/MS
[57]	Somatic embryogenesis	Embryonic vs. non-embryonic calli ( <i>Cyclamen persicum</i> )	Precipitation	2DE; DIGE	MALDI-TOF/TOF MS/MS
[58]	Somatic embryogenesis	Time-course study on zygotic and somatic embryos ( <i>Cyclamen persicum</i> )	Precipitation	2DE	MALDI-TOF/TOF MS/MS

Table 1 (continued)

Reference	Subject	Sample	Extraction	Separation	Identification
[61]	Somatic embryogenesis	Time-course study ( <i>Acca sellowiana</i> )	Phenol extraction	2DE	MALDI-TOF/MS
[62]	Somatic embryogenesis	Embryonic vs. non-embryonic calli ( <i>Vitis vinifera</i> )	Precipitation	2DE	MALDI-TOF MS
[66]	Somatic and zygotic embryogenesis	Somatic vs zygotic embryos ( <i>Phoenix dactylifera</i> )	TCA–acetone–phenol	2DE	MALDI-TOF/TOF MS/MS
[63]	Microspore-derived embryogenesis	Time-course study ( <i>Brassica napus</i> )	Precipitation	2DE	Q-TOF MS/MS
[60]	Zygotic embryogenesis	Time-course study ( <i>Phoenix dactylifera</i> )	TCA–acetone–phenol	2DE	MALDI-TOF/TOF MS/MS
[63]	Zygotic embryogenesis	Time-course study ( <i>Coffea arabica</i> )	Phenol extraction	2DE	MALDI-TOF/TOF MS/MS
[65]	Zygotic embryogenesis	The comparison of different organs ( <i>Coffea arabica</i> )	Phenol extraction	2DE	MALDI-TOF/TOF MS/MS
[67]	Zygotic embryogenesis	Time-course study ( <i>Araucaria angustifolia</i> )	Precipitation	2DE	LC–MS/MS
[79]	Zygotic embryogenesis	Transformed vs. wild type sample ( <i>Zea mais</i> )	Precipitation	2DE	LC–MS/MS
[68]	Zygotic embryogenesis	The comparison of different organs ( <i>Jatropha curcas</i> )	Precipitation	2DE	MALDI-TOF LC–MS/MS
[70]	Seed germination	Time-course study ( <i>Lepidium sativum</i> )	Extraction in lysis buffer	2DE	LC–MS/MS
[71]	Seed germination	Time-course study ( <i>Oryza sativa</i> )	Phenol extraction	2DE	MALDI-TOF-MS
[76]	Seed germination	Time-course study; phosphoproteome ( <i>Zea mais</i> )	Precipitation	Gel free	LC–MS/MS
[77]	Seed abortion	Normal vs. aborted seeds, time-course study ( <i>Dimocarpus longan</i> )	Phenol extraction	2DE	MALDI-TOF/TOF MS/MS
[78]	Root development	The comparison of different tissues ( <i>Zea mais</i> )	Precipitation	2DE	LC–MS/MS
[80]	Root development	Pericycle cells ( <i>Zea mais</i> )	Extraction in lysis buffer	2DE	LC–MS/MS
[81]	Leaf, root and stem proteome	The comparison of different organs ( <i>Oryza sativa</i> )	Precipitation	2DE	MALDI-TOF MS LC–MS/MS
[82]	Leaf and flower development	Time-course study; comparison of different organs ( <i>Glycine max</i> )	TCA; phenol extraction; different solubilization buffers	2DE	MALDI-TOF MS
[83]	Bud dormancy	Time-course study ( <i>Pinus sylvestris</i> )	Precipitation	2DE	LC–MS/MS
[84]	Potato tuber development	Time-course study ( <i>Solanum tuberosum</i> )	Extraction in lysis buffer	2DE	LC–MS/MS
[85]	Potato tuber development	Time-course study ( <i>Solanum tuberosum</i> )	Precipitation	2De	LC–MS/MS
[86]	Potato tuber development	Transformed vs. wild type sample ( <i>Solanum tuberosum</i> )	Extraction in lysis buffer	2DE	MALDI-TOF MS
[87]	Potato tuber development	( <i>Solanum tuberosum</i> )	Precipitation	2DE	MALDI-TOF/TOF MS/MS
[88]	Potato tuber aging	Time-course study ( <i>Solanum tuberosum</i> )	Extraction in lysis buffer	2D DIGE	MALDI-TOF/TOF MS/MS
[43]	Maize rachis development	Rachis, developmental stages ( <i>Zea mais</i> )	Phenol extraction	2DE and gel free	LC–MS/MS
[89]	Maize rachis development	Control vs. treated sample ( <i>Zea mais</i> )	Phenol extraction	2D DIGE	MALDI-TOF/TOF MS/MS

<sup>a</sup> Semi-quantification was applied on the base of coverage and the ratio between detected AA in the peptide and all AA in the band.

specialized plant tissues and cells. Last but not least, laser-capture microdissection enables the analysis of cell-type-specific protein profiles and it can contribute to more specific dissection of plant development, as it was recently shown by Dembinski et al. [80].

## 6. Conclusion

Plant proteomics is very dynamic research area. At the present, the proteomics approaches are complemented with

molecular cell biology, genetic and biochemical methods, which bring novel systemic and functional information. This is also true for studies with developmental context. With advances in mass spectrometry instrumentation and in sample preparation (robotic sample prep systems, cell fractionation methods, chemical drug treatments, etc.), there is no doubt that the near future will bring both more complex and focused studies gaining deeper knowledge about particular pathways and processes operating in individual organs, tissues, cell types and subcellular compartments during plant development.

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