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Comparative Analyses of Extracellular Matrix Proteome: An Under-Explored Area in Plant Research

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1. Introduction

Within their social milieu, cells are petite and deformable, enclosed in a flimsy plasma membrane which swerves from their default spherical shape to more polar shapes due to the local deposition, complex interactions and the remodelling of the extracellular matrix (ECM). Consequently, multicellularity has evolved, albeit independently in plants and animals. Although animals are truly multicellular, plants are supracellular organisms because their immobile cells divide via phragmoplast-based incomplete cytokinesis, which results in the formation of cytoplasmic cell-to-cell channels known as plasmodesmata (Baluska et al., 2003). The ECM in plants, often referred as the cell wall, is integrated into the apoplast—a structurally coherent superstructure extending throughout the plant body. In lieu, plant cells are not fully separated and both the plasma membrane and endoplasmic reticulum traverse cellular borders through plasmodesmata (Baluska et al., 2003; Fincher, G. 2009.). The ECM is a fundamental component of the microenvironment of both animal and plant cells that has been substantially expanded during evolution. Throughout the plant kingdom, the formation and regulation of the ECM architecture has been shown to have the potential to influence many conduits of development, position-dependent differentiation, patterning and totipotent cell niches, besides environmental stress response and pathobiology (Brownlee & Berger, 1995; Degenhardt & Gimmer, 2000; Wilson, 2010). Furthermore, it has been reported that the ECM plays an important morphoregulatory role during somatic embryogenesis and organogenesis in plants, besides its pivotal role in cellular osmo- and volume-regulation (Šamaj et al., 1999; Rose et al., 2004). The plant ECM has biomechanical and morphogenetic functions with the immense ability to turn cells into hydraulic machines which establish a crucial functional difference between cell walls and other cellular surface structures. It encloses the cell hermetically and constrains the hydrostatic pressure evoked by osmotic gradients between the cell and its environment which controls cellular osmo- and volume-regulation (Peters et al., 2000; Cosgrove, D. J. 2005). Plasticity in the ECM allows the cellular uptake of massive amounts of water into

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a central vacuole while rigidity in the ECM determines the conductance of enormous amounts of water and dissolved solutes through vascular bundles. The secretion of an ECM by one cell can also influence the neighbouring cells, conceivably the best exemplified paracrine interaction known in the plant kingdom (for a review, see Brownlee, 2002). Beyond their paramount importance in the generation of form, cell walls are frequently considered 'growth-controlling' (Wolf et al., 2009). Cells devoid of the ECM inevitably lose their polar shape and the loss of cellular polarisation prevents cell-to-cell interactions and communication. The ECM/cell wall is evolutionary and inherently bestowed with information that can be both stored and relayed to cell interior via templating processes. It serves as the first line mediator in cell signalling for perceiving and transmitting extra- and intercellular signals in many cellular pathways. Communication between the cytoplasm and the cell wall is necessary and evident because of events such as cell expansion (Cosgrove, 1997, Schröder, F et al 2009), mechanical stress (Kumar et al., 2006; Telewski, 2006), environmental perturbation (Gail McLean et al., 1997; Thelen, J. and Peck, S. 2007) and pathogen infection (Hammond-Kosack & Jones, 1996) which lead to altered biosynthesis and the modification of wall components and downstream cytoplasmic events. In addition, it can act as a substrate for migration and has also been recognised as a surrogate for providing inputs into cell behaviour (Hall et al., 2002), although the available data is rather scarce for higher plants and critical linker molecules between the cytoskeleton and the ECM are still missing. Thus, the ECM/cell wall primarily serves a dual function, as a cell support system and for signalling during development and stress. The ECM/cell wall must therefore be dynamic as cells divide and elongate, modulating its composition and architecture during its synthesis and after it has been deposited. The wall function is a multi-step, complex process and the underlying mechanisms governing these steps are not fully understood.

Proteome research holds promise of understanding the molecular basis of the ECM function using an unbiased comparative and differential approach. We and others have identified several hundred plant proteins that include both predicted and non-canonical ECM components, presumably associated with a variety of cellular functions; viz. cell wall modification, signal transduction, cellular transport, metabolism, cell defence and rescue, all of which impinge on the complexity of ECM proteins in crop plants (Bhushan et al., 2006; Telewski, 2006). In recent years, reports have also been published focusing on changes in the ECM proteome in varied cellular events (Jones et al., 2004; Irshad et al., 2008, Bhushan et al., 2007, Cheng et al., 2009; Pandey, et al., 2010, Bhushan et al., 2011). The proteins that have been identified reveal the presence of complex regulatory networks that function in this organelle. Currently, we are focusing on disease-responsive ECM proteomes in order to understand the ECM-related pathobiology in plants. Although over the past few years there have been rapid advances in cell wall proteome research, the study of the complexity of ECM proteins remained secondary, irrespective of the fact that they correspond to about 10% of the ECM's mass and are comprised of several hundred different molecules with diverse functions. Moreover, a vast array of post-translational modifications to these proteins adds diversity to the structure and ligand-binding properties of matrix components, leading to their differential activity. Therefore, characterisations of the ECM proteome in plants hold the promise of increasing our understanding about the gene's function.

In this report, we begin by summarising the essential and unique features of the ECM and we discuss recent findings concerning the regulation and biochemistry of it, with specific emphasis on the fundamental role of ECM proteins in development, environmental stress and signalling by analysing the ECM's proteomes. Furthermore, we report here the comparative analysis of ECM proteomes towards crop specificity, organ-based, developmental and environmental adaptations based on our own findings, the available literature and databases focusing on ECM proteins in view of the current understanding and perspectives of the ECM's functions.

1.1 Exploring the sink and link in ECM

Ubiquitously present, the ECM/cell wall is composed of different molecules with diverse functions to meet the specialised requirements of different tissues. It is a dynamic milieu, having homeostatic properties and a reservoir for bioactive molecules, such as carbohydrates and proteins. Long before the determination of comprehensive chemical differences between plant and animal ECMs, Boerhaave proposed in the early 18th century that fermented plant material which is rich in carbohydrate is acidic whereas putrefied animal material which is rich in protein is basic (ammoniacal) (Rose, 2003). It alludes only briefly to the differences and similarities between the ECMs of higher plants and animals. Consequently, proteins are largely responsible for the chemical transformation properties that distinguish plants from animals. The ECM in higher plants and higher animals consists of a mixture of fibrous and amorphous components. In higher animals, a protein-based collagen - elastin, a fibronectin fibre matrix is infiltrated by mucopolysaccharides, peptidoglycans and calcium phosphate, whereas in higher plants, non-protein cellulose and protein-based extensin fibre matrices are infiltrated by a varied assortment of non-nitrogenous hemicelluloses, pectins and lignins and, to a much smaller extent (on a mass basis), by various structural proteins and enzymes (Irshad et al., 2008). Similarities in ECM design may be apparent as it is likely that ancient functional protein domains and carbohydrate backbones have been used in a variety of arrangements and combinations to affect the function of convergent biological structures. On the contrary, as stated by Darwin the "web of wall molecules have a long evolutionary history," and it is therefore relevant that different family members show highly regulated and specific patterns of the expression of ECM components in an evolutionary context. In addition to protein heterogeneity and the presence of various metals as linkers, carbohydrate compositions can vary between cell types and even within one wall of a given cell, suggesting that the cell wall serves as a sink of variability in terms of macromolecules or microelements. On a fresh mass basis, the vegetative growth of all organisms (70-90% water) is predominantly owing to water uptake, but on a dry mass basis the vegetative growth of plants differs markedly from that of animals (Rose, 2003). During differentiation, plant cells increase in size from typically 10^2 mm^3 (volume of a meristematic cell) to up to 10^7 mm^3 (e.g., a xylem vessel). This increase in cellular volume requires the addition of building materials in the form of cell wall polymers and membranes. While new cell wall materials are incorporated, the existing material is deformed and stretched mechanically. The force for this deformation is supplied by the turgor pressure (Geitmann, 2010). The ECM serves as the first line mediator in cell signalling to perceive and transmit extra- and intercellular signals in many cellular pathways. ECM proteins constitute more than just a structural framework but they also play a variety of roles in growth and development, defence against environmental stresses as well as giving structural support.

1.1.1 The ECM protein sink: A dynamic framework for multiple functions

Earlier it was believed that ECM proteins were large and complex, with multiple distinct domains, and were highly conserved among the different taxa (Hall & Cannon, 2002). However, it is not necessary that proteins be large or complex in order to generate strong, stable fibrils and intermediate filament proteins. The conserved domains are now known to be arranged in specific juxtapositions, sometimes controlled by highly regulated alternative splicing (Hynes, 2009), indicating thereby that the specific domains and architectures of ECM proteins contain information of biological importance and evolutionary value. In plants, abundant wall proteins include those rich in hydroxyproline or proline (HRGPs, PRPs), glycine (GRPs) and arabinogalactan (AGPs). Expansins, which relax the linkages of the wall during cell elongation, play a crucial role in development. Peroxidases, methyltransferases, galactosidases, glycanases and proteases have also been identified as the cell wall resident proteins having an N-terminal targeting sequence. Perhaps the protein most expected to be similar to their metazoan counterparts in the plant cell wall is aggrecan, which binds hyaluronan orthologs. At least three classes of hydroxyproline-rich glycoproteins exist in higher plants, namely extensins, arabinogalactans and solanaceae lectins (Hall & Cannon, 2002,). Extensins, which comprise 5-10% of wall proteins, are assumed to play a role in the structure of plant cell walls and may, therefore, be important in controlling growth. Increasing evidence suggests that the level of extension is developmentally regulated. It also accumulates upon wounding and pathogen attack, suggesting its involvement in plant defence (Cassab, 1998). The fact that extensins and collagens are hydroxyproline containing glycoproteins means that they may have common evolutionary precursors (Chen & Verner, 1985). In addition, the primary cell wall includes numerous enzymes, viz. endoglucanases, xyloglucan endotransglycosylases and a number of other glycosyl transferases that alter carbohydrate linkages and modify secreted cell wall components. Tetraspanin - one of the important classes of ECM protein in higher plants but absent from unicellular eukaryotes - is known as the secretory carrier membrane protein, important for synaptic vesicle recycling in stigma-pollen interaction. Other cell wall proteins, some of which are heavily glycosylated, have been proposed as structural cell wall components and have been implicated in mediating multiple aspects of plant development (Irshad et al., 2008). Germin is another ECM protein that signals the onset of growth and determines plant immunity. A chronic theme proverbial to the class of ECM-cytoskeleton linker proteins of plant cells is that these mechano-transducing transmembrane molecules communicate and interact preferentially with the actin cytoskeleton on the cytoplasmic side of the plasma membrane. Generally, the actin cytoskeleton has been optimised during eukaryotic evolution for acting as a structural scaffold for diverse signalling complexes (Baluska et al., 2003). Bruce Kohorn classified putative plant-specific linker molecules in four categories, focusing on the four most appealing candidates: cell wall-associated kinases (WAKs), arabinogalactan proteins (AGPs), pectins and cellulose synthases. Progress made during the last three years has resulted in additional candidates, including formins, plant-specific class VIII myosins, phospholipase D and callose synthases. Unexpectedly, formins represent a new candidate for a putative ECM-cytoskeleton linker in plant cells. Current bioinformatic analyses show that there is one plant-specific group of formins not only abundant in cell wall but also moonlighting in cytosol.

1.1.2 ECM proteins: Cross talk in signalling and stress

ECM senses and physiologically responds to environmental stress via signalling pathways. Signalling events are clearly not linear and induce many different reactions, including stress-related processes that crosstalk with hormone signalling pathways. It is known that cell wall stress provokes a transient depolarised distribution of the cell wall biosynthetic enzyme glucan synthase and its regulatory subunit RHO1, possibly as a mechanism to repair general damage to the wall. Both environmental and patho-stress are thought to cause wall weakening which in turn transduces a signal to the interior of the cell as a homeostatic mechanism to repair the wall. Various kinases mediate the stress-induced synthesis of ECM proteins to combat cell wall interfering factors, such as pathogens, osmotic stress, dehydration and other environmental stresses. Recently, it has been found that wall-associated kinase (*WAK)* expression was induced when *Arabidopsis* plants were infected with a pathogen or stimulated by exogenous SA or its analogue INA. *WAK1* mRNA induction requires the positive regulator NPR1/NIM1 (Cheng et al., 2009). It provides a direct link between a protein kinase that could mediate signals from the ECM to the events that are precipitated by pathogen infection. It also suggests that while pathogen infection induces protective hangs in cells, these changes can be detrimental if certain cellular components, such as WAK1, are not present in sufficient amounts (Jones et al., 2004). In osmotic, salinity and dehydration stress, the expansion ability of the cell wall decreases. Correlated with this weakening was a substantial decrease in the proportion of crystalline cellulose in the primary cell wall while the amount of insoluble proteins (such as HRGPs) associated with the wall was increased relative to other wall components (Sakurai et al., 1998).

2. Methodology and strategy

We have compared the ECM proteome of six plants, namely *Arabidopsis thaliana*, *Cicer arietinum Medicago sp*, *Oryza sativa*, *Zea mays* and *Brassica napus*. The *modus operandi* in investigating the cell wall proteomes of available crops was the extensive literature and availability of relevant databases (wallprotDB, Phosida, UniPro, ProtAnnDB pep2proandSwissprot) search. The CWPs identified in these works were classified into functional categories. This classification is only tentative, since the biological role of many of the proteins identified has not been established experimentally. Furthermore, we applied a cross-species comparison on the available datasets. When analysing proteomes within the specified group of plants, a logical strategy was used to maximise efficiency and the overall comparative results. Thus, it was imperative to first evaluate the available proteomes, followed by an analysis of organ-specific proteomes of the model plant *Arabidopsis*. Once the organ-specific differential proteomes of the model plant were analysed, we then tentatively evaluated the developmental proteomics of rice at various leaf stages so as to understand the acquisition of major pathways involved in the development of the cereal. We then moved on to assessing the stress-responsive plant proteomes in order to understand the overlap and specificity amongst different environmental and patho-stress. These comparative studies were customised for specific protein families. For example, when the environmental stress-responsive proteomes were compared, the parallel analysis of the proteomes of different clades of vascular plants were performed, viz. *Arabidopsis* vs. maize for osmotic stress, and chick pea vs. rice for dehydration. Similarly, in case of patho-stress, *Arabidopsis* and *Brassica* proteomes were compared. It is to be noted that protein consensus can be obtained across any combination of proteomes based on the type of extraction procedure.

2.1 Description of tools

An outline of the procedure and an illustration of the data that can be generated with the methodology are shown in Figure 1. Each proteomic study is described through a simplified flowchart showing its different steps, from plant material to protein identification. As illustrated in Figure 1, two types of methods can be used to prepare a CWP fraction. Non-destructive methods leave the cells alive and allow the elution of CWPs from cell walls using different buffered solutions, while destructive methods start with tissue grinding, thus mixing CWPs and intracellular proteins (Boudart et al., 2005; Bayers et al., 2006). The CWP fraction needs to be fractionated in order to allow for the identification of proteins by mass spectrometry (MS). Proteins can be directly submitted to enzymatic digestion with the appropriate proteases, such as trypsin, or to chemical treatment to get peptides of the appropriate mass (usually between 750 and 4000 Da). Alternatively, proteins are separated prior to cleavage into peptides. Since most CWPs are basic glycoproteins which are poorly resolved by bi-dimensional electrophoresis (2D-E), the most efficient means to separate them are either mono-dimensional electrophoresis (1D-E) (Boudart et al., 2005) or else cationic exchange chromatography followed by 1D-E of protein fractions eluted with a salt gradient (Irshad et al., 2008). The identification of proteins can then be done either by peptide sequencing through liquid chromatography (LC) coupled to MS (LC-MS/MS) or by peptide mass mapping using the matrix-assisted laser desorption/ionisation-time of flight (MALDI-TOF) MS followed by *in silico* analyses. At the bioinformatics end, custom ECM protein databases markedly increase the identification of extensively modified peptides. New generations of mass spectrometers will help meet the demand for high-throughput identification and the localisation of biologically significant peptide modifications.

3. Results and discussion

Proteomics has turned out to be an imperative benefactor for studying the acquaintance of plants' ECM structure and functions by allowing the identification of proteins present in this cellular compartment. It is a well known fact that the field of proteomics is evolving from the cataloguing proteins under static conditions to comparative analyses. Defining proteins that change in abundance, form, location or other activities may indicate the presence and functional significance of a protein. Whereas comparative ECM proteome research is quite advanced in animals (Zhu et al., 2007) and yeast (Kim et al., 2007), there is less information as to plants. The identification and cataloguing of plant ECM proteomes in recent years raises the following important questions: What are the essential plant ECM proteins? Do ECM proteins show clade specificity in vascular plants? What are those organ-specific cell wall proteins, if any? Does the cell wall developmental proteomics of one of the clades yield any astonishing or prolific results? How does ECM protein remodelling during environmental and/or patho-stress provide new perspectives? Are some of the ECM proteins unexpected? And, last but not the least, what sort of post-translational modifications have so far been characterised in CWP? Here we analyse and compare the experimental results of the thus far available proteomes so as to elucidate the dynamics of plant ECM /cell wall proteins.

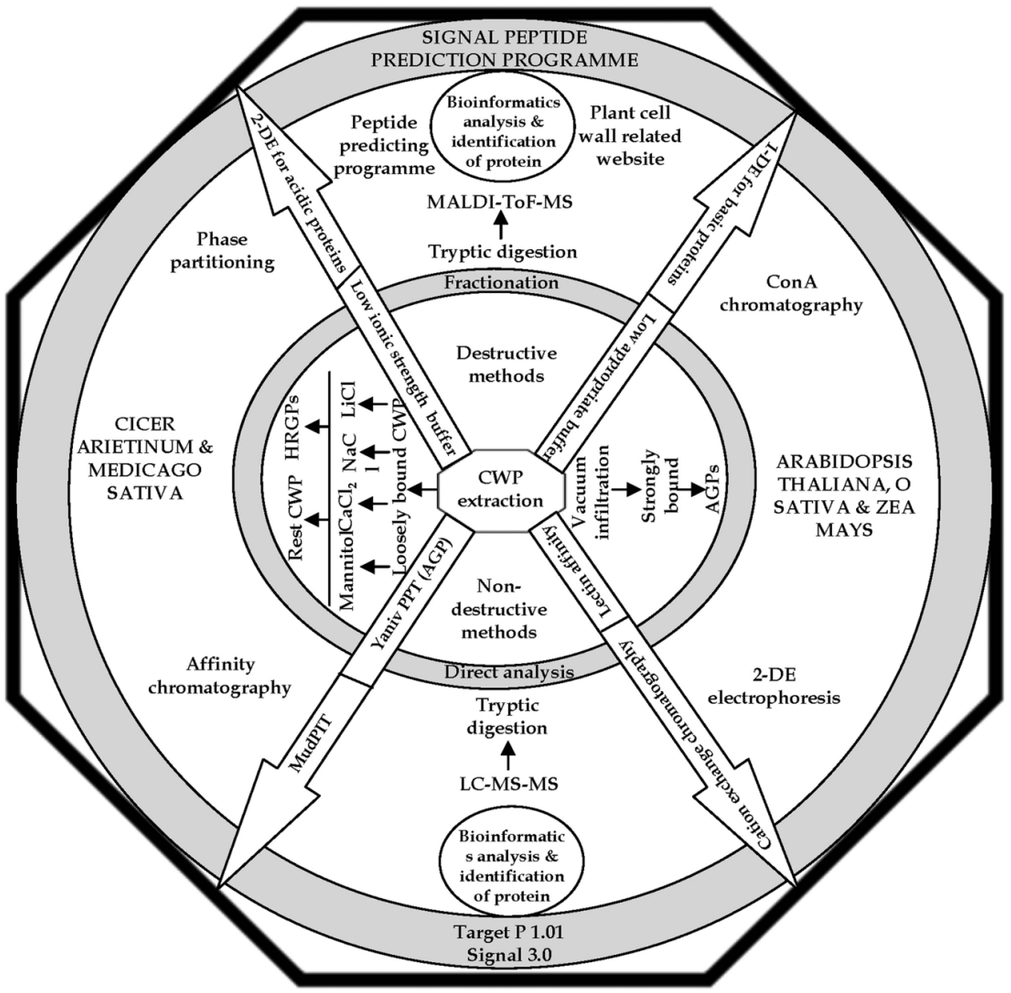


Fig. 1. A flowchart illustrating the overall experimental strategy for the analysis of the ECM proteome.

3.1 Analysis of ECM proteome dynamics in plants: social class vs. diversity

ECM/cell wall design and protein composition has been shown to differ between two major clades, viz. the monocots and dicots of vascular plants. Results have mainly been obtained with the model plants *Arabidopsis thaliana* (Liepman et al., 2010; Basu et al., 2006; Bayer et al., 2006; Borderies et al., 2003; Chivasa et al., 2002; Feiz et al., 2006; Jamet et al. 2008a), *Medicago sativa* (Soares et al., 2007; Watson et al., 2004), and crop plants for, e.g., *Oryza sativa* (Choudhary et al., 2010), *Brassica napus* (Basu et al., 2006) *Zea mays* (Zhu et al., 2006) and *Cicer arietinum* (Bhushan et al., 2006). Around 500 CWPs of *Arabidopsis*, representing about one third of its estimated cell wall proteome, have been described (Liepman et al., 2010) while 219, 143, 102, 58 CWPs were identified in rice, chickpea, maize and Brassica, respectively. Our comparative analysis of different species in relation to their function showed that a high percentage of proteins were found to be unique to each proteome: 87% in *A. thaliana*, 82% in *B. napus*, 84% in *C. arietinum*, 76% in *M. sativa*, 80% in *O. sativa* and 71% in *Z. mays*, with only peroxidase and glycosyl hydrolase being the social class of proteins present ubiquitously in all (Fig. 2).

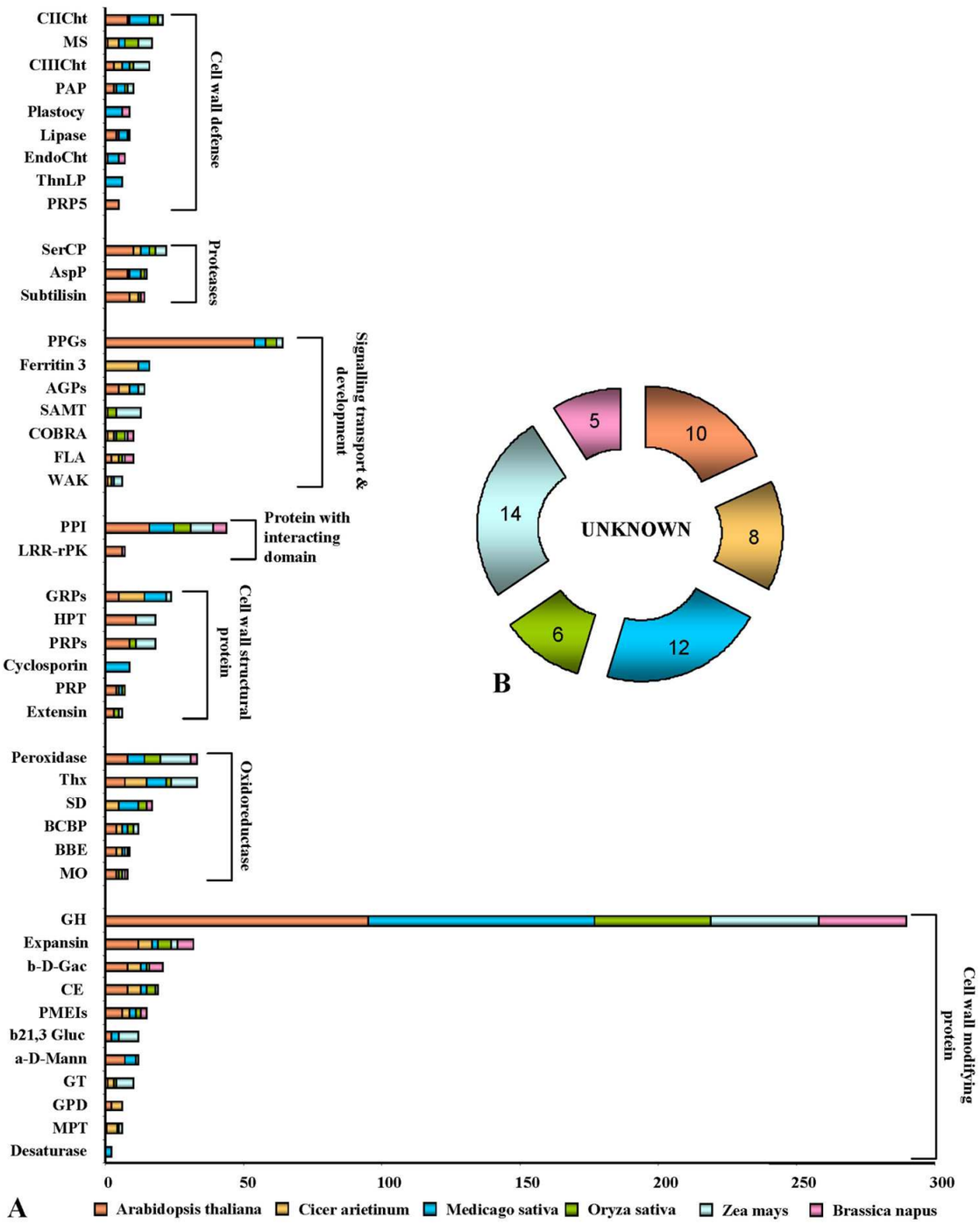


Fig. 2. Cross-species comparison of ECM proteomes. The functional classification of the identified proteins was made according to the biological processes in which they are involved. The length of the bar indicates the number of proteins present in a particular species, such as *Arabidopsis thaliana*, *Cicer arientum*, *Medicago sativa*, *Oryza sativa*, *Zea mays* and *Brassica napus*. The pie chart inset represents the fraction of unknown protein classes in each of these plants.

The available ECM proteome of the six plants compared in Figure 2 varied in molecular weight from 8.9 to 133.8 kDa and had a spread of p*I* values from 5.2 to 10.1. Seventy-six percent of the ECM proteins were basic in nature, concordant with the acidic environment of the wall. Between monocots and dicots, it was found that rice and *Arabidopsis* have a similar number of cell wall-related gene families and members within each family, even though rice has a far greater number of genes than Arabidopsis. This implies that similar numbers of genes are required for wall construction and maintenance, at least among Angiosperms (Yokoyama et al 2004). However, the cell wall proteome data of *Arabidopsis* is better explored than rice and therefore comparison of their proteome may not yield the postulated results as defined by genome analysis. When the maize cell wall proteome was compared with that of *Arabidopsis*, the results revealed an evolutionary divergence as well as tissue specificity, with few conserved proteins (Fig. 2). The protein network of maize (Zhu et al., 2006) revealed the predominance of the inhibitors of hemicellulose-degrading enzymes from monocots, such as endoxylanase inhibitors, and the *Arabidopsis* protein network (Slabas et al., 2004; Peck, 2005) was found to be rich in xyloglucan endoglucanase-inhibiting proteins and glycine rich protein as cell wall remodelling or biosynthetic enzymes. Comparison of the functional classes of cell wall proteins amongst dicot species like *Arabidopsis, Brassica, Medicago* and *Cicer* confirms the dynamic nature of the cell wall, as exemplified by the presence of cellulose synthase and peroxidase in all dicots. However, surprisingly the protein turnover rate of these enzymes are greater in *Medicago*. A more comprehensive investigation of the studied legume proteomes revealed that the proportion of proteins involved in cell wall modification is three times greater in *Medicago (*99 proteins*)* than in *Cicer* (28 proteins).

This may be due to the fact that ionically bound and soluble ECM proteins can be separated with ease from *Medicago* as compared with *Cicer*. Additional variation of cell wall proteomes in *Cicer* and *Medicago* is provided by the presence of ferritin in the former and Polypolyglutamatesynthase in the latter, illustrating that nature invented vastly different solutions to a common problem, viz. transport and storage. When the studies on the legumes like *Cicer* and *Medicago* were compared to *Arabidopsis* belonging to the Brassicaceae family (Fig. 2), it can be readily observed that the protein machinery of the wall for activating the wall-modifying enzymes is diverse between the two families as well as between the members of the same family, leguminosae. Investigation between *Arabidopsis* and *Brassica* proteomes by MudPIT, using a homology-based search, unambiguously identified 16 proteins which were common to the 52 proteins of *Arabidopsis*. When the cell wall proteomes of *Oryza* (145) and *Zea* (128) were compared, less diversity was observed in Poaceae (Pandey et al., 2010; Zhu et al., 2006) except for the fact that one of the CWP expansins - HPT - is expressed by a moderate amount in maize, whereas in rice PRP is represented by a moderate number (Fig. 2). It may be assumed that the divergence in the resulting proteomes of the vascular plants is due to the presence of the different design of their wall based on their carbohydrate composition. It is known that type I carbohydrates - which typically contains xyloglucan and/or glucomannan and 20–35% pectin - are found in all dicotyledons whereas type II carbohydrate rich in arabinoglycan are only characteristic of the Poaceae family in the monocot, suggesting the occurrence of clade-specific ECM proteins that would bind to their cognitive carbohydrate molecules. Most intriguing are the remaining 10% of ECM proteins that do not have any similarity to the known proteins in other organisms. The challenge is to elucidate their biological role within the cell wall.

3.2 Discerning organ-specific ECM proteomes in *Arabidopsis thaliana*

We further analysed the organ-specific proteome of *Arabidopsis thaliana*, namely root, stem, leaves, etiolated hypocotyl, etiolated seedlings from liquid and cultured media, and protoplast- and leaf-derived cell suspension (Miller & Fry, 1992; Feiz et al., 2006; Minic et al., 2007, Irshad et al., 2008; Minic et al., 2008). Comparative analysis (Fig. 3) revealed that cell wall modifying proteins, structural proteins and proteins involved in signalling and development constitute 58% of the ECM's proteins in mature stems (71) and dark-grown hypocotyls (147) with high and moderate expression. However, it was intriguing to note that most of these CWPs identified by the proteomics study originate from genes whose level of transcripts was low (between 37% and 58%) or below the background (between 18% and 25%) as reported in Minic et al., 2007 indicating thereby the importance of the post-transcriptional regulation of organ-specific ECM proteomes. A further 29 and 54 cell wall modifying proteins were identified in the roots and leaves respectively, in which members of the hydroxyproline-rich glycoprotein family and other major structural proteins were not detected. For a few protein sequences within a particular organ, there also exists a certain degree of heterogeneity in terms of the occasional amino acid substitution as well as their appearance at different molecular weights. The former may be explained due to the origin of these protein species from different genes and the latter by post-translational modifications, such as glycosylation. Expansin, a cell wall modifying component, was the most dominant class in all the major organs, while well-known cell wall enzymes like glycoside hydrolase, pectin methylesterases, peroxidases and glycosyl transferases were represented by several members of the same family (Fig. 3). The analysis of protoplast and suspension-cultured cell derived proteomes in *Arabidopsis* and rice showed the relative abundance of the GH family of ECM proteins. They might be involved in the modification of mixed glycan polymers, only found in monocot cell walls during the regeneration of the cell wall in the protoplast. However, the role of GH family of proteins has not yet been elucidated in *Arabidopsis*. A moderate number of carbohydrate esterases were identified in the ECM proteome of the cell suspension culture, etiolated hypocotyl and leaves while a novel family of HRGP, called LRR-extensin proteins (LRX), has only been found in the case of cell suspension cultures. The only organ in which a few salt-extractable structural proteins were identified is etiolated hypocotyl, possibly because such proteins are not yet completely insolubilised from other organs. Proteins having domains of interaction with proteins or polysaccharides are well-represented in all organs, and especially in rosettes. As expected from the fact that GH represents almost 20% of the identified CWPs (Fig 2 and 3), proteins acting on cell wall polysaccharides are also the category with the highest diversity within each organ. Oxidoreductases are particularly numerous in cell suspension cultures, probably due to the mechanical stress produced by continuous spinning and the oxidative stress that occurs in the liquid media culture. At least 20% of the identified CWPs represent a social class in one organ not found in the others. This may be partially linked to the high redundancy in the number of genes encoding each CWP family, presumably differentially-regulated during organ development (Fig. 3).

3.3 Exploring the variability of the developmental stage specific ECM proteome

A cornerstone of evolution is associated with the diversity of individuals within a population. This diversity is generally understood to arise at the genetic level and leads to characteristics that may be advantageous or disadvantageous within the context of the

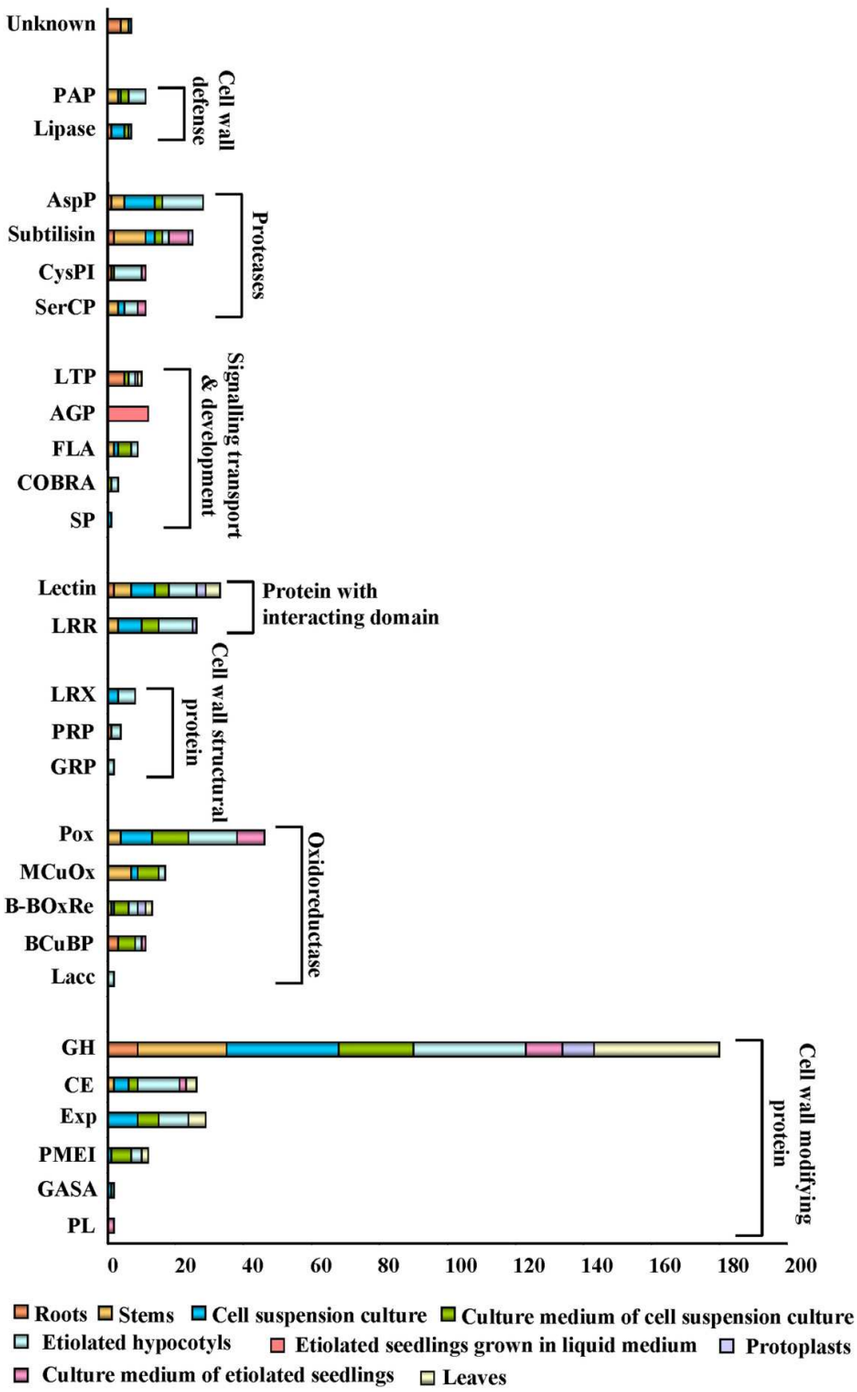


Fig. 3. The organ-specific comparative ECM proteome in *Arabidopsis*. The functional classification of the identified proteins was according to the biological processes in which they are involved. The length of the bars indicates the number of proteins present in a particular organ or culture, such as roots, the stem, the cell suspension culture, the culture medium of the cell suspension culture, etiolated hypocotyls, etiolated seedlings grown in the liquid medium, the culture medium of the etiolated seedlings, protoplast and leaves.

environment (Taraszka et al., 2005). The emerging field of developmental proteomics, in which large mixtures of proteins are characterised in a single experimental sequence, may allow for the assessment of variability or similarity in an individual at the level of the proteome (Hunter et al., 2002). The developmental proteomics of rice is perhaps the least studied, but its importance was realised when the proteome of rice at 5 days and the third and fourth leaf stages were analysed (Jung et al 2008; Chen et al., 2009). When we compared the existing dataset, even though the proteomes were found to be similar, some of the CWPs which were unknown earlier were uniquely present at a particular developmental stage. For example, COBRA and Leucine rich repeat extensins were found only in the third leaf stage while the polysaccharide lyase appeared in the fourth leaf stage (Fig. 4). Although ECM proteins which regulate development and expansion form the major class, very few have been functionally characterised so far. Such a protein, COBRA (COB), anchored to the extracellular surface of the plasma membrane by a glycosyl phosphatidylinositol (GPI) moiety is thought to regulate and link oriented-cell expansion in root cells (Brady, 2007). Another protein, LRX1, a chimeric leucine-rich repeat/extensin is also expressed in root hair cells. The interaction between the cell wall and the LRX1 protein is important for proper root hair development and expansion (Diet et al., 2006). A family of secreted proteins called SCAs (stigma/stylar cysteine-rich adhesion) was identified as a pollen tube adhesion molecule for the wall material of the style found in the lily (Baumberger et al., 2001). One of the ECM protein family Arabinogalactan-proteins (AGPs) belonging to the category of HRGP consists of a rather small and highly glycosylated protein moiety which has been found to play vital role in cell wall development (Gillmor et al., 2005). THESEUS1 (THE1), which is a member of the subfamily of the *Catharanthus roseus* protein kinase1-like receptor kinases also has efficacy in cell wall integrity, sensing and development (Hematy et al., 2007). Thus, the resulting cell wall proteomes were different, showing in another way that the cell wall structure and composition are regulated during development. However, the biological functions of most CWPs involved in development have not yet been experimentally studied.

3.4 *In silico* protein profiling of comparative ECM stress proteomes

The plant cell wall or the extracellular matrix (ECM) is the first compartment that senses stress signals, transmits them to the cell interior and eventually influences the cell fate decision (Ellis et al., 2002), and thus it can be envisaged that ECM proteomes primarily regulate the environmental and patho-stress response in plants. We analysed the cell wall proteomes of *Arabidopsis* and maize in response to osmotic stress (Kachroo et al., 2001; Amaya et al., 1999), and the dehydration responsive ECM proteomes of chickpea and rice (Bhushan et al., 2007; Pandey et al., 2008; Choudhary et al., 2009; Pandey, et al., 2010; Bhushan et al., 2011). Interestingly, a great deal of divergence in the protein classes amongst these organisms was observed (Fig. 5A).

To our surprise, except for peroxidase, serine protease and subtilisin none of the ECM proteins was found to be common in all the organisms under both kinds of the stresses studied. The families of antimicrobial peptides such as thionins, defensins and knottin-like peptides have been found in the dehydration-responsive proteome of chickpea, while it was found that rice DRPs comprised of antimicrobial peptides such as oryzacystatin, thioredoxin and oligopeptidase. The *Cicer* dehydration-responsive protein network showed the exclusive presence of glycine-rich protein, methionine synthase, ferritin, tubby-like protein

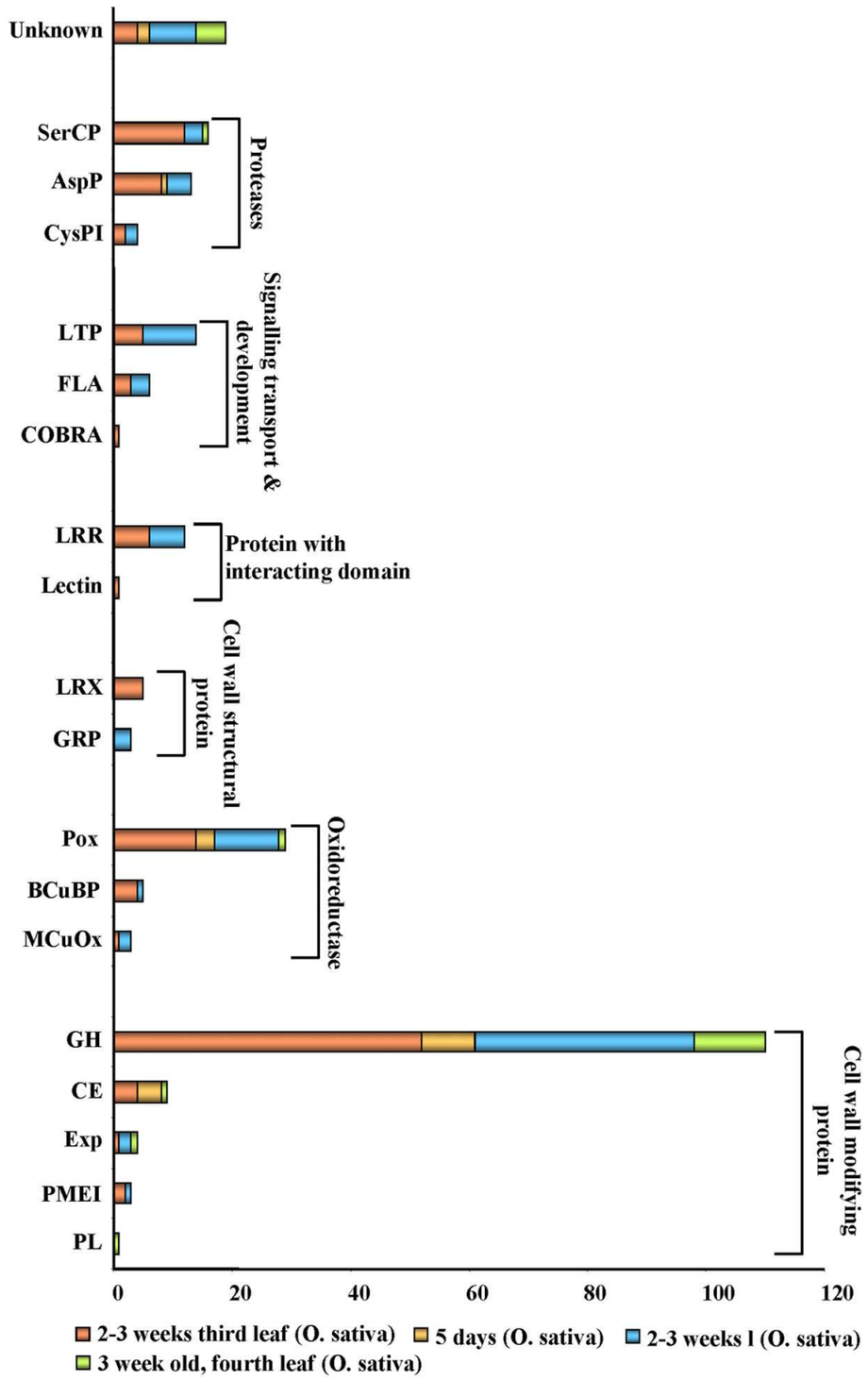


Fig. 4. The developmental stage specific comparative ECM proteome in rice. The functional classification of the identified proteins was according to the biological processes in which they are involved. The length of the bars indicates the number of proteins present in a particular leaf stage/day of suspension culture.

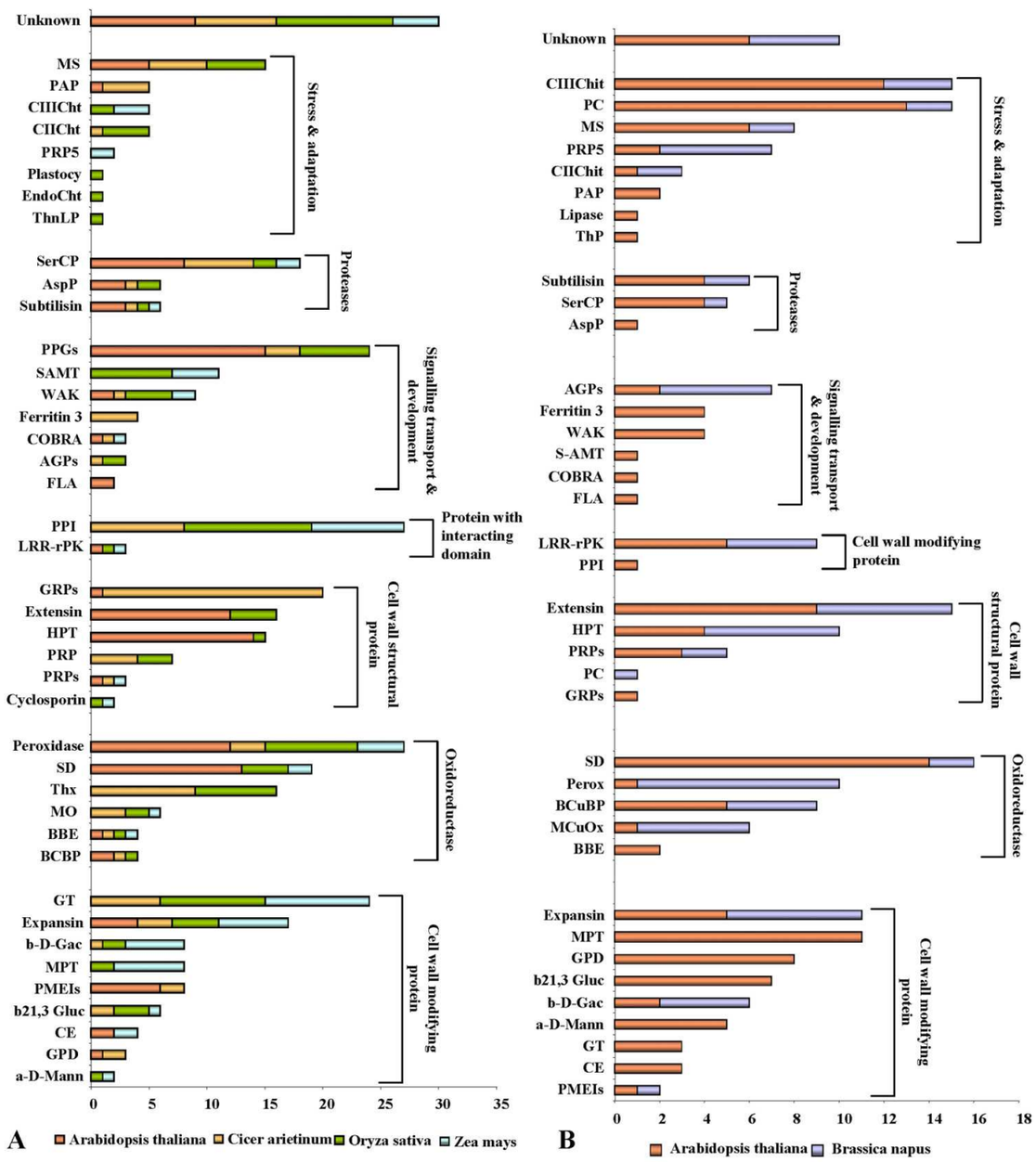


Fig. 5. Comparative stress proteome: A comparison of various functional classes of the extracellular matrix protein in environmental stress (A) and in patho-stress (B). The functional classification of the identified proteins was according to the biological processes in which they are involved. The length of the bars indicates the number of proteins present in a particular stress.

and leucine aminopeptidases. Another important finding was the presence of falacinin-like AGPs during osmotic stress in *Arabidopsis*, but not in other cases. Extensin, hydroxyproline transferase and carbohydrate esterase were predominantly found during the dehydration response but were absent in response to osmotic stress. Interestingly, rice as well as maize cell wall proteomes under both types of abiotic stresses revealed the presence of class III chitinase, plastocyanin, S-adenosylmethionine transferase and cyclosporine, suggesting their clade-specific expression. Our analysis revealed the presence of monocot and dicot peroxidases having specific protein sequences that clearly demonstrate the diversity of the identical CWPs in two divisions of angiosperm. This may be attributed to the evolution of orthologs vs. paralogs.

Moreover, plant cell walls constitute the first stage of defence against invading pathogens. The endogenous wall metabolism might facilitate pathogen infection, either because wall substrates are made more physically accessible to pathogens or because the plant enzymes convert wall polymers into appropriate nutritional substrates for the invading micro-organism. In addition to the crucial role of CWPs in growth and development, these proteins or peptides are also involved in plant defence mechanisms in response to patho-stress. Earlier, a number of ECM proteins have been shown to play a crucial role in plant defence against microbes (Sakurai, 1998), including pathogenesis-related (PR) proteins, chitinases and endo-b-1,3-glucanases, that are known to directly interact with pathogens (Jung et al., 2004; Jones et al., 2006). However, plants also deploy a repertoire of proteins in the wall that act as a surveillance system to allow the early detection of an impending pathogen assault. We analysed the cell wall proteomes of *Arabidopsis* and *Brassica napus* in response to fungal stresses (Ndimba et al., 2003; Floerl et al., 2008), and elicitor-induced ECM proteome of *Zea mays* (Chivasa et al., 2005). The common ECM proteins identified in fungal stress were jacalin-related, LRR-containing proteins, chitinase, thaumatin-like proteins, esterase/lipase thioesterase and the GLIP1 lipase. On contrary the, S-AMT, COBRA, FLA, BBE, CE and GRPs were found to be exclusive in the case of *Arabidopsis-Fusarium* interactions, suggesting that the cell wall is a dynamic milieu and responds differently in response to different pathogen within the divisions or in between the divisions of the angiosperm (Fig. 5B). Likewise, in order to assess the generality of the cell wall proteome of *A. thaliana* (Oh et al., 2005) and *B. napus* (Floerl et al., 2008) under patho-stress were compared. The results indicate that the fungal stress-induced changes in CWPs were diverse in both of the plants except for the oxidoreductases, stress- and adaptation-related proteins and structural proteins. Meanwhile signalling-, transport- and development-related proteins were induced mostly in *A. thaliana*, except for the AGPs which were commonly present in both of the proteomes (Fig. 5B). Thus, *B. napus* may depend exclusively on AGPs-mediated stress signalling responses, whereas diverse signalling pathways operate in *A. thaliana.* Pathogen elicitor-induced changes in maize ECM proteomes revealed the involvement of lipases, esterases and thiols similar to the response of *Arabidopsis* to pathogen invasion**.** However, how monocot cell wall proteins respond to pathogens still needs further investigation in order to comprehend the monocot-dicot difference in response to pathogen invasion. In addition, a number of extracellular proteins and peptides have been identified that contribute to signalling and the recognition of not only pathogens but also other cell-type responses, such as in pollen–pistil interactions and the phosphate deficiency proteome of *Arabidopsis* (Kachroo et al., 2001; Tran & Plaxton, 2008).

The comparative analysis of clade and organ-specific, developmentally-regulated, stress-responsive plant ECM proteomes revealed the presence of certain proteins that were unexpected, either in their abundance, form, number or else localisation. These unexpected or non-canonical proteins suggest the constant remodelling of cell wall proteomes. The exact function and specificity of these candidates can only be comprehended once they are functionally characterised.

4. Conclusion and perspectives

In this study, cross-species as well as cross-condition comparisons of ECM proteomes in vascular plants illustrates the divergence in protein profiles within only a few social classes. Across species, cell wall modifying proteins (23%) represent the largest category, followed by oxidoreductase (19%) and cell wall structural protein (18.5%). In total, 213 and 110 glycoside hydrolase were found in the organ specific proteome of Arabidopsis and development specific proteomes of rice, respectively. Oxidoreductase constitutes the second largest category in both these cases. Furthermore, the dehydration responsive comparative proteome in legumes, chickpeas and cereals, rice showed both genotypic- and crop-specific adaptation. As expected, the proteins involved in cell-wall remodelling were found to be the most predominant across all conditions. Nonetheless, a large number of proteins were unique or novel to each of the plant species, organs, stages of development and different stresses. It may be thought that the ubiquitously present classes of proteins are the essential proteins for sustenance while the unique classes bring out the condition-specific special function. The differences in terms of protein pattern and protein function appear to encompass both genetic and physiological information. It may be speculated that the differential proteome is shaped by the cellular environment and the ecological niche of the corresponding organism. The divergence may arise due to codon bias, amino acid composition and protein length. However, a much more comprehensive survey of the ECM proteomes in several plants will ultimately draw a more complete picture of the social class vs. protein diversity in this organelle. We are witnessing a significant but inadequate progress in the understanding the ECM proteomes of various crops of agricultural importance. Our understanding of ECM composition, organisation and homeostasis has been greatly enhanced through targeted biochemical and genetic approaches. Unbiased 'discovery' methods, such as proteomics, have only recently gained traction in the field of matrix biology. To date, a key word search using "ECM proteome" retrieves only 43 results in a pubmed search, emphasising the need for in-depth study in the field of the plant ECM proteome. Our future efforts will focus on the development and analysis of comparative ECM proteomes towards an understanding of crop- and genotype-specific adaptation as an important amendment for the determination of protein networks influenced by the internal and external cues associated with the complex cellular biochemical and physiological process that bring about phenome variation.

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6. Abbreviations

Polysaccharide lyase, PL; gibberellin acid-stimulated Arabidopsis (GASA) protein, GASA; pectin methyl esterase inhibitor, PMEI; Expansin, Exp; carbohydrate esterase, CE; glycoside hydrolase, GH; Laccase, lacc; blue copper binding protein, BCuBP; berberine-bridge oxido-reductase, B-BOxRe; multicopper oxidase, MCuOx; Peroxidase, Pox; glycine-rich protein, GRP; proline-rich protein, PRP; leucine-rich repeat extensin, LRX; LRR protein, LRR;signal eptidase, SP; COBRA-like family, COBRA; fasciclin-like arabinogalactan protein, FLA; arabinogalactan protein, AGP; lipid transfer protein, LTP Ser carboxypeptidase, SerCP; Cys protease inhibitor, CysPI; Asp protease, AspP; purple acid phosphatase, PAP; glycosyl transferases, GT; a-D-mannosidases, a-D-Mann; b-D-galactosidases, b-D-Gac; b21,3 Glucanase, b21,3 Gluc; glycerophosphodiesterases, GPD; Mannose-1-phosphateguanyltranferase, MPT; berberine-bridge enzyme, BBE; Superoxide dismutase, SD; Putative cyclosporin, PC; Proline-rich proteins (PRPs), PRPs; Hydroxyl proline transferase, HPT; Putative protease inhibitor, PPI; LRR-receptor protein kinases; LRR-rPK; S-adenosylmethioninetransferaae, S-AMT; Wall- associated kinase, WAK; Arabinogalactan-proteins, AGPs; Thaumatin-like protein, ThP; Class II chitinase, CIIChit; Pathogenesis-related protein 5, PRP5; methionine synthase, MS; Class III chitinase, CIIIChit; threonine-hydroxyproline-rich glycoprotein, THRGP;

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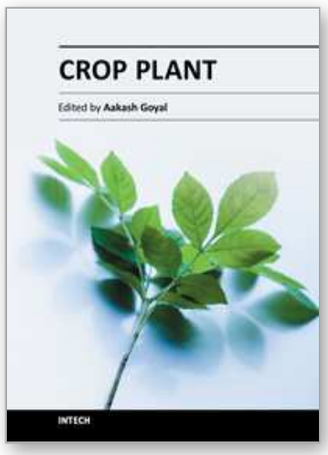
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This book provides us a thorough overview of Crop Plant with current advance in research. Divided into two section based on the chapters contents. Chapter 1 provides information about markers and next generation sequencing technology and its use. Chapter 2 is about how we can use Silicon for Drought tolerance. Chapter 3 is to deal with the major problem of rising CO2 and O3 causing environmental pollution. Chapter 4 covers the phenomena of RNAi and its use, application in crop science. Chapter 5 is a review for boron deficiency in soils and how to deal with it for better crops. Chapter 6-10 provide some information regarding recent works going on in crop science.

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