Softening-up mannan-rich cell walls

María del Carmen Rodríguez-Gacio , Raquel Iglesias-Fernández , Pilar Carbonero and Ángel J. Matilla

Abstract

The softening and degradation of the cell wall (CW), often mannan enriched, is involved in several processes during development of higher plants, such as meristematic growth, fruit ripening, programmed cell death, and endosperm rupture upon germination. Mannans are also the predominant hemicellulosic CW polymers in many genera of green algae. The endosperm CWs of dry seeds often contain mannan polymers, sometimes in the form of galactomannans (Gal-mannans). The endo-β-mannanases (MANs) that catalyse the random hydrolysis of the β-linkage in the mannan backbone are one of the main hydrolytic enzymes involved in the loosening and remodelling of CWs. In germinating seeds, the softening of the endosperm seed CWs facilitates the emergence of the elongating radicle. Hydrolysis and mobilization of endosperm Gal-mannans by MANs also provides a source of nutrients for early seedling growth, since Gal-mannan, besides its structural role, serves as a storage polysaccharide. Therefore, the role of mannans and of their hydrolytic enzymes is decisive in the life cycle of seeds. This review updates and discusses the significance of mannans and MANs in seeds and explores the increasing biotechnological potential of MAN enzymes.

Introduction

Photosynthetic eukaryotes have a carbohydrate-rich cell wall (CW), which is a highly complex and dynamic cell compartment that performs various mechanical and biochemical functions and protects the cell from extreme osmotic conditions (Cosgrove, 2005; Sarkar et al., 2009; Burton et al., 2010; Sørensen et al., 2010). Genes encoding enzymes involved in the biosynthesis of CW components may have been transferred during the endosymbiotic process from bacteria to plants (Popper et al., 2011). This biosynthesis takes places mainly in the Golgi complex and in the plasma membrane (Lerouxel et al., 2006; Fincher, 2009; Burton et al., 2010).

The CW is comprised of a primary (PCW) and, sometimes, a secondary cell wall (SCW), depending on the specialization and the cellular ability to expand or divide. Structurally, the PCW is made up of proteins and a scaffolding of cellulose [β(1→4)-glucan chains organized in para-crystalline microfibrils] embedded in a matrix of cross-linking hemicellulosic glycans and pectins. The hemicellulosic glycans are mainly made up of xyloglucans, xylans, and β-mannans that are characterized by having a backbone of β(1→4)-linked monomers with occasional lateral α(1→6) sugar branchings. In land plants, especially in the Poaceae, β(1→3;1→4)-glucans are quantitatively important hemicelluloses (Smith and Harris, 1999). The relative proportions of each sugar type in the PCW vary among different plant species and in different tissues and organs (Cosgrove, 2005; Burton et al., 2010; Scheller and Ulvskov, 2010). Once cell growth has ceased and the differentiation process occurs, a SCW can be laid between the PCW and the...
plasma membrane. The SCW is more rigid and thicker than the PCW, and contains predominantly cellulose, xylan, lignin, pectin, and/or mannan. SCWs are prominent in xylem, sclerenchyma, and fibres (Knox, 2008; Vogel, 2008; Marcus et al., 2010; Albersheim et al., 2011). In recent years, progress in knowledge of the CW composition and structure has been achieved due to new sets of tools such as carbohydrate-binding modules (CBMs), used in immunolocalization studies, and micro-spectroscopical techniques (Knox, 2008; Lee et al., 2011).

Cell expansion in meristematic tissues is a major and irreversible cell process requiring PCW remodelling and synthesis (Somerville et al., 2004; Knox, 2008; Albersheim et al., 2011). This growth is not only caused by water uptake, driven by differences in osmotic pressure, but also involves different processes of hydrolysis, and rearrangement or disassembly of the structural polymers, carried out by hydrolyases, expansins, transglycosylases, or reactive oxygen species (ROS; Cosgrove, 2005; Schopfer, 2006; Fincher, 2009; Burton et al., 2010). This CW softening process is not exclusive to the meristematic growing cells; in fruit ripening, endosperm rupture upon germination, and programmed cell death, an important CW dismantling process takes place (Sampedro and Cosgrove, 2005; Gadjev et al., 2008; Moreira and Filho, 2008; Payasi et al., 2009; Pirello et al., 2009; Weitbrecht et al., 2011).

In this review, we present an update on the composition, metabolism, and putative biological functions of mannans and mannanases (MANs) with special emphasis on seed germination. Some of the potential biotechnological implications are also considered.

### The mannan family in photosynthetic organisms

The mannans are a set of heterogeneous glycans widespread among green algae and terrestrial plants (Fig. 1A). Structurally, these polysaccharides contain β(1→4)-linked residues of either mannose (Man) or a combination of glucose (Glc) and Man. This backbone may also be substituted with α(1→6)-linked galactose (Gal) short side chains. Several types of mannans have been characterized: (i) pure mannans that contain >95% Man and that can adopt para-crystalline structures; (ii) glacto-mannans that contain >5% Gal; (iii) gluco-mannans with Glc and Man residues in the backbone; and (iv) (Gal–Glc)-mannans (Handford et al., 2003; Wang et al., 2006; Scheller and Ulvskov, 2010). The Man:Gal ratio varies from 1:2 to 20:1; the amount of Gal residues in the mannan polymer influences its solubility, viscosity, and interactions with other CW polysaccharides; as the Man:Gal ratio diminishes, the solubility of the polymer increases, by preventing the formation of insoluble para-crystalline structures among the mannan chains. The Man:Gal ratio in Gal-mannans tends to be relatively constant during seed development; however, deviations from this can occur as in Senna occidentalis where there is an increase in the Man:Gal ratio from 2.3 to 3.3 during the last 5–10 d of endosperm development (Edwards et al., 1992).

Plants and algae have a complex phylogenetic history, including the acquisition of genes responsible for carbohydrate synthesis and modification, through a series of endosymbiotic events. Organisms that share photosynthesis and CW do not form a monophyletic group, but they contain some common CW components (Table 1; Popper et al., 2011).

### Mannans in algae

Man-rich hemicelluloses are found in the CW of the Charophycean green algae that are the most probable ancestor of terrestrial plants, and in the Bryophytes (Fig. 1A). However, the ferns (Pteridophytes and Equisales) possess mannans showing lower amounts of Gal- and Glc-mannans in the PCW than in the SCW (Popper and Fry, 2004; Harris, 2005; Popper, 2008; Popper et al., 2011). Mannans are the predominant skeletal CW polymers in several green algae and some of them are of great industrial importance (Sittikijyothin et al., 2005; Ciancia et al., 2007; Dunn et al., 2007; Pauly and Kegestra, 2008; Estévez et al., 2009; Popper and Touhy, 2010). Linear β(1→4)-mannans are able to form para-crystalline microfibrils in the CW of all red algae of the fillum Rhodophyta (Table 1) and they are present in several siphonaceous green algae (Chlorophyta) including the single-cell Acetabularia (Dasycladales), Codium (Bryopsidales), and Haliclytre (Polyphylales).

The CW of the giant alga Acetabularia is a well known example of heterogeneity since it is mannan rich during the vegetative phase, while it is enriched in cellulose during the reproductive phase (cysts; Dunn et al., 2007). Mannan-rich CWs are likely to be more extensible than cellulose-rich CWs. The CWs of marine organisms are less rigid than those of terrestrial plants, since the former are adapted to the buoyant water environment, such as Acetabularia and other algae with a similar mannan-enriched CW composition. Mannans in algae can display a variable degree of polymerization, between 20 and 10 000 monomers, as in Codium fragile CWs (Mackie and Sellén, 1969).

The CWs of the red algae, such as Porphyra umbilicalis and Bangia atropurpurea, are composed of three unique polysaccharides: β(1→4)-mannans, β(1→3)-xylans, and poryphans. In B. atropurpurea, the soluble amorphous β-mannans are the main CW polysaccharides, detected using a fluorescence-labelled CBM that uses a MAN from Vibrio which has a specific ability to bind the soluble amorphous β-mannans but not those which are para-crystalline (Tanaka et al., 2009; Umemoto and Araki, 2010).

### Mannans in vascular plants

Monocotyledonous plants contain Glc-mannans instead of xyloglucans, but the major hemicellulosic components are mixed-linked glucans and xyans (Stone, 2006). Likewise, the (Gal–Glc)-mannans are the major components of the
Fig. 1. Mannans and mannan synthases in Plantae. (A) Schematic outline of the abundance of mannans from Charophycean green algae to vascular plants. (B) Percentage of mannans in the CW of higher plants. (C) Glc-mannans in the CW of different tissues of cereal seeds.

SCW of coniferous plants (Fig. 1B, C; Ebringerová et al., 2005; Benová-Kákosova et al., 2006). In the wood of these plants, the mannan polysaccharides are the most abundant cross-linking glycans (Maeda et al., 2000). In the Gymnosperms (especially in the softwoods), the xylem is very rich in (Gal-Glc)-mannans (15-20% of wood) compared with hardwoods (3-5%). Glc-mannans are also present in the CWs of coniferous seeds and to a lesser extent in those of some hardwoods. Although mannans are abundant in both PCW and SCW of plants, the Glc-mannans are present in low amounts in dicotyledonous plants (Fig. 1A-C).

In general, Angiosperm seeds contain in their CWs Man-rich hemicelluloses as structural or storage components. In Arabidopsis thaliana inflorescences, mannan polysaccharides are localized in the thickened SCW of xylem elements and in the interfascicular fibres, and also in the leaf xylem. In some cases, Glc-mannans are abundant in epidermal thickened outer CWs, and these probably have a role in defence against pathogens (Handford et al., 2003). Gal-mannans can be abundant in the CW of storage tissues, notably those from the endosperm of leguminous seeds, such as the locust bean (Ceratonia siliqua), guar (Cyanopsis tetragonoloba), or tara gum (Caesalpinia spinosa). In Aloe barbadensis, a storage linear polysaccharide located within the parenchyma cells corresponds to the acetylated mannan known as acemannan or carrysin (Femenia et al., 1999). O-Acetylation is a common modification of CW polysaccharides, including mannan. Although the enzymes involved in this biochemical reaction are far from being identified, a knock-out Arabidopsis mutant in the Reduced Wall Acetylation2 (RWA2) gene has decreased levels of acetylated CW polymers, including xylloglucans and pectins (Manabe et al., 2011). Recently, it has been shown that this acetylation disturbs the immunological probes for mannan determination (Marcus et al., 2010).

Two main roles have been assigned to mannans: (i) structural, as para-crystalline or cross-linking polymers that bind cellulose; and (ii) as storage reserves in seed endosperm CWs and in vacuoles, as well as in vegetative tissue CWs (Reid et al., 2003; Handford et al., 2003; Gidley and Reid, 2006; Liepman et al., 2007; Tomini et al., 2007; Buckeridge, 2010; Burton et al., 2010; Scheller and Ulvskov, 2010). Besides their well-known structural and storage functions, mannans can also be involved in oligosaccharide signalling, zygotic embryogenesis, and vascular cell differentiation (Benová-Kákosová et al., 2006; Liepman et al., 2007; Moreira and Filho, 2008; Goubet et al., 2009). The mannans play an essential role in higher plants, as evidenced by the embryo-lethal phenotype in the single Arabidopsis csla7 mutant that is lacking the major Glc-mannan synthase activity in the embryo (Goubet et al., 2003, 2009).

The synthesis of mannans in plant CWs appears to be catalysed by proteins encoded by certain members of the cellulose synthase family. The CEllulose Synthase gene superfamily (CESA) of A. thaliana includes a subfamily encoding the catalytic subunits of the enzyme cellulose synthase, and eight other subfamilies of CEsullase Synthase-like (CESA-like; CSLA-F) genes. Certain members of the CSLA subfamily encode mannan synthases, and the CSLF subgroup encodes β-glucan synthases. A CSLA family member from guar (a eudicot) has been shown to synthesize mannans and Glc-mannans when expressed in transgenic soybean cells (Dhugga et al., 2004; Liepman et al., 2007).

Hemicellulosic glycans are mainly synthesized and assembled in the Golgi cisternae, and the fusion of the Golgi
vesicles with the plasma membrane results in the deposition of the polysaccharides into the apoplast and their eventual incorporation into the CW (Lerouxel et al., 2006; Fincher, 2009; Scheller and Ulvskov, 2010). However, the biosynthesis of \( \beta(1 \rightarrow 3) \)-glucan (i.e. callose) is widely accepted to occur at the plasma membrane. By using monoclonal antibodies against a range of CW polysaccharides, Wilson et al. (2006) have detected mannan in barley endosperm but they did not find \( \beta(1 \rightarrow 3;1 \rightarrow 4) \)-glucans either in the Golgi or in the Golgi-derived vesicles. Is the Golgi apparatus the only place for the complete synthesis of the CW matrix polysaccharides, or are there other possibilities?

Recently, a two-phase assembly system has been proposed to account for the synthesis of CW glucans. During the first phase, linked to the Golgi complex, a population of \( \beta(1 \rightarrow 4) \)-oligoglucosides would be synthesized by enzymes of the CSL family, and then transferred to the plasma membrane by exocytosis. The second phase of the process, taking place in the apoplast, would imply the modification of the \( \beta(1 \rightarrow 4) \)-oligoglucoside chain, by a callose synthase encoded by a member of the CSL gene family, by a xyloglucan-endotransglycosylase, or by another CSLF isoenzyme, through \( \beta(1 \rightarrow 3) \)-linkages (Burton and Fincher, 2009; Doblin et al., 2009; Burton et al., 2010).

**Endo-\( \beta \)-mannotanases**

MAN enzymes are ubiquitous in nature and synthesized by a vast variety of organisms including bacteria (mainly Gram-positive bacteria), fungi, and plants (Dhawan and Kaur, 2007; Table 2; Supplementary Tables S1, S2 available at *JXB* online). MANs belong to families 5 and 26 of the glycosyl hydrolase superfamily, each containing >70 entries in the CAZY database (Carbohydrate-Active enZymes Database; http://afmb.cnrs-mrs.fr/CAZY). However, plant MANs are all included in family 5. The classification of the MAN proteins is based on their amino acid sequences and on their structural and mechanistic similarities (Shallom and Shoham, 2003).

Degradation of the mannan polymers depends on the pattern of substitutions of their backbones, and their complete hydrolysis requires the synergistic action of endo- and exo-acting enzymes. MAN (EC 3.2.1.78), \( \beta(1 \rightarrow 4) \)-mannotanases (EC 3.2.1.25), and \( \beta(1 \rightarrow 4) \)-glucosidases (EC 3.2.1.21) are the major enzymes involved in mannan hydrolysis. MANs are endohydrolases that catalyse the cleavage of \( \beta(1 \rightarrow 4) \) bonds in the mannan backbone; \( \beta(1 \rightarrow 4) \)-mannotanases and \( \beta(1 \rightarrow 4) \)-glucosidases are exohydrolases that attack the non-reducing end of the mannan polymer, releasing Man or Glc units, respectively (Moreira and Filho, 2008). Acetyl mannan esterases (EC 3.1.1.6) and \( \alpha(1 \rightarrow 6) \)-galactosidases (EC 3.2.1.22) are also needed to remove acetate or the galactosyl residues of the mannan polymers (Shallom and Shoham, 2003; van Zyl et al., 2010).

The size of the MAN genes and the primary structure of their corresponding proteins are quite different (Dhawan and Kaur, 2007); however, eight amino acid residues, important for the catalytic activity of the enzyme, are conserved in all the members of family 5 MANs (Sakon et al., 1996). From the total of 76 MAN proteins characterized so far, only eight are from plants (Table 2; Supplementary Tables S1, S2 at *JXB* online). LeMAN4 from tomato is the only plant MAN whose three-dimensional structure has been determined (Bourgault et al., 2005). Although at least 20 different MANs have been purified from fungi and five from the animal kingdom (Supplementary Table S2), >65 putative MAN genes from

### Table 1. CW polymers in higher plants and algae

<table>
<thead>
<tr>
<th>KINGDOM</th>
<th>SUBKINGDOM</th>
<th>PLAN (VIRIDiplANTAE)</th>
<th>CHAROPHYCEAE</th>
<th>ALL CLASSES</th>
<th>RHODOPHYTA</th>
<th>CHROMISTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>POLYSACCHARIDES</td>
<td>CLASS</td>
<td>EMBRYOPHYTEAE</td>
<td>CHAROPHYCEAE</td>
<td>ALL CLASSES</td>
<td>EMBRYOPHYTEAE</td>
<td>CHAROPHYCEAE</td>
</tr>
<tr>
<td>Para-cristalline fibrils</td>
<td>cellulose</td>
<td>cellulose</td>
<td>cellulose</td>
<td>cellulose</td>
<td>cellulose</td>
<td>cellulose</td>
</tr>
<tr>
<td>Cross-linking glycans</td>
<td>xyloglucans</td>
<td>xyloglucans</td>
<td>xyloglucans</td>
<td>xyloglucans</td>
<td>xyloglucans</td>
<td>xyloglucans</td>
</tr>
<tr>
<td>Carboxylic matrix</td>
<td>pectins</td>
<td>pectins</td>
<td>pectins</td>
<td>pectins</td>
<td>pectins</td>
<td>pectins</td>
</tr>
<tr>
<td>Sulphated matrix</td>
<td>ulvans</td>
<td>ulvans</td>
<td>alginates</td>
<td>alginates</td>
<td>alginates</td>
<td>alginates</td>
</tr>
</tbody>
</table>

Adapted from Popper et al. (2011) with permission.
Table 2. Endo-β-mannanases (MANs) from photosynthetic eukaryotes belonging to glycosyl hydrolase family 5 in the GenBank database (http://www.ncbi.nlm.nih.gov/)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Protein/Gene Name</th>
<th>Genebank accession</th>
<th>Expression</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Eudicot</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rosids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citrus maxima subsp.</td>
<td>CitrMAN</td>
<td>AC097666.1</td>
<td>Seedlings</td>
<td>-</td>
</tr>
<tr>
<td>melo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malus x domestica</td>
<td>MalMAN1</td>
<td>DQ149433.1</td>
<td>Germinating seeds</td>
<td>-</td>
</tr>
<tr>
<td>Glyceria max</td>
<td>GmMAN1</td>
<td>AY588667.1</td>
<td>Vegetative tissue</td>
<td>-</td>
</tr>
<tr>
<td><strong>Populus trichocarpa</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Vitis vinifera</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Arabidopsis thaliana</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lepidium sativum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Asterid</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leucaena</td>
<td>LeuMAN1</td>
<td>A595459.1</td>
<td>Germinating seeds</td>
<td>-</td>
</tr>
<tr>
<td><strong>Solomonum lycopersicum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Oxalis ferox</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coffea arabica</td>
<td>CaMAN8</td>
<td>AU222994.1</td>
<td>Germinating seeds</td>
<td>-</td>
</tr>
<tr>
<td>Lactuca sativa</td>
<td>LcMAN1</td>
<td>AY508148.1</td>
<td>Germinating seeds</td>
<td>-</td>
</tr>
<tr>
<td>Actinidia arguta</td>
<td>AaMAN1</td>
<td>AF387627.1</td>
<td>Germinating seeds</td>
<td>-</td>
</tr>
<tr>
<td>Vitis vinifera</td>
<td>VvMAN1</td>
<td>AY508148.1</td>
<td>Germinating seeds</td>
<td>-</td>
</tr>
<tr>
<td><strong>Monocot</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Musa acuminata</td>
<td>MaMAN2</td>
<td>-</td>
<td>Fruit softening</td>
<td>-</td>
</tr>
<tr>
<td>Hordeum vulgare</td>
<td>HvMAN1</td>
<td>AY508148.1</td>
<td>Germinating seeds</td>
<td>-</td>
</tr>
<tr>
<td>Oryza sativa japonica</td>
<td>OsMAN6</td>
<td>AY508148.1</td>
<td>Germinating seeds</td>
<td>-</td>
</tr>
</tbody>
</table>

**Chlorophyta (green algae)**

| Chlorophyta vulgaris C-169 | MAN8 | BAA95163.1 | -                              | Maki et al., 2000 (Biosci Biotechnol 90, 431-436) |

The phylogenetic classification of the Eudicots is followed as shown in Linkies and Leubner-Metzger (2011). Underlined are those MAN proteins that have been enzymatically characterized. An asterisk indicates the only three-dimensional structure known for a plant MAN (LeMAN4).
photosynthetic eukaryotes have been annotated. From these, 54 are from eudicotyledonous and 10 from monocotyledonous species; only one MAN gene has been reported from the green alga Chlorella vulgaris (Maki et al., 2000). More than 20% of plant MANs appear to have a role in seed germination and post-germination processes (Table 2).

The deduced signature sequence of Arabidopsis, rice, and poplar MAN is [AGS]W-[EQ]L-[MI]-N-E-P-[RHQ]-[CS]. MANs cleave the O-glycosidic bond through a ‘retaining mechanism’, for which two catalytic residues, one acid/base and one nucleophile, are critical. In LeMAN4, these two residues are E204 and E318, which are conserved in plant MANs (Bourgault et al., 2005; Yuan et al., 2007). Besides the catalytic module, MAN, like other known glycosylases, also have a CBM which allows the targeting of the enzyme to its specific polysaccharide substrate within the heterogeneous and dense plant CW (McLean et al., 2002; Boraston et al., 2004; Michel et al., 2009). This specific recognition motif can enhance hydrolysis of insoluble polymers, bringing the catalytic domain near to the substrate and allowing the disruption of polysaccharide clusters (Guillén et al., 2010). There are three types of CBMs: (i) type A has a flat hydrophobic surface composed of aromatic residues that interact with the flat surfaces of the para-crystalline polysaccharides such as cellulose or chitin; (ii) type B binds amorphous xylans, β(1→3)-glucans, β(1→3;4)-glucans, β(1→4)-mannans, glucomannans, and galactomannans; and (iii) type C or lectin-like CBMs that only bind monodi- or trisaccharides (Guillén et al., 2010). CBMs are grouped into 55 families (CAZY database: http://afmb.cnrs-mrs.fr/CAZy), showing notable variations in substrate specificity (Boraston et al., 2004). The CBMs can be localized at the N- or C-terminal end of the carbohydrate-active enzymes (CAZymes); in some cases, tandem CBMs can comprise modules with different substrate specificities (Juge et al., 2002; Abe et al., 2004; Guillén et al., 2010).

A mannan-transglycosylase (MTH) activity, besides its main hydrolytic activity, has been established for tomato LeMAN4 (Carrington et al., 2002; Schröder et al., 2006), and recently this MTH activity has also been described in the Aspergillus niger MAN (van Zyl et al., 2010). MTH activity has also been detected in flowers, fruits, and seedlings of Arabidopsis, in flowers and in the starchy endosperm (Fig. 1C; Guillon et al., 2011). It is interesting to quote the germative strategy of fenugreek (Trigonella foenum-graecum) seeds where the water-imbibing capacity of the endosperm-localized Gal-mannan provides a mechanism to protect the germinating embryo from desiccation if dry conditions follow the initial seed imbibition (Buckeridge, 2010).

**Mannans and MAN in seeds**

**Functional properties of mannans in seeds**

The seed, the dispersal unit of higher plants resulting from sexual reproduction, ensures the survival and perpetuation of the species. Upon seed germination, the radicle protrudes through the previously weakened seed coat (testa) and endosperm (if present). This weakening process takes place in the CW and is mainly carried out by several CW hydrolytic enzymes, expansins, ROS, and the radicle turgor pressure (Holdsworth et al., 2008; Nonogaki et al., 2010; Iglesias-Fernández et al., 2011a, c; Weitbrecht et al., 2011). Mannans appear to be the main polysaccharides in tomato endosperm CWs (>60%), and indirect evidence of the presence of mannans in seeds derives from reverse genetic experiments with knock-out MAN lines of A. thaliana and from MAN enzymatic activity analyses in Sisymbrium officinale upon germination (Groot et al., 1988; Dahal et al., 1997; Iglesias-Fernández and Matilla, 2010, 2011a).

In the seeds of the leguminosae family, Gal-mannans serve mainly as storage components; however, in the endosperm CWs of Schizolobium amazonicum seeds, a linear mannan has a structural role (Pietkowicz et al., 2001). The endosperm cells of the leguminous seeds of Cyamopsis tetragonoloba, Ceratonia siliqua, and Caesalpinia spinosa have thickened CWs enriched with Gal-mannan very heavily substituted with Gal (30–96%; Gidley and Reid, 2006). All these seeds contain large reserves of CW-derived storage carbohydrates deposited in the endosperm SCW, eventually resulting in cells with a reduced cytoplasm and a thick CW; however, during seed formation, Gal-mannans are included transiently in the PCW (Somerville et al., 2004; Davis et al., 2010; Scheller and Ulvskov, 2010). At maturity, the Gal-mannans accumulated in the guar seed endosperm constitute >90% of its weight (Reid, 1995). In these seeds, the highly expressed guar mannan synthase (CtMS5) has an endosperm-specific expression and phylogenetically is closely related to CSLA of Arabidopsis and rice (Fig. 2; Dugga et al., 2004).

Mannans are also detected in high amounts in the storage endosperm and in the aleurone CWs of the wild grass Brachypodium distachyon, a model species for temperate cereals, whose genome has been sequenced recently (International Brachypodium Initiative, 2010; Guillen et al., 2011). Seeds from other monocotyledonous plants such as wheat, barley, and rice also contain Gle-mannans both in the aleurone and in the starchy endosperm (Fig. 1C; Williams et al., 2001; Rhodes et al., 2002). Palm (Phoenix dactylifera) seed endosperm has extremely thick CWs, composed almost entirely of linear mannan, conferring hardness that protects the seeds against mechanical damage (Reid, 1995).

The hydrophilic properties of mannan, especially the galactosyl side branches that are the most hydrophilic part of the molecule, are also important to the ecology of the seed because of their role in water retention and prevention of desiccation of the seed embryo (Penfield et al., 2001; Albersheim et al., 2011). It is interesting to quote the germinative strategy of fenugreek (Trigonella foenum-graecum) seeds where the water-imbibing capacity of the endosperm-localized Gal-mannan provides a mechanism to protect the germinating embryo from desiccation if dry conditions follow the initial seed imbibition (Buckeridge, 2010).
Role of MAN enzymes in the germination of endospermic seeds

The angiosperm seeds consist of the diploid embryo and the triploid endosperm surrounded and protected by the maternal tissue of the testa. The permanence of the endosperm varies depending on the species (Olsen, 2001; Linkies et al., 2010). It may persist as a major storage structure (i.e. endospermic legumes, cereals, and Ricinus communis) or as a thin layer surrounding the embryo (e.g. A. thaliana, Glycine max, L. sativa, and Gossypium herbaceum), or it may be completely absorbed during seed development, disappearing in the dry seed (e.g. P. sativum, Phaseolus sp., and Aesculus hippocastanum). Solanaceous species (e.g. S. lycopersicum and Nicotiana tabacum) have mature seeds with a multilayer aleurone (Petruzelli et al., 2003), whereas seeds with a thin aleurone of one to three cell layers include lettuce (L. sativa, Asteraceae), and the Brassicaceae species Lepidium sativum, S. officinale, and A. thaliana (Iglesias-Fernández and Matilla, 2010; Linkies and Leubner-Metzger, 2012). In some endospermic seeds such are N. tabacum (tobacco), L. sativum (garden cress), S. officinale (hedge mustard), and A. thaliana, it is well documented that germination progresses in two visible and temporally separated steps, with testa rupture and endosperm rupture being two differentially regulated events (Petruzelli et al., 2003; Iglesias-Fernández and Matilla, 2010; Weitbrecht et al., 2011). In the endospermic seeds, the endosperm seems to be the predominant constraining structure to radicle emergence (Nonogaki et al., 2007). However, this role is exerted in the Cucurbitaceae (e.g. muskmelon) by the perisperm, and in the Pinaceae (e.g. Piceae spp.) by the mega-gametophyte (Bewley, 1997).

Several CW remodelling proteins and ROS are also involved in the endosperm weakening (Müller et al., 2009). These proteins include MANs, endo-β(1→3)-glucanases, chitinases, peroxidases, expansins, etc. (Bewley, 1997; Wu et al., 2001; Koornneef et al., 2002; Leubner-Metzger, 2003; Bailly, 2004; Iglesias-Fernández et al., 2011a; Morris et al., 2011; Matilla-Vázquez and Matilla, 2012). In many endospermic seeds (e.g. locust bean, guar, lettuce, tomato, and pepper), MANs are produced in the lateral endosperm following germination, mobilizing the mannan reserves that
contribute to the early growth of the seedlings (Nonogaki and Morohashi, 1999). These isoenzymes are different from those produced in the micropylar endosperm, which are involved in the endosperm cap weakening prior to radicle emergence (Voigt and Bewley, 1996).

**Eudicotyledonous seeds**

*Arabidopsis thaliana* (Rosids): In the model plant *A. thaliana*, whose genome has been completely sequenced (Arabidopsis Genome Initiative, 2000), the MAN gene family is represented by eight members, all with canonical signal peptides, and only four of them (*AtMAN2, AtMAN5, AtMAN6*, and *AtMAN7*) are expressed in germinating seeds. The transcript accumulation of these genes is restricted to the micropylar endosperm and the radicle, disappearing soon after radicle emergence. Knock-out mutants in *AtMAN5, 6*, and *7* have a lower germination rate than the wild type, particularly those in *AtMAN6*, suggesting a putative role for these genes in seed germination; a possible cooperation between the radicle and the endosperm MAN activities prior to radicle protrusion has also been hypothesized (Iglesias-Fernández et al., 2011a, b). In other species of the Brassicaceae family, such as *S. officinale* L., MAN activity increases sharply in dry after-ripened seeds and before radicle emergence, and this activity is ethylene and gibberellin dependent (Iglesias-Fernández and Matilla, 2009). In *L. sativum*, transcripts of *LesaMAN7* accumulate in the micropylar endosperm and, to a lesser extent, in the radicle prior to protrusion; after endosperm rupture, these transcripts are accumulated in the cotyledons (Morris et al., 2011). However, although the MAN expression studies and the knock-out mutants are valuable tools for understanding the germination process, these studies should be reinforced by establishing the characterization and the spatial location of mannans in the CWs of these seeds.

*Solanum lycopersicum* (Asterids): During tomato seed germination, three genes encoding MANs (*LeMAN1, 2*, and *3*) are expressed sequentially in different parts of the endosperm (Nonogaki et al., 1998a). *LeMAN1* and *LeMAN3* are synthesized in the lateral endosperm after germination and have been implicated in the mobilization of mannan reserves in CWs (Nonogaki et al., 1998b; Belotserkovsky et al., 2007; Gong and Bewley, 2007). *LeMAN2* expression and its enzyme activity take place in the micropylar area prior to radicle emergence, and most probably is involved in the weakening of the micropylar endosperm to facilitate radicle emergence (Nonogaki et al., 1998a, b; Toorop et al., 2000; Gong and Bewley, 2007). This two-peak pattern of MAN activity is also present in other species such as *Daucus carota* and *Sesbania virgata* (Homrichhausen et al., 2003; Lisboa et al., 2006). In other Solanaceae species, the accumulation of the MAN proteins and transcripts in the micropylar endosperm is mediated by phytochrome (Arana et al., 2006).

*Lactuca sativa* (Asterids)

The micropylar endosperm in lettuce seeds exerts a notable germination constraint for radicle protusion. However, the MAN activity in lettuce endosperm is associated with reserve mobilization closely following radicle emergence rather than prior to endosperm weakening (Wang et al., 2004). Therefore, hemicellulases different from MAN should be more critical for the weakening of the micropylar endosperm and the induction of germination in these seeds.

**Other eudicotyledonous species**: In other species, such as *Glycine max* (soybean), *Coffea arabica* (coffee), and hard-seeds, e.g. *Ceratonia siliqua* (carob), *Senna obtusifolia* (Chinese senna) and *Trigonella foenum-graecum* (fenugreek), MANs are likely to have a role in the degradation of mannan-rich reserves during seedling growth as occurs in lettuce. In soybean, four genes (*GmMAN1–GmMAN4*) have been described as expressed in seeds, but only *GmMAN1* shows a MAN-hydrolase activity and is specifically expressed in cotyledons of 14-day-old seedlings. In coffee, two MAN genes (*CaMANA* and *CaMANB*) are only expressed after seed germination (Marraccini et al., 2001; Lin et al., 2011).

**Monocotyledonous seeds**

In contrast to the extensive investigation of MAN in eudicotyledonous seeds, only a few works have been published in monocots, mainly in cereal seeds (DeMason et al., 1985; Dirk et al., 1995; Wang et al., 2005; Hrmova et al., 2006).

*Oryza sativa*: In the complete sequenced genome of rice (International Rice Genome Sequencing Project, 2005), the MAN gene family is represented by nine members (*OsMAN1–OsMAN8* and *OsMANP*). Four rice MAN genes (*OsMAN1, OsMAN2, OsMAN6*, and *OsMANP*) are expressed in germinating and germinated rice seeds prior to radical emergence, three transcripts are expressed in the scutellum (*OsMAN1, 2*, and *6*) and two in the aleurone layer (*OsMAN1* and *OsMANP*; Yuan et al., 2007). Three isoforms of the enzyme (pI 8.86, pI 8.92, and pI 8.98) are found in the starchy endosperm and aleurone layer of germinating seeds, but only two of them are expressed in the scutellum. MAN proteins are also present in dry seeds. The amount of this protein decreases in the scutellum, but increases in the aleurone layer during germination (Ren et al., 2008).

*Hordeum vulgare* (barley): In barley coleoptiles, the mannan-containing polysaccharides are minor CW components (<0.5% of total CW polysaccharides; Gibeaut et al., 2005). HvMAN1 has been purified from extracts of 10-day-old barley seedlings and its corresponding gene has been detected in the scutellum of germinated barley grain, but the highest
Biotechnological potential of MAN enzymes

The biotechnological potential of MANs is quite extensive, and currently they are used in different types of industry such as the pulp/paper and textile industry, coffee manufacturing, feed and food production, etc. Moreover, MANs are being tested for their potential in second- generation biofuel production. Consequently, there are many studies describing biochemical and kinetic parameters of MAN from different origins, mainly from microorganisms and yeasts (Supplementary Tables S1, S2 at JXB online). Site-directed mutagenesis and other protein engineering techniques are being used to improve the catalytic efficiency or the resistance to hot temperatures and extreme pHs of MANs. Improving MAN expression in heterologous systems, to find suitable enzymes for these industries, is also being pursued (Wen et al., 2009).

One of the applications of MANs is their use in the enzymatic pre-bleaching of softwood pulps. The currently used alkaline pre-treatment causes an environmental pollution problem that could be solved using an enzymatic pre-treatment (Dhawan and Kaur, 2007). The majority of pulps are derived from softwoods, where 20% of hemicelluloses are (Gal–Glc)-mannans. In this context, MANs would be excellent candidates for use in the enzymatic bleaching, particularly the thermophilic and alkalophilic MANs (Yanhe et al., 2004). Currently, studies in this field are searching for a suitable cocktail of different hydrolytic enzymes (xylanases, MANs, α-galactosidases, etc.) to improve bleaching (Clarke et al., 2000).

In order to obtain biofuel from lignocellulosic (LC) biomass, a first thermochemical or biochemical processing is necessary (Larson, 2008). The thermochemical process uses high temperatures and high pressures. The biochemical process implies the conversion of LC materials to biofuels in four major steps (Balat et al., 2008): (i) a chemical or mechanical pre-treatment which promotes the physical disruption of the LC matrix; (ii) the enzymatic hydrolysis of the cellulose and hemicellulose to fermentable sugars; (iii) fermentation of the sugars to bioethanol; and (iv) the distillation of the ethanol. MANs can be useful in the two first steps particularly when the LC biomass is rich in mannans, as occurs in softwoods (Do et al., 2009).

MANs together with other degrading enzymes (cellulases, proteases, lipases, amylases, pectinases, and xyloglucanases) are employed in detergent composition as stain removal boosters, including laundry, personal cleansing, and oral/dental products (Kirk et al., 2002). However, the use of MANs in cleaning compositions requires enzymes with high activity at basic pHs; in this context, MANs produced by fungi are being explored (Wen et al., 2009). MAN preparations are also employed in combination with cellulases, xylanases, and pectinases to reduce the viscosity of coffee extracts (Nunes et al., 1998, 2006; Gorgens et al., 2006). The treatment of mannan-rich meals with MANs results in facilitating feed digestion, decreasing the intestinal viscosity, and improving weight gain in several farm animals (Dhawan and Kaur, 2007). Another important application of MAN enzymes is for enhancing the flow of oil and gas drillings (Politz et al., 2000).

Future perspectives

In the near future, our knowledge about plant mannan-rich CWs will most probably enlarge, since new interdisciplinary technologies such as the use of monoclonal antibodies against several CW carbohydrate epitopes together with confocal microscopy and computer-based image analyses are being applied to clarify the CW structure and composition. The transcriptional and post-transcriptional regulation of MAN genes will help to elucidate its dual functional role upon germination. In the model plant A. thaliana, molecular platforms can be used to this aim: DNA microarrays, libraries of arrayed transcription factors, ChIP-seq and RNA-seq facilities, knock-out gene collections, etc. These platforms are quickly being extended to other plant species at the same time as their genomes are available, thanks to the new high-throughput sequencing technologies.

From a biotechnological point of view, a more systematic analysis of MAN enzymes is needed, mainly to optimize the physical disruption of the LC matrix of softwoods and to improve the enzymatic cocktail to hydrolyse the cellulose and hemicellulose mixtures to fermentable sugars. In this regard, cloning of the mannanase genes, expression of these genes in bacteria or yeast, and determination of the kinetic parameters of the corresponding enzymes and their optimum pH, etc. are needed. Eventually, exon shuffling and/or site-directed mutagenesis will help to improve the biotechnological potential of plant MANs.

Supplementary data are available at JXB online.


This work was financially supported by grants CGL2009-11425 and BFU2009-11809 from the Ministerio de Ciencia e Innovación (MICINN, Spain). RI-F is supported by a Juan de la Cierva post-doctoral contract (JCI-2010-07909) from...
We thank G. Revilla and J. Sampedro (USC, Spain) for critical reading of the manuscript.


Do B-C, Dang TT, Berrin JG, Haltrich D, To KA, Sigoillot JC, Yambayai M. 2009. Cloning, expression in Pichia pastoris, and characterization of a thermostable GH5 endo-1,4-β-mannosidase from Aspergillus niger BK01. Microbial Cell Factories 8, 59–70.


domain of glucoamylase from *Aspergillus niger*: overview of its structure, function, and role in raw-starch hydrolysis. *Biologia Bratislava* 57, 233–245.


Leubner-Metzger G. 2003. Functions and regulation of \(\beta\)-1,3-
glucanases during seed germination, dormancy release and after-
ripening. *Seed Science Research* 13, 17–34.


Lisboa CGS, Tonini PP, Tiné MAS, Buckeridge MS. 2006. Endo-


function mutation of REDUCED WALL ACETYLATION2 in Arabidopsis leads to reduced cell wall acetylation and increased resistance to *Botrytis cinerea*. *Plant Physiology* 155, 1068–1078.


Ren YF, Bewley JD, Wang XF. 2008. Protein and gene expression patterns of endo-β-mannanase following germination of rice. Seed Science Research 8, 139-149.


Tonini PP, Lisboa CGS, Silva CO, Mazzoni-Viveiros SC, Buckeridge MS. 2007. Testa is involved in the control of storage


