Expression of Chia4-Pa chitinase genes during somatic and zygotic embryo development in Norway spruce (*Picea abies*): similarities and differences between gymnosperm and angiosperm class IV chitinases

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Abstract

The developmental pathway of somatic embryogenesis in Norway spruce involves proliferation of proembryogenic masses (PEMs), PEM-to-somatic embryo transition and further development of the somatic embryos. It has previously been shown that extracellular signal molecules, including arabino-galactan proteins, lipo-chitooligosaccharides and chitinases, regulate somatic embryogenesis. The Chia4-Pa1 gene from Norway spruce is described here. The Chia4-Pa1 encodes a typical basic class IV chitinase, although the intron–exon organization of this gymnosperm chitinase is different from that in angiosperm class IV chitinases. The Chia4-Pa1 belongs to a small gene family with highly similar members, and the expression pattern of Chia4-Pa1 cannot be distinguished from that of other Chia4-Pa members. Upon withdrawal of plant growth regulators, i.e. during a treatment that stimulates PEM-to-somatic embryo transition and massive programmed cell death, a significant increase in transcription and translation of Chia4-Pa genes takes place. The expression pattern analysis revealed that Chia4-Pa genes are expressed in a subpopulation of proliferating cells and at the base of the somatic embryo. Furthermore, in seeds, Chia4-Pa genes are expressed in the megagametophyte in the single cell-layered zone surrounding the corrosion cavity. Taken together these results suggest that the Chia4-Pa expressing cells have a megagametophyte signalling function and that CHIA4-Pa stimulates programmed cell death and promotes PEM-to-somatic embryo transition.

Key words: Chitinase, embryogenesis, endosperm, evolution, gymnosperms, *Picea abies*.

Introduction

The early events in embryogenesis are the most critical for plant body pattern formation. In embryo-defective mutants of angiosperms, deviations during early embryogenesis lead either to immediate developmental arrest and abortion or to a progressive accumulation of errors in morphogenesis. In gymnosperms, embryo-defective mutants are not available. Instead, the regulation of embryo development is studied using somatic embryogenesis. In recent years, embryogenic cultures of Norway spruce [*Picea abies* (L.) Karst.] have been used extensively for studying the regulation of embryo development (von Arnold *et al.*, 2002). The possibility of comparing early embryo development in angiosperms and gymnosperms is particularly interesting since these two groups separated about 300 million years ago.

Somatic embryogenesis of Norway spruce can be divided into two distinct phases. The first phase is
represented by proembryogenic masses (PEMs). In the presence of auxin and cytokinin, PEMs proliferate, changing both cellular organization and number of aggregated cells. The second phase corresponds to PEM-to-somatic embryo transition, which occurs after withdrawal of plant growth regulators (PGRs) (Filonova et al., 2000b). Further development of the somatic embryos follows the developmental pattern described for the zygotic embryo development. PEM-to-somatic embryo transition is a key step determining the yield and quality of mature somatic embryos in Norway spruce (Bozhkov et al., 2002). This transition is associated with massive programmed cell death (PCD) taking place in PEMs that give rise to somatic embryos (Filonova et al., 2000a). The signal pathway that triggers PCD is normally suppressed by a constant supply of PGRs or signal molecules (Dyachok et al., 2002; Jacobson et al., 1997).

Chitinases are enzymes that hydrolyse β-1,4-N-acetyl-D-glucosamine (GlcNAc) linkages. Those with lysozyme activity also cleave β-1,4 linkages between GlcNAc and N-acetylmuramic acid. They are present in a broad range of organisms including bacteria, fungi, plants, and animals. According to their primary structure, chitinases are divided into seven classes (classes I–VII). (For a schematic description of the various chitinases see Fig. 1.) Plant chitinases and lysozymes are likely to have arisen from one coancestor by divergent evolution (Monzingo et al., 1996). The protein genealogy of chitinases shows that class I and class II chitinase genes evolved from the same ancestral gene (Araki and Torikata, 1995; Shinshi et al., 1990). Moreover, a basic class II chitinase is a putative ancestor of basic class I and acidic class II chitinase genes (Ohme-Takagi et al., 1998). It has also been proposed that chitinases in class IV, which are phylogenetically related to class I and II chitinases (Araki and Torikata, 1995; Gomez et al., 2002; Hamel et al., 1997), evolved from a class I chitinase gene by four deletions in the coding sequence (Araki and Torikata, 1995).

Among many other functions, chitinases can stimulate embryo development (van Hengel et al., 1998) and seed development (Van Damme et al., 1999). Besides chitin, the primary substrate for chitinase, which is not present in plants, chitinases can also hydrolyse arabinogalactan proteins (AGPs) (van Hengel et al., 2001), rhizobial Nod factors (Staelhelin et al., 1994) and other lipo-chitooligosaccharides (LCOs) (Dyachok et al., 2002). Since the enriched pattern of proteins found in cell suspensions includes seed-specific proteins, it has been assumed that some somatic cells show endosperm properties (Kragh et al., 1993). It has been hypothesized that endosperm and embryo interact during their development (Berger, 1999). The evidence supporting endosperm–embryo interaction is derived from the carrot system. The EP3 chitinase that is expressed in the endosperm of carrot, rescue somatic embryos of carrot ts11 variant (van Hengel et al., 1998).

**Materials and methods**

**Plant material**

Embryogenic cell lines of Norway spruce [*Picea abies* (L.) Karst.] were stored in liquid nitrogen and successively thawed and re-established (Bozhkov et al., 2002). Three of the cell lines, A95:88:22 (A22), A95:61:21 (A21) and A95:88:17 (A17), were of A-type and produced normal mature cotyledonary embryos when given a maturation treatment. The number of mature embryos was higher for cell lines A22 and A17 than for cell line A21. One cell line was of B-type (B41) and produced a low number of abnormal cotyledonary embryos in response to the maturation treatment. Cultures were maintained as described previously (Dyachok et al., 2002). In order to induce PEM-to-somatic embryo transition, the cultures were transferred to liquid medium lacking 2,4-dichlorophenoxyacetic acid (2,4-D) and 6-benzylaminopurine (BA) (Filonova et al., 2000b). After 1 week the cultures were plated on maturation medium containing 30 µM abscisic acid (ABA) (Filonova et al., 2000b).

**The Chia4-Pa cDNA sequence**

The cDNA was prepared as described in Ingouff et al. (2001). Based on the sequence of *PgChi-1* from white spruce (*Picea glauca*; L42467) two primers were designed: CHI3, 5'-ATAAGAATGCG-GCCGCTATGGGAGTAGTAGTGATAGTAAAAATC-3' and CHI2, 5'-ATAAGAATGCGCCGCTTACAGGAGGAGGACATTGGCTCC-3'. The full length sequence of *Chia4-Pa* was obtained from cDNA from proliferating embryogenic cultures of Norway spruce cell line A22 using Chia4-Pa1S, 5'-GATCTTACGGGAATTATTGGC-3' and the adapter 5'-GACTCGAGTGCACATCG-3' oligonucleotides. The PCR products were amplified with the Expand High Fidelity PCR System (Boehringer Mannheim) using cDNA from proliferating embryogenic cultures of Norway spruce as a template. PCR was carried out for 35 cycles of 94 °C for 20 s, 55 °C for 30 s and 72 °C for 90 s. The amplification products were cloned in the pGEM-T-easy vector (Promega) and sequenced.
**Expression pattern of a gymnosperm class IV chitinases**

Total plant DNA and RNA were isolated according to Chang et al. (1993). For RNA blot analysis 15 µg of total RNA was separated by gel electrophoresis and blotted to a Hybond-N+ nylon membrane (Amersham). Total DNA (15 µg) was digested with BamHI, EcoRI, EcoRV, and HindIII, separated on 1% agarose gel and transferred to Hybond-N+ nylon membrane, as described by Sambrook et al. (1989). Purified PCR product corresponding to the ORF of Chia4-Pa1 cDNA (amplified using CH3 and CH2 primers) was labelled with digoxigenin by using DIG RNA Labelling Kit T3/pGEM-T-Easy (Promega). The sense and antisense RNA probes corresponding to the catalytic domain of Chia4-Pa1 were labelled by using random priming labelling kit (Pharmacia). Hybridization was performed overnight at 42 °C in 5× SSC, 4× Denhardt’s solution, 0.1% SDS, 40% formamide, 1% dextran sulphate, and 100 µg ml⁻¹ denatured salmon sperm DNA. The membranes were washed in 2× SSC, 0.1% SDS for 2×15 min at room temperature and in 0.1× SSC, 0.5% SDS for 20 min at 65 °C.

**Phylogenetic analysis**

The cDNA sequences of 25 chitinase genes with known intron pattern were aligned using ClustalW (Thompson et al., 1994) and further refined manually (complete alignment available from the authors on request). Phylogenetic trees were constructed by the Neighbor-Joining (NJ) and maximum parsimony (MP) methods. NJ analyses were carried out in MEGA2 (Kumar et al., 2000) by using the Kimura 2-parameter and LogDet parallel correction distance. The MP searches were conducted in PAUP version 4.0b (Swofford, 1999), using heuristic searches with 10 random additional replicates and TBR branch swapping. Nodal support was estimated using bootstrap analyses based on 1000 replicates.

**The Chia4-Pa1 mRNA in situ hybridization**

Proliferating cultures containing various stages of proembryogenic masses, maturing embryos and germinating seeds were fixed as described for Ingouff et al. (2001). A 615 bp PCR fragment corresponding to the catalytic domain of Chia4-Pa1 was cloned into pGEM-T-Easy (Promega). The sense and antisense RNA probes were labelled with digoxigenin by using DIG RNA Labelling Kit T3/pGEM-T-Easy (Promega). Hybridization was performed overnight at 42 °C in 5× SSC, 4× Denhardt’s solution, 0.1% SDS, 40% formamide, 1% dextran sulphate, and 100 µg ml⁻¹ denatured salmon sperm DNA. The membranes were washed in 2× SSC, 0.1% SDS for 2×15 min at room temperature and in 0.1× SSC, 0.5% SDS for 20 min at 65 °C.

**Extracellular protein extractions and western blot analysis**

Proteins were extracted from media that were conditioned for 7 d by extracellular protein extractions and western blot analysis. Extracellular protein extractions and western blot analysis were performed as described for Ingouff et al. (2001).

**Immunolocalization of CHIA4-Pa**

Germinating seeds were fixed as described in Ingouff et al. (2001). Sections of 4–6 µm were incubated for 20 min in xylene (100%) and then rehydrated through a series of ethanol baths (99, 95, 70, 50, and 30%, v/v) for 1 min each. Then, the slides were washed in PBS (20 min) and stored dry until use. For Norway spruce cell lines A22, A21 and B41, whole mounts of material and sections were used. Samples were taken at the stage of proliferation (7 d after subculturing) and maturation (2 weeks and 4 weeks of ABA treatment). Half of the samples were immediately fixed in 4% (v/v) formaldehyde in PBS as previously described (Filonova et al., 2000). Immunolocalization of CHIA4-Pa chitinases in fixed and unfixed plant material was carried out as described for the western blot analysis, except that the primary antibody was diluted 1:1000.

**Results**

**Isolation of the Chia4-Pa1 gene and phylogenetic analysis**

The Chia4-Pa1 sequence was isolated from cDNA from proliferating embryogenic cultures of Norway spruce. The first set of primers was designed based on the sequence of the class IV chitinase gene (L42467) from white spruce. An amplified fragment of 831 base pairs (bp) covered the complete ORF of Chia4-Pa1. Another set of specific primers was designed and used to amplify the untranslated regions of the Chia4-Pa1 gene. The 24 bp of 5’-UTR and 96 bp of 3’-UTR were obtained by 5’- and 3’-RACE screening, respectively, using cDNA from proliferating embryogenic cultures as a template. The sequence of the Chia4-Pa1 cDNA can be found in Genbank, accession number AY270018.

To analyse the genetic relationships of plant chitinases, phylogenetic trees were constructed based on class I, II, IV, and VII chitinase genes. Class III, V and VI genes were excluded from the comparison because of their markedly different primary structure. The phylogenetic analysis revealed the presence of several subgroups. Class IV chitinases form a highly supported subclass comprising two sister groups. One group consists of Chia4-Pa1 and the other gymnosperm gene from white spruce, while the other comprises angiosperm chitinases from Arabidopsis.
and carrot (Fig. 2A). This grouping was present irrespective of the method used in the phylogenetic analysis (see Materials and methods).

Sequencing of the corresponding genomic region showed that the \textit{Chia4-Pa1} gene contains two introns within its ORF, numbered 2 and 3 (Fig. 2B). The position of the introns was compared with that from other known plant chitinases. An intron pattern identical to that of the \textit{Chia4-Pa1} gene exists in some members of class I and II chitinases, while all known angiosperm class IV and VII chitinases have the intron at the position number 2, and lack the intron at position 3. Thus, the intron±exon organization of the \textit{Chia4-Pa1} gene does not follow the pattern present in closely related class IV and VII chitinase genes. Instead, \textit{Chia4-Pa1} has an identical intron pattern with some dicot class I and gymnosperm class II chitinases. Introns in class I, II, IV, and VII chitinase genes are present at a few positions determined by the 5′- and 3′- boundaries of the conserved motifs (Fig. 2B).

Interestingly, analysis of angiosperm class IV genes revealed the presence of the 5′- and 3′- boundary at position number 3, even though intron number 3 is absent.

A gel-blot of Norway spruce genomic DNA (pool of different individuals) digested with four different restriction enzymes was probed with the PCR fragment of \textit{Chia4-Pa1} (see Materials and methods). Under stringent conditions four or five bands (non-cutter or cutter enzyme, respectively) were detected (data not shown). This multiband pattern suggests that \textit{Chia4-Pa1} belongs to a small gene family. Partial sequencing confirms the presence of other, highly similar chitinase genes, for example \textit{AY270019}, although, at this stage, the possibility cannot be excluded that they are different alleles of the same gene. Owing to the high similarity (over 96%) among the \textit{Chia4-Pa} genes, the conclusion about the expression pattern of the \textit{Chia4-Pa1} gene had to be expanded to the whole \textit{Chia4-Pa} family.

Northern blot analysis using a PCR fragment of \textit{Chia4-Pa1} as a probe revealed a single 1 kb transcript (Fig. 3A). In proliferating embryogenic cultures of Norway spruce the \textit{Chia4-Pa} genes were expressed at a low level in all the tested lines. After withdrawal of PGRs the expression of \textit{Chia4-Pa} increased significantly in all cell lines (Fig. 3A). Similar results were obtained in three independent experiments.

The \textit{Chia4-Pa1} gene encodes a predicted protein of 276 amino acids organized into a signal peptide (26 aa), a highly conserved chitin-binding domain (33 aa), a hinge region (13 aa), and a catalytic domain (204 aa). The primary structure, predicted pI value of 9.3 and molecular weight of 25.9 kDa, of the mature CHIA4-Pa1 are typical for chitinases belonging to class IV chitinases (Fig. 1). A database BLAST search (Altschul et al., 1997) revealed that the CHIA4-Pa1 had a high similarity to plant
Extracellular chitinases in embryogenic cultures of Norway spruce

The CH4 antibody (raised against class IV chitinase from sugar beet) was used for the detection of CHIA4-Pa1. The recognition of CHIA4-Pa1 by CH4 antibody was tested by western blot analysis using the GST–CHIA4-Pa1 fusion protein. The GST antibody cross-reacted with the control (GST) and GST–CHIA4-Pa1 proteins. By contrast, the anti-CH4 recognized the GST–CHIA4-Pa1, but not the GST (data not shown), which confirmed that the CH4 antibody recognized CHIA4-Pa1.

Proteins secreted by proliferating embryogenic suspension cultures of Norway spruce were subjected to SDS-PAGE and western blot analysis. A representative separation is shown in Fig. 3B. Proteins recognized by anti-CH4 were present in all the tested lines. Two bands (26 and 28 kDa) were in accordance with those previously reported in Norway spruce (Egertsdotter and von Arnold, 1998). However, the amount of secreted 26/28 kDa chitinases varied between cell lines and could be correlated with the type of cell line and its ability to produce somatic embryos. The cell line A22, which produces a high yield of mature somatic embryos, secreted more 26/28 kDa chitinases of class IV and I. The deduced CHIA4-Pa1 amino acid sequence showed 73% similarity to the CH4 chitinase from sugar beet.

Expression pattern of a gymnosperm class IV chitinases

In order to compare the localization of the CHIA4-Pa proteins with the expression pattern of the encoding genes, in situ mRNA hybridization and immunolocalization of CH4-related proteins were performed at different stages of embryo development (Figs 4, 5). No signal was detected in the controls (Figs 4G, 5B). In proliferating embryogenic cultures, Chia4-Pa mRNA was detected only in subpopulations of cells (data not shown). It was not possible to relate this subpopulation to a specific cell type or to a specific developmental stage of PEMs. However, owing to the fixatives used during sample preparation, most of the highly vacuolated cells were destroyed, so it is not known if Chia4-Pa genes are expressed in suspensor cells. The CHIA4-Pa proteins were detected on the surface of unfixed embryonal masses, but no signal could be detected in fixed material, indicating that CHIA4-Pa are bound by weak ionic forces. Therefore, whole mounts of living materials were incubated with anti-CH4 and appropriate secondary antibodies. After addition of NBT/BCIP substrate a purple precipitation appeared. Owing to the high background caused by the presence of endogenous phosphatases, it was difficult to observe signals in PEMs and suspensor cells.

During early embryogeny, strong expression of the Chia4-Pa genes was found in a few cells, mostly at the base of the embryonal mass (Fig. 4A, B). This expression pattern became more distinct at the beginning of late embryogeny when Chia4-Pa were mainly expressed in the outer part of the root cap region (Fig. 4C). At the time of early embryogeny, CHIA4-Pa were localized at the surface of the embryonal mass (Fig. 5A).

During late embryogeny and maturation, the expression of Chia4-Pa was down-regulated in the root-cap and up-regulated in the cortex, in a ring of cells reflecting the positions of emerging cotyledonary ridges (Fig. 4D, E, F). In maturing somatic embryos, the CHIA4-Pa proteins were localized at the surface of the somatic embryo, but not in the root cap region (Fig. 5C). Later, in mature somatic embryos, the CHIA4-Pa signal became at first limited to small patchy areas localized at the surface of the embryo, and finally declined. Interestingly, abnormal embryos from
line B41 did not show any signals (data not shown). Cross-sections of maturing somatic embryos revealed the presence of CHIA4-Pa in the shoot meristem region (Fig. 5D).

In seeds, the Chia4-Pa genes were strongly expressed in the inner part of the megagametophyte, in a single cell layer surrounding the corrosion cavity (Fig. 4H). During germination, Chia4-Pa were also expressed in the megagametophyte, both in the cell layer surrounding the corrosion cavity and in the micropylar region (Fig. 4I). The CHIA4-Pa proteins were localized in the micropylar region of the megagametophyte, but not in the cell layer surrounding the corrosion cavity (Fig. 5E, F).

Discussion

Evolution of class IV chitinases in plants

It has been proposed that class I and II chitinase genes evolved from the same ancestral gene (Araki and Torikata, 1995; Shinshi et al., 1990). Moreover, Araki and Torikata (1995) suggested that genes of low molecular weight class VII (formerly class II-L) and class IV chitinases evolved from the high molecular weight genes (class I and II chitinases). According to Hamel et al. (1997) the derivation of the class IV lineage from a common ancestral sequence would have occurred before the separation of monocots and dicots, estimated to have taken place around 200 million years ago. According to the results presented in this paper, which are in agreement with work by Gomez et al. (2002), class IV chitinases probably evolved from class I or II chitinases more than 300 million years ago, i.e. before the separation of angiosperms and gymnosperms. In this case, the derivation of the class IV lineage from a common ancestral sequence occurred even earlier than Hamel et al. (1997) suggested.

It has previously been shown that the phylogenetic classification of some proteins is supported by the exon-
intron structures of the corresponding genes (Ingouff et al., 2001). In chitinase genes of classes I, II, IV, and VII, there are up to two introns which are present at positions determined by highly conserved boundaries. It is most likely that intron number 3 was lost during evolution while intron number 1 was gained. The phylogenetic analysis revealed that the Chia4-Pa1 gene belongs to a highly supported subclass including other class IV chitinases. However, the intron-exon structure suggests that, in this respect, Chia4-Pa1 is more similar to class I and II chitinases than to angiosperm class IV and VII chitinases. The analysis of Arabidopsis genes revealed that all known class IV chitinases have the same gene structure, with one intron at the position number 2 (data not shown). It is tempting to assume that gymnosperm class IV genes have introns at positions 2 and 3, while angiosperm class IV chitinases possess an intron only at position number 2 and lack intron number 3. However, information from other gymnosperm class IV genes is required.

Increased expression of Chia4-Pa correlates with PEM-to-somatic embryo transition

A significant increase in transcription and translation of Chia4-Pa takes place upon withdrawal of PGRs in embryogenic cultures. Withdrawal of PGRs stimulates PEM-to-somatic embryo transition and concomitant activation of PCD (Filonova et al., 2000a). The EP3 and AtEP3/AtchitIV chitinases were proposed to be involved in regulating PCD in carrot and Arabidopsis (Passarinho et al., 2001; van Hengel et al., 1998). From these data, it is suggested that ChIA4-Pa mediate PEM-to-somatic embryo transition by direct or indirect activation of PCD.

It has previously been shown that AGPs and LCOs as well as chitinases can stimulate somatic embryogenesis in Norway spruce (Dyachok et al., 2002; Egertsdotter and von Arnold, 1998). The identity of cells or tissues might be reflected by the AGPs present in the cellular matrix (Nothnagel, 1997). Recently, van Hengel et al. (2001) showed that AGPs contain GlcNAc and Glc groups in a form that can be hydrolysed by chitinases. Moreover, the presence of AGPs that are sensitive to a chitinase treatment and which can also stimulate somatic embryogenesis was reported in carrot (Van Hengel et al., 2002) and in Carribean pine (Domon et al., 2000). Interestingly, van Hengel et al. (2001) also found that the susceptibility of certain AGPs to endochitinase activity increases during embryo development. So far, the identity and localization of substrate for CHIA4-Pa chitinases remain unknown. However, it is likely that CHIA4-Pa chitinases migrate towards the place where a substrate, for example AGP, is localized. The cover film of CHIA4-Pa bound to the surface of embryonal masses might act on AGPs present in epidermal cell walls, causing either loosening of the cell walls or protection of the embryonal masses from signal molecules causing proliferation.

Chia4-Pa is expressed in ‘nurse cells’

The in situ mRNA analysis showed that Chia4-Pa genes are expressed in a subpopulation of cells in proliferating embryonic cultures, and during embryo development in cells at the base of the embryonal mass. Expression of chitinase genes in cells close to the developing embryo, but not in the embryo itself, was also reported for EP3 and AtEP3/AtchitIV (Passarinho et al., 2001; van Hengel et al., 1998).

In seeds the Chia4-Pa genes are predominantly expressed in the single cell zone surrounding the corrosion cavity. These data are in agreement with those in van Hengel et al. (1998) where EP3 mRNA was detected in a few inner layers of the integuments (early stages) and in the endosperm (late stages). By contrast, the Arabidopsis AtEP3/AtchitIV, an orthologue of EP3, is not expressed in the integument or in the endosperm (Passarinho et al., 2001). However, Arabidopsis has only the remains of
Endosperm and, therefore, might lack the cell-layer where the EP3 chitinase is expressed.

Failure of endosperm development usually results in embryo abortion (Birchler, 1993). The importance of an interaction between the embryo and the megagametophyte nursing organ in conifers, has been shown in pine (Filonova et al., 2002). Moreover, in Arabidopsis, the maternal MEA allele is required for proper endosperm and embryo development. Mutation in MEA causes precocious endosperm formation before fertilization and prolonged endosperm nuclear proliferation after fertilization (Kiyosue et al., 1999). Defective functioning of the endosperm causes, at least to some extent, mea embryo abortion (Kinoshita et al., 1999). Chitinases that promote somatic embryogenesis are mainly expressed by other cells than those in the embryo proper (Passarinho et al., 2001; van Hengel et al., 1998, 2001). Moreover, since the expression pattern is similar in a gymnosperm (Norwegian spruce) and in an angiosperm (carrot) this function appears to be evolutionarily conserved. In addition, in Drosophila, chitinase-like molecules (the IDGF transcripts) are detected in the nurse cells and the oocyte, but are absent from follicle cells. The fat body may, therefore, be an important source of growth factors that support peripheral tissue growth during insect development (Kawamura et al., 1999). Moreover, in mammals, chitinase-like proteins such as oviducin and YK1-40 are involved in fertilization and morphogenesis, respectively (Bleu et al., 1999). Therefore, the signalling function of maternal tissue seems to be highly conserved among plants (gymnosperm and angiosperm) and animals.

In conclusion, it is shown that Chia4-Pa1 encodes a basic class IV chitinase. Chia4-Pa1 is a member of a small family, the Chia4-Pa. The results suggest that CHIA4-Pa chitinases regulate the differentiation of somatic embryo from PEMs by promoting PCD. The Chia4-Pa are expressed in ‘nurse cells’, but not in the early embryo proper.

Supplementary data

For a table detailing the intron–exon structure of class IV chitinases please refer to Journal of Experimental Botany online.

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