Additional file 7

Glycoside hydrolases (GHs) are involved in the metabolism of various carbohydrates containing compounds present in plant tissues, but the majority of the GH enzymes are involved in cell wall polysaccharide metabolism (Cosgrove, 2005; Minic and Jouanin, 2006; Minic, 2008). In Arabidopsis (*Arabidopsis thaliana* L.), cell wall associated β-glucosidase (βG, EC 3.2.1.21), which breaks down polysaccharides to soluble sugars, is induced by starvation (Lee et al., 2007) and senescence (Mohapatra et al., 2010). In barley (*Hordeum vulgare* L.), hydrolysis of β-linked oligosaccharides results in cell wall degradation in endosperm during seed germination (Leah et al., 1995).

For studying the role of cell wall hydrolyzing enzyme βG in the cell wall breakdown, the Scots pine βG gene (KM046994) was sequenced. The predicted βG protein shows 97% identity with the βG protein identified previously from lodgepole pine (*Pinus contorta* Dougl. var. latifolia Engelm.) xylem (Dharmawardhana et al., 1995). No significant difference could be detected with the number of βG mRNA transcripts between early and late embryogenesis or the mature seeds and megagametophyte. However, the average βG mRNA transcripts levels showed somewhat similar trend to that observed with CAT transcripts, being higher in early embryogeny and in megagametophyte tissues than in late embryogeny or mature embryos, respectively (Figure S7A). During the early embryogeny, βG expressed strongly in the megagametophyte cells in the arrow shaped region in the front of the corrosion cavity preparing to die (Figure S7B, D). At the late embryogeny, βG expressed strongly in the cells of the nucellar layers (Figure S7F, G). The specificity of the antisense βG probe was confirmed by the absence of signals in the sections hybridized with the sense βG probe (Supplementary Figure S8).


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**Figure S8.** βG expression in developing Scots pine seeds. (A) The expression of βG in developing seeds at the early and late embryogeny and in the embryos (e) and megagametophytes (m) of mature seeds. The expression was based on mRNA copy numbers generated with the absolute Q-RT-PCR analysis and values presented were normalized using the expression at the early embryogeny. (B) The localization of βG mRNAs (blue signal) in a developing Scots pine seed at the early embryogeny. (C) The localization of βG mRNAs at the late embryogeny. (D) Intense βG expression in the megagametophyte cells in the ESR at the early embryogeny. (E) Weak βG expression in the cells of the nucellar layers at the early embryogeny. (F, G) Intense βG expression in the cells of the nucellar layers at the late embryogeny. asr=arrow-shaped region, cc=corrosion cavity, e=embryo, esr=embryo surrounding region, m=megagametophyte, nc=nucellar cap, nl=nucellar layers, nt=cellular nucellus, se=subordinate embryo, sr=suspensor remnants. Bars: (G) 20 μm, (D) 50 μm, and (B, C, E, F) 100 μm.
Figure S9. The sections hybridized with the sense βG probe in a developing Scots pine seed at the early embryogenesis (A) and at the late embryogenesis (B). asr=arrow-shaped region, cc=corrosion cavity, e=embryo, esr=embryo surrounding region, m=megagametophyte, mm=megasporangial membranes, nc=nucellar cap, nl=nucellar layers, nt=cellular nucellus, sr=suspensor remnants. Bars: 100 μm.