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Coleorhiza-enforced seed dormancy: a novel mechanism to control germination in grasses

Thomas Holloway, Tina Steinbrecher, Marta Pérez, Anne Seville, David Stock, Kazumi Nakabayashi and Gerhard Leubner-Metzger

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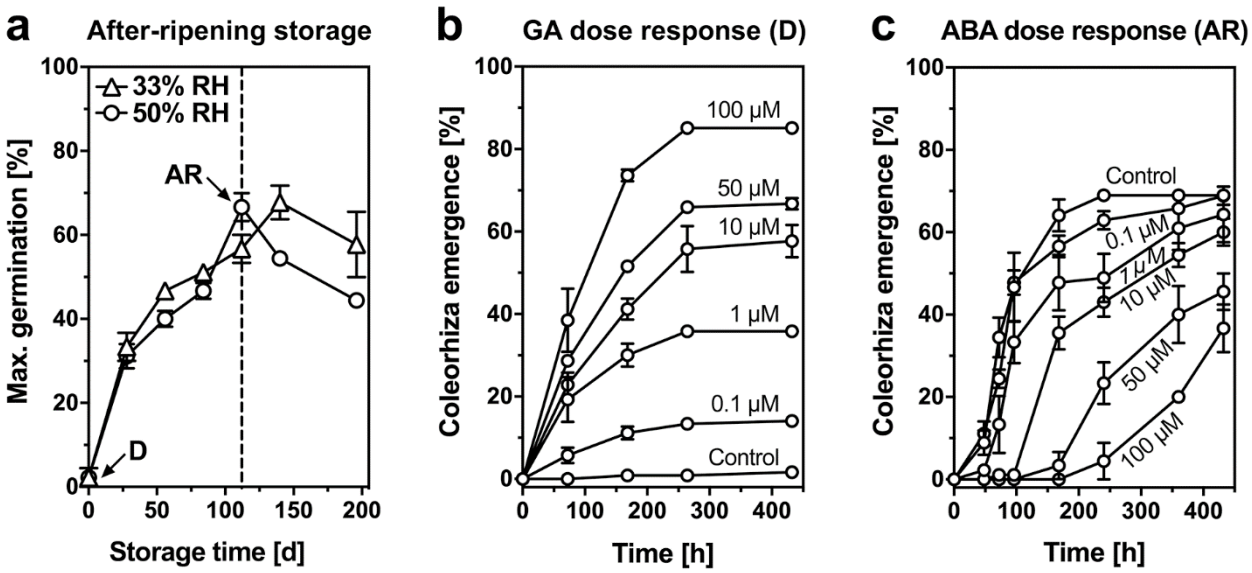

Fig. S1 After-ripening and hormonal control of *Avena fatua* diaspore germination. **(a)** After-ripening assay showing the effect of storage time on the maximum germination of D diaspores at 33 and 50% relative humidity (RH); the dashed line indicates when maximal after-ripening was achieved. **(b)** The dormancy breaking effect of different doses of gibberellin A_{4+7} (GA) on coleorhiza emergence of D diaspores. **(c)** The inhibitory effect of different doses of ABA on coleorhiza emergence in AR diaspores. Mean values ± SEM for triplicates each of 30 diaspores are presented. D, dormant; AR, after-ripened.

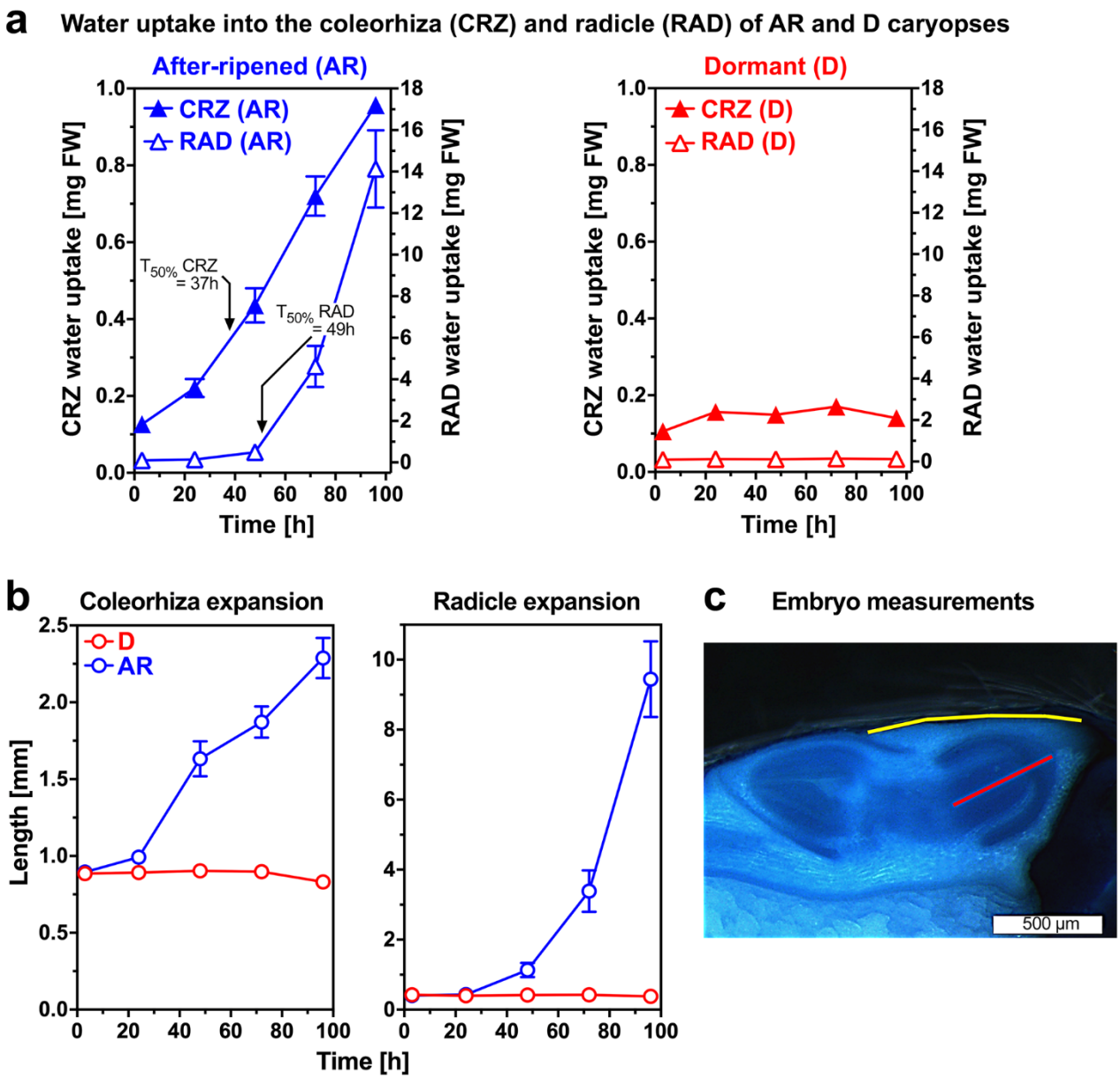

Fig. S2 Water uptake and organ expansion in D and AR coleorhizae and radicles. **(a)** Water uptake into coleorhizae (CRZ) and radicles (RAD) of AR (*left*) and D (*right*) caryopses. **(b)** Measurement of organ length over time for D and AR coleorhizae and radicles. **(c)** An example image showing an embryo stained with methylene blue. For radicle length measurements, the length from the centre point of the base of the radicle to the apex of the root cap was measured (*red line*). For measurements of coleorhiza length, the periphery of coleorhiza was measured form the apex of the epiblast to the apex of the coleorhiza and epiblast was measured (*yellow line*). Mean values ± SEM for ~30 individuals per treatment are presented. D, dormant; AR, after-ripened; CRZ, coleorhiza; RAD, radicle.

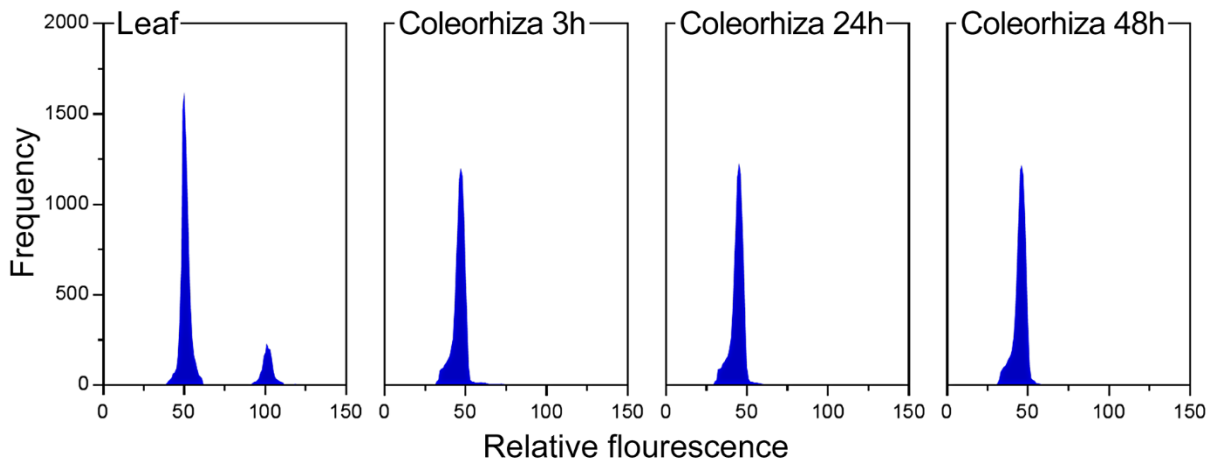

Fig. S1 Flow cytometric analysis of DNA contents in leaf and coleorhiza tissue. Gain-adjusted frequency histograms showing embryonic leaf tissue and coleorhiza tissue isolated from AR caryopses imbibed for 3, 24 and 48 hours. The presence of two peaks in the leaf tissue shows nuclei with 2n DNA contents, indicating DNA replication is occurring and this tissue is proceeding through the cell cycle. The absence of this peak in the coleorhiza samples shows that this tissue is not proceeding through the cell cycle. Measurements were made for ~20 individuals on >9,000 nuclei per sample.

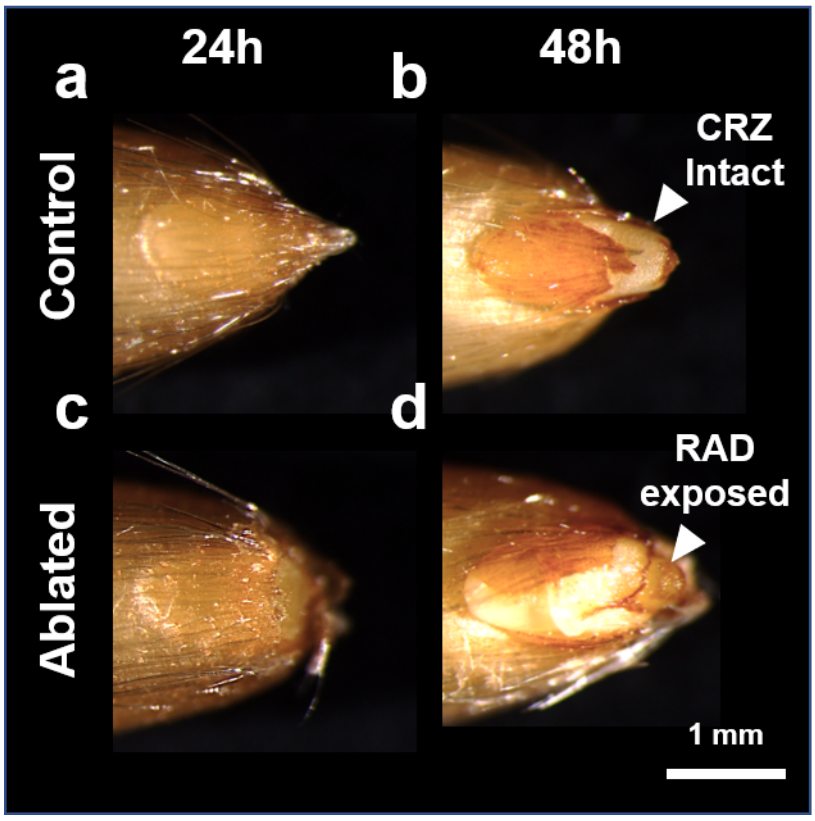

Fig. S4 Images showing ablated coleorhizae. **(a,b)** Control after-ripened caryopsis with no ablation treatment. **(c,d)** Caryopses with ablated coleorhizae. Caryopses have been imbibed for 24 hours **(a,c)** and 48 hours **(b,d). (b)** Non-ablated treatment (control) at 48 hours exhibits the intact (unruptured) coleorhiza covering the radicle. **(d)** Ablation treatment at 48 hours, the coleorhiza is compromised and leaves the radicle exposed.

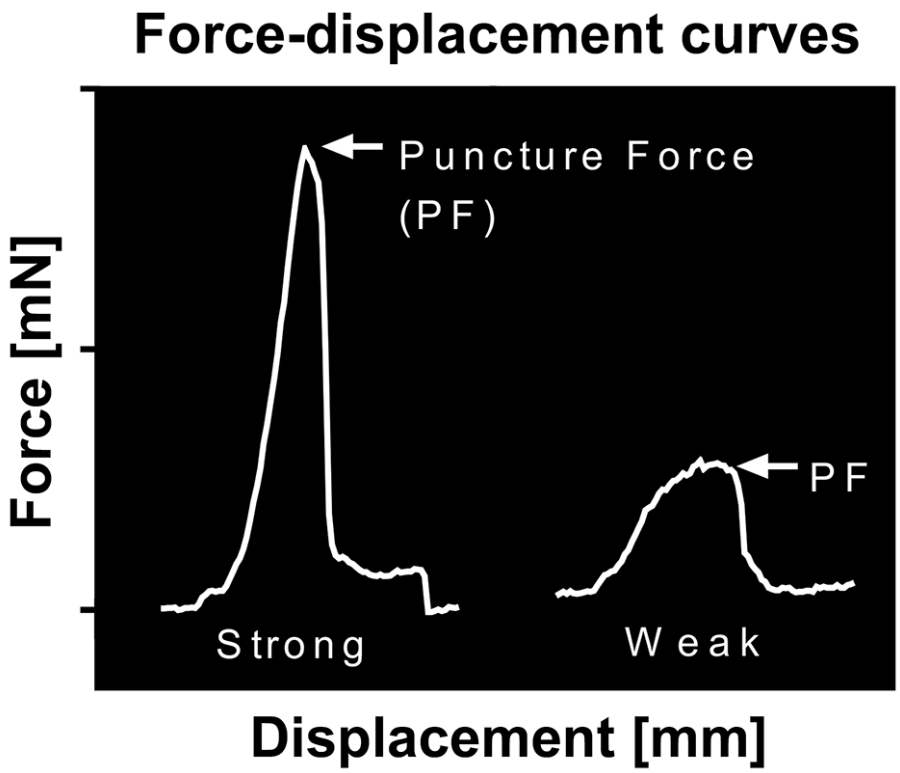

Fig. S5 Methodology to analyse mechanical properties of the *Avena fatua* coleorhizae by puncture force analysis. Example force-displacement curves for strong and weak coleorhizae. For further details about the device used see Figure 5 in the review 'The biomechanics of seed germination' by Steinbrecher and Leubner-Metzger, *J Exptl Bot* 68:765-783 (2017).

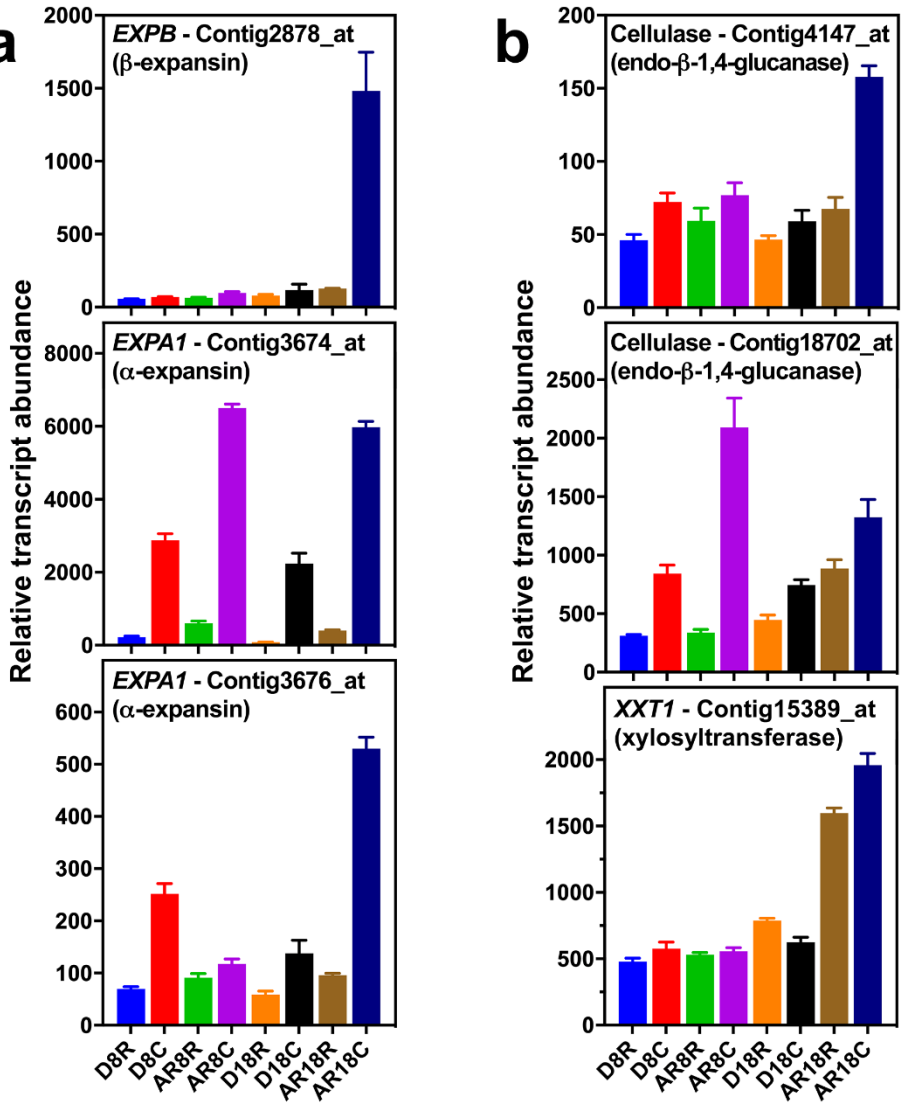

Fig. S6 Transcript expression pattern of barley cell wall remodelling genes (CWRP) genes. Mean transcript abundance values ± SEM for dormant (D) and after-ripened (AR) *Hordeum vulgare* coleorhizae (C) and radicles (R) imbibed at 8 and 18 hours were extracted from publicly available datasets (Barrero et al., *Plant Physiol* 150:1006-1021, 2009). Barrero and colleagues published that several CWRP were differentially expressed in these barley tissues. **(a)**, Expansins: They found Contig2878_at *EXPB* (*top panel*), a β-expansin, to be specifically upregulated in AR coleorhizae. We show here that also two α-expansins of the *EXPA1* group (*middle and bottom panels*) are up-regulated in AR coleorhizae. When compared to D coleorhizae their transcript abundance is at least 2-fold higher. **(b)**, Cellulases (endo-β-1,4-glucanases) and xylosyltransferases (XXT): We found in the barley datasets that two cellulases (*top and middle panels*) are upregulated in AR coleorhizae as compared to D coleorhizae. Further to this, *HvXXT1* (*bottom panel*) transcripts accumulate in AR coleorhizae, as well as *HvXXT3* (Contig6858_at) transcripts. Together this suggests that cell wall remodelling associated with coleorhiza rupture has the cellulose-xyloglucan network as target; the cellulose-xyloglucan network is also the target for α-expansins.

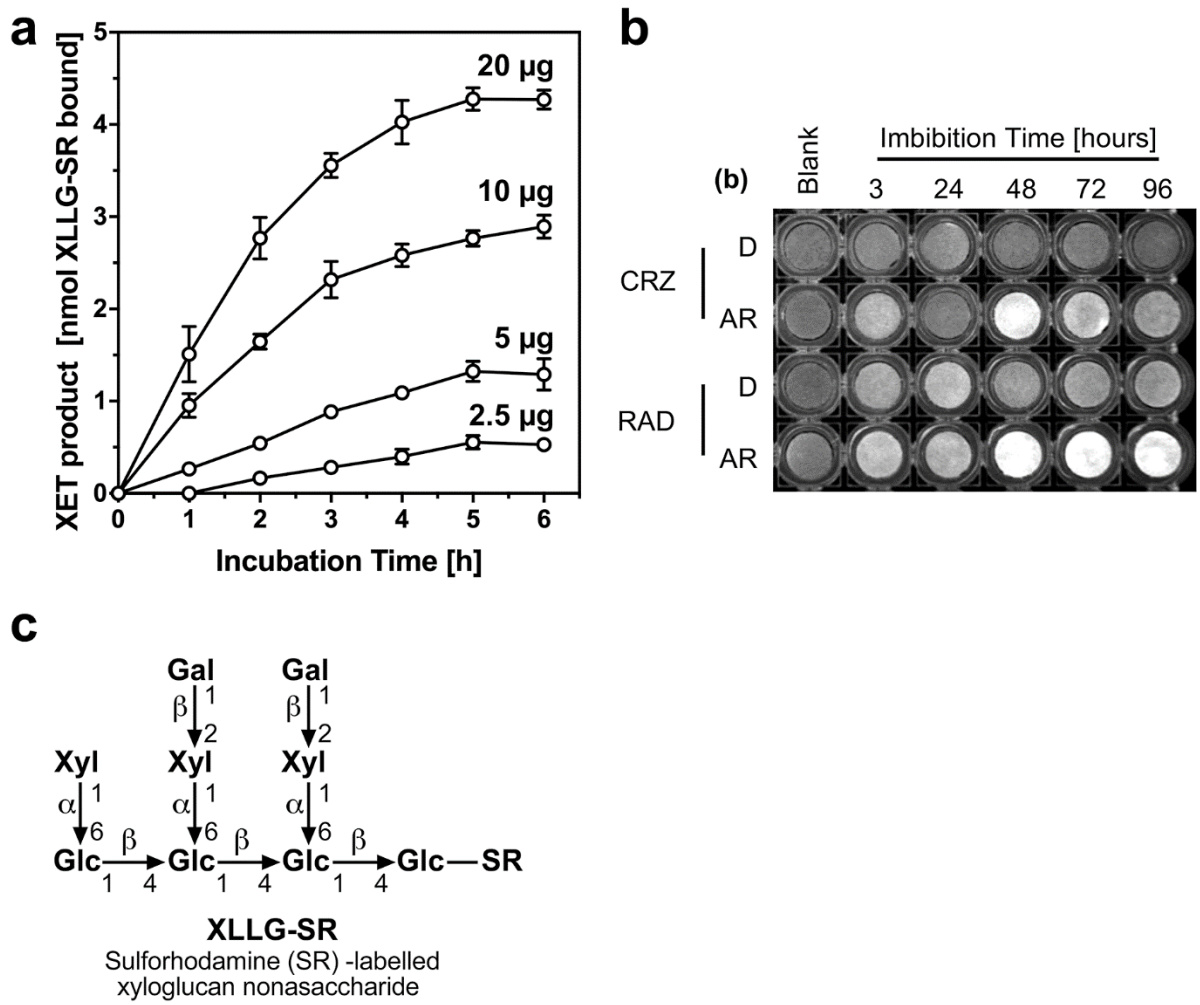

Fig. S7 Optimisation of the XET activity assay and xyloglucan oligosaccharide used. **(a)** The effect of different total protein loadings and incubation times on measured XET activity from isolated embryos from 48 hour imbibed AR caryopses. **(b)** Example UV transilluminator image of the XET activity assay showing 20 µg total protein loaded with a 3-hour assay incubation time from dormant (D) and after-ripened (AR) coleorhiza (CRZ) and radicles (RAD) isolated from caryopses imbibed for different lengths of time. Mean values ± SEM for triplicates of ~30 individuals are presented. **(c)** Structure of fluorescence-labelled xyloglucan oligosaccharide XLLG.

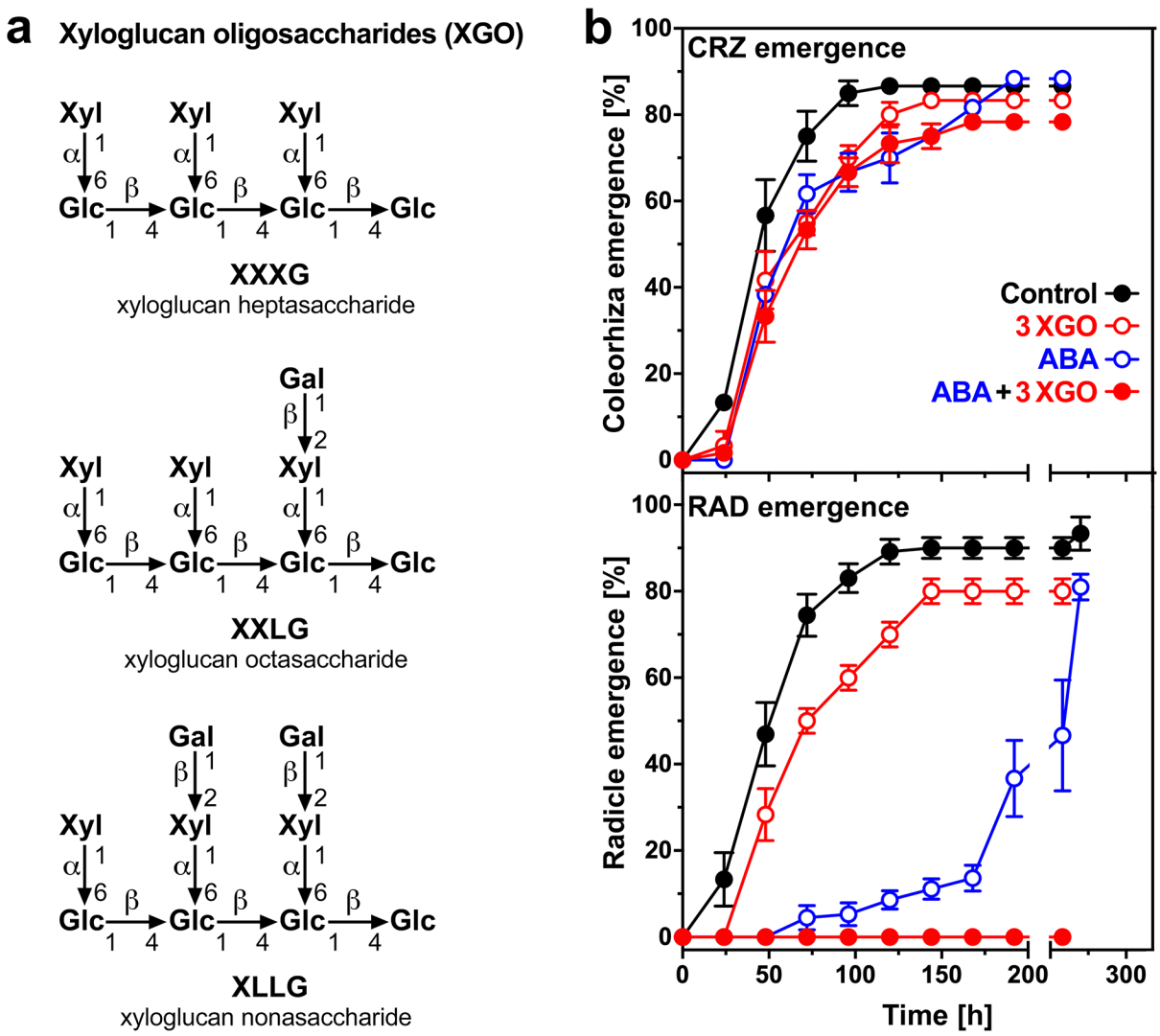

Fig. S8 The effect of the xyloglucan oligosaccharides (XGO) on *Avena fatua* germination. **(a)** The structures of the XGOs used in the experiments: XXXG was used in the experiment presented in Figure 6; the 3 XGO mixture (XXXG, XXLG, XLLG) was used in the experiment presented here in (b). **(b)** The effects of 50 µM ABA, 0.1% (w/v) of the 3 XGO mixture and a combination of both ('ABA+ 3 XGO') on coleorhiza (CRZ) and radicle (RAD) emergence of after-ripened (AR) caryopses. Note that the combination 'ABA+ 3 XGO' blocked coleorhiza rupture by RAD emergence (germination) in the same way as shown for 'ABA+ XXXG' in Figure 6. Mean values ± SEM for triplicates each of 30 caryopses are presented.

Table S1. Statistical analysis of stress applied to D and AR coleorhizae during biomechanical measurements

Summary Data[a]

Treatment	Number of measurements (N)	Mean stress (mN * mm^{-2})	SEM
AR 24h	33	126.7	6.3
AR 48h	26	91.5	8.4
D 24h	22	359.9	9.8
D 48h	35	394.6	8.1

One-way ANOVA with Tukey's Multiple Comparisons Test[b]

Tukey's multiple comparisons test; DF=112	Mean difference	95% CI of diff.	Significance summary	Adjusted P value
AR 24h vs. AR 48h	35.2	5.692 to 64.77	*	0.0125
AR 24h vs. D 24h	-233.2	-264.2 to -202.2	****	<0.0001
AR 24h vs. D 48h	-267.8	-295.2 to -240.5	****	<0.0001
AR 48h vs. D 24h	-268.4	-301 to -235.8	****	<0.0001
AR 48h vs. D 48h	-303.1	-332.2 to -273.9	****	<0.0001
D 24h vs. D 48h	-34.7	-65.31 to -4.017	*	0.0199

[a] Summary data for the estimated stresses applied to the coleorhizae during biomechanical measurements. Stress was calculated from puncture force by approximating coleorhizae surface area to that of a cone. AR, after-ripened; D, dormant.

[b] Results from a one-way ANOVA and Tukey's Multiple Comparisons Test comparing the mean stress of all treatments, showing significant differences between all treatments. DF, Degrees of Freedom; CI, Confidence Interval; *, P<0.05; **** P<0.0001.

Table S2. Primer sequences used for cloning and RT-qPCR.

Avena cDNA	Forward primer (5´→3´)	Reverse primer (5´→3´)	Amplicon length
Cloning primers			
CL1533	TGCAGGGTAGTAGGCCTGAT	AGGCGTGCCACAATTAAGGA	1038 bp[c]
3140	CACATCCAGGAAGCAGAGCA	TGCTTCAATTCGGACGGACA	907 bp[c]
20183	GCAGCTCAAGCTCGT	AGTAGAGCCGCATGG	283 bp[c]
CAC	CGACGAGGAGACGATGCC	TATCATGCCAGACGCCGTAC	1350 bp[c]
PP2A	GCCGCTGTATCCAATTGCTG	CAAGTTGTTCGCAGCAGCTT	1307 bp[c]
RT-qPCR primers			
CL1533[a]	ACCAGTCTCTCTCTCCGGAC	ACAGGTAGAAGGCGGTGTTG	167 bp
3140[a]	GCCGACTTCCACACCTACAA	TTCTTGAACGTCCTCACCGG	86 bp
20183[a]	TACACTCTGCACACCAACGT	TTCCCCTCCAGGTTCCTGAA	176 bp
CAC[b]	GGTACATGCCCTGTTCGACA	CGGCCTCCACAACAAACTTG	130 bp
PP2A[b]	GGCTAGCATCTGGTGAGTGG	GTGGCAGCAAACTTCCCAAG	184 bp

[a] Candidate gene

[b] Reference gene

[c] Sequences were submitted to GenBank (BankIt2382318); the following names and accession numbers (in brackets) were assigned: *AfXTH_1533* (MT993831), *AfXTH_3140* (MT993832), *AfXTH_20183* (MT993833), *AfCAC_10394* (MT993834), *AfPP2A_6627* (MT993835).