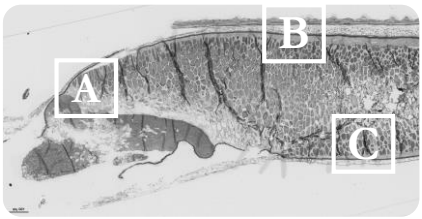
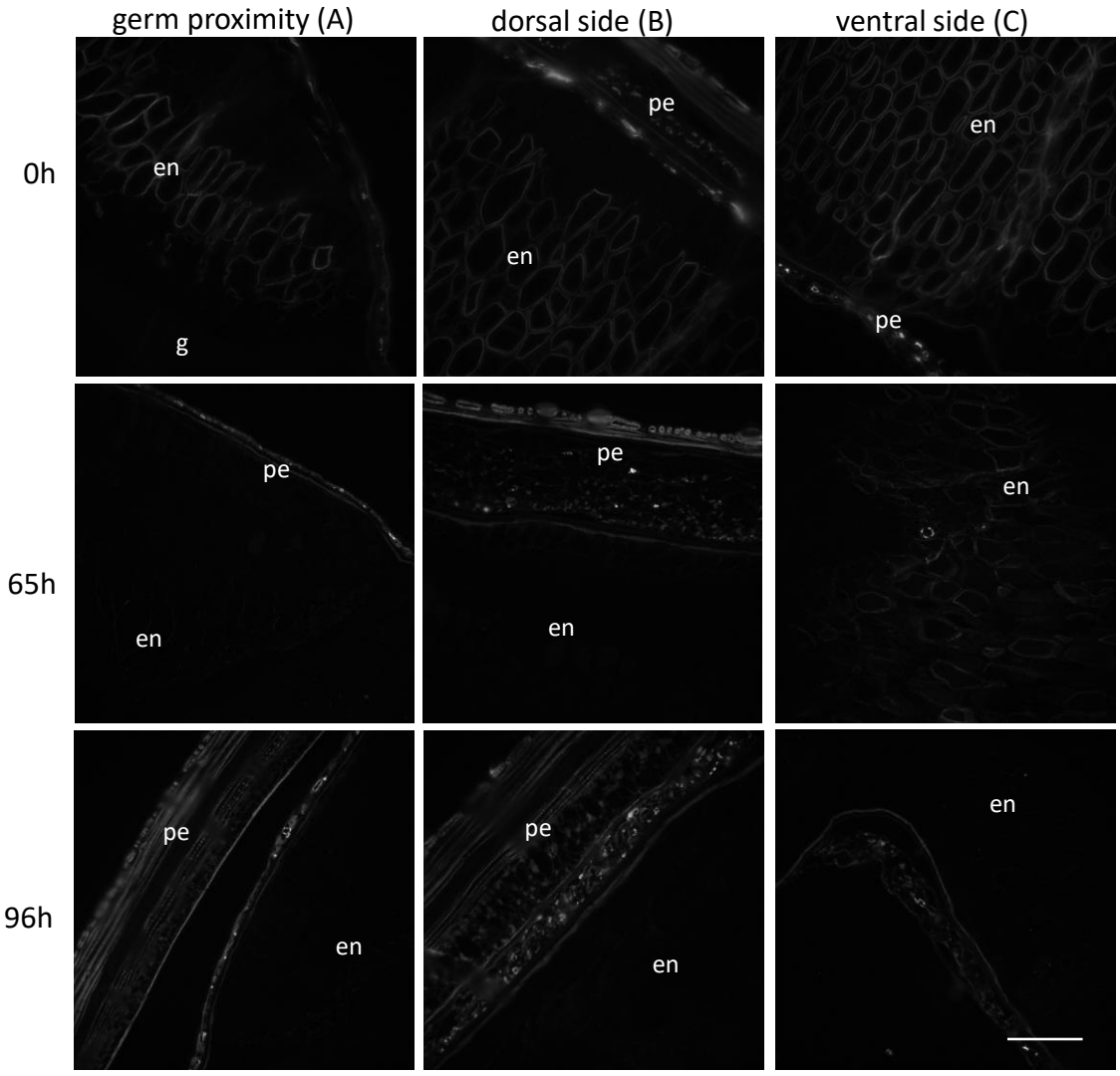
**Supplementary Figure S1**

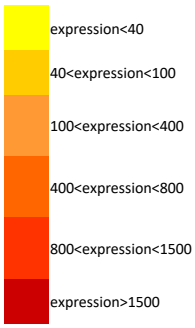



Immunolabeling control of wild type germinated grains (0, 65 and 96 hours after imbibition) pretreated with lichenase to remove MLG and using antibodies specific to MLG. Scale bar = 50µm. g : germ; pe : pericarp; en : endosperm.

## Supplementary Figure S2

| | GH17 | | | | | GH51 | GH9 | GH16 | GH13 | CSLF6 | CSLH1 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Bradi2g22222 | Brad12g22224 | Bradi2g22226 | Bradi2g60490 | Bradi2g27140 | Bradi4g43710 | Bradi5g14580 | Bradi1g27867 | Bradi3g58010 | Bradi3g16307 | Bradi5g10130 |
| Pre-anthesis ovary | 0.0 | 0.0 | 0.0 | 0.2 | 3.0 | 38.0 | 50.0 | 0.4 | 0.2 | 316.0 | 0.3 |
| Young grain (1-3 DAA) | 0.0 | 0.0 | 0.0 | 4.0 | 29.0 | 77.0 | 64.0 | 0.8 | 0.5 | 397.0 | 0.2 |
| Middle length grain (3-8 DAA) | 0.0 | 0.0 | 0.0 | 0.2 | 9.0 | 75.0 | 76.0 | 2.0 | 3.0 | 819.0 | 0.1 |
| Full length grain (8-15 DAA) | 0.0 | 0.0 | 0.0 | 0.1 | 27.0 | 62.0 | 89.0 | 4.0 | 12.0 | 940.0 | 0.1 |
| Mature grain (15-20 DAA) | 0.8 | 2.0 | 0.0 | 3.0 | 149.0 | 981.0 | 46.0 | 1.0 | 1.0 | 536.0 | 1.0 |
| Mature grain without embryo | 0.1 | 0.6 | 0.1 | 6.0 | 84.0 | 1000.0 | 33.0 | 2.0 | 2.0 | 618.0 | 0.9 |
| Germinating grain | 1052.0 | 2847.0 | 1520.0 | 849.0 | 329.0 | 1161.0 | 219.0 | 56.0 | 2223.0 | 213.0 | 2.0 |
| Young seedling | 0.1 | 2.0 | 4.0 | 19.0 | 468.0 | 46.0 | 148.0 | 43.0 | 0.2 | 498.0 | 10.0 |



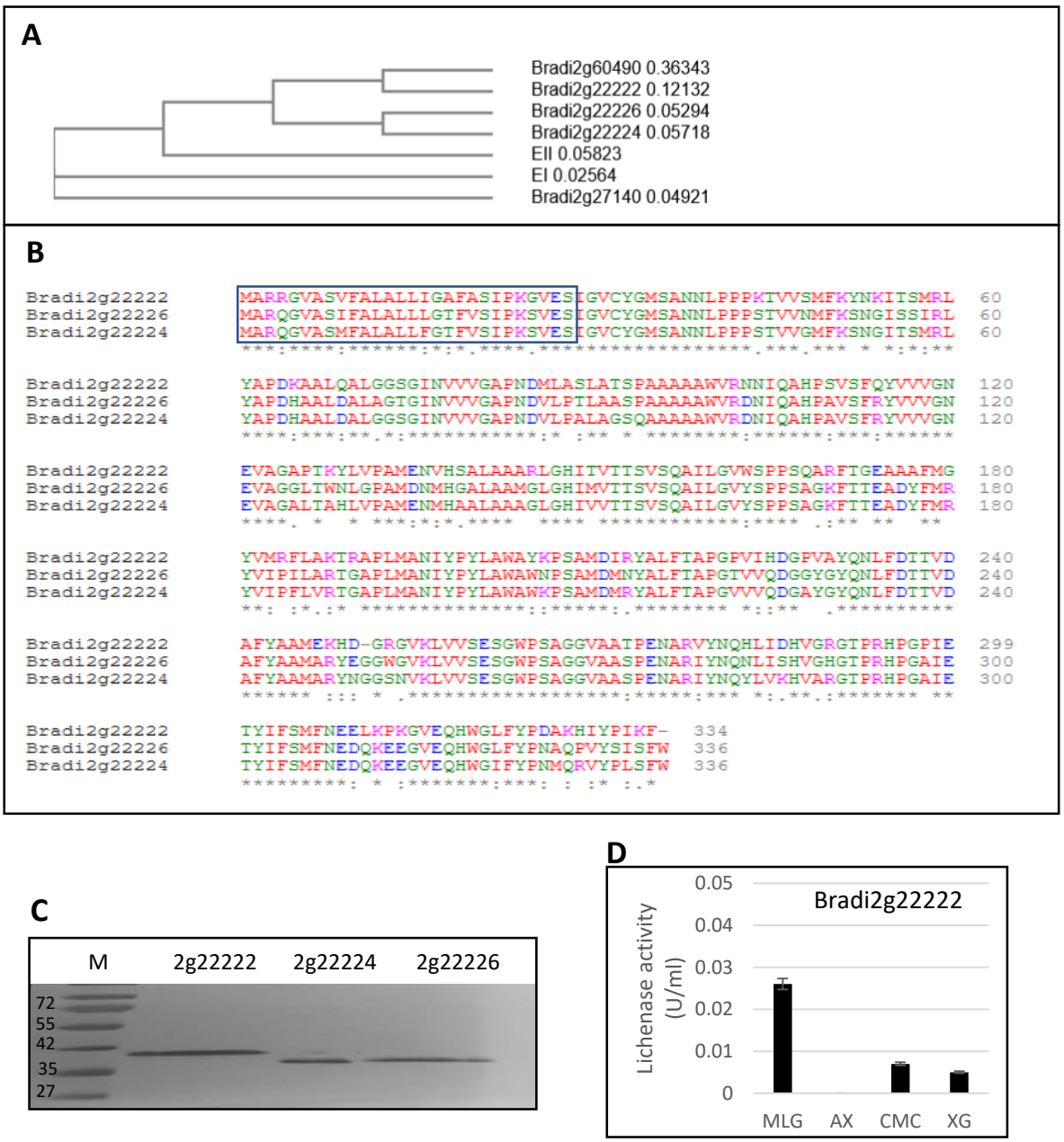

Expression level (average expression values) of genes putatively involved in sugar metabolism of Brachypodium grain according to the *Brachypodium distachyon* eFP Browser (http://bar.utoronto.ca; Sibout et al., 2017 [35])

**Supplementary Table S1**

Primer lists used for gene cloning and qRT-PCR

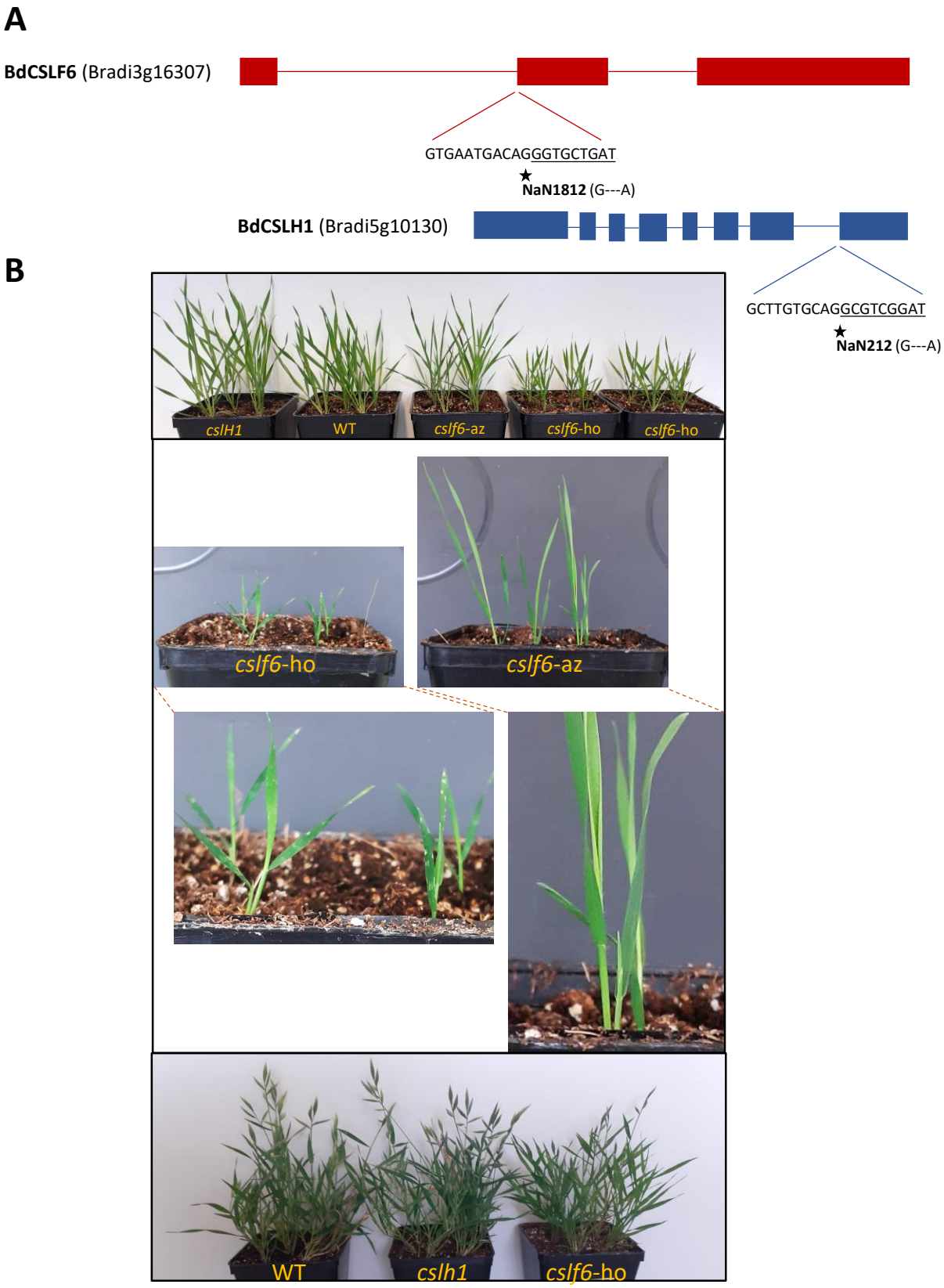
| | Accession | Forward primers | Reverse primers |
|---|---|---|---|
| primers used for cloning | Bradi2g22222 | CCGGAATTCATCGGCGTGTGCTACGGC | GCTCTAGAGCAAATTTGATGGGGTAGATGTG |
| | Bradi2g22224 | CCGGAATTCATCGGGGTGTGCTACGGC | GCTCTAGAGCCCAGAAGCTGAGGGGGTAGAC |
| | Bradi2g22226 | CCGGAATTCATCGGGGTGTGCTACGGC | GCTCTAGAGCCCAGAAGCTGATGGAGTAGAC |
| primers used for qPCR | Bradi2g22222 | CCAAAAGGCGTGGAGTCCATC | AGGCTGGCAAGCATGTCGTT |
| | Bradi2g22224 | CCAAAAGCGTGGAGTCCATCG | CGACGACGTTGATGCCACTGC |
| | Bradi2g22226 | CCAAAAGCGTGGAATCCATCG | TAGCGTGGGCAGCACGTCGTT |
| | Bradi2g27140 | CACAAAGCGTGGAGTCGATCG | ATGTTGGAGAGCACGTCGTT |
| | Bradi2g60490 | CTGTTCCTACGAGCGTGCAATC | GGCGATGTACTTGATGTTGACC |
| | Bradi4g43710 | GAT AAC GAT CGC ACG TGG AAC C | CCGAAGTTCACAATCTTCACTCT |
| | Bradi5g14580 | ACGCCCAGAAATCCGGCAAG | CCCTCACATGGTCGTACTCGC |
| | Bradi1g27867 | GGCTTTCTACCTGTCGAACAACG | CAGAGGTGGAAGCGCATCTC |
| | Bradi3g58010 | ACAAGGTCATGCAGGGCTAC | AGGTGCTCGATCTCCTCCTT |
| | PP2A | AGCGCTGGTGGAATCTGAAA | TGGACACGATCTAAGCACCG |
| | SDH | TTCAACAGCATGGATGGCC | ATCTTCGGTTGCAGAGCTCCT |

**Supplementary Figure S3**



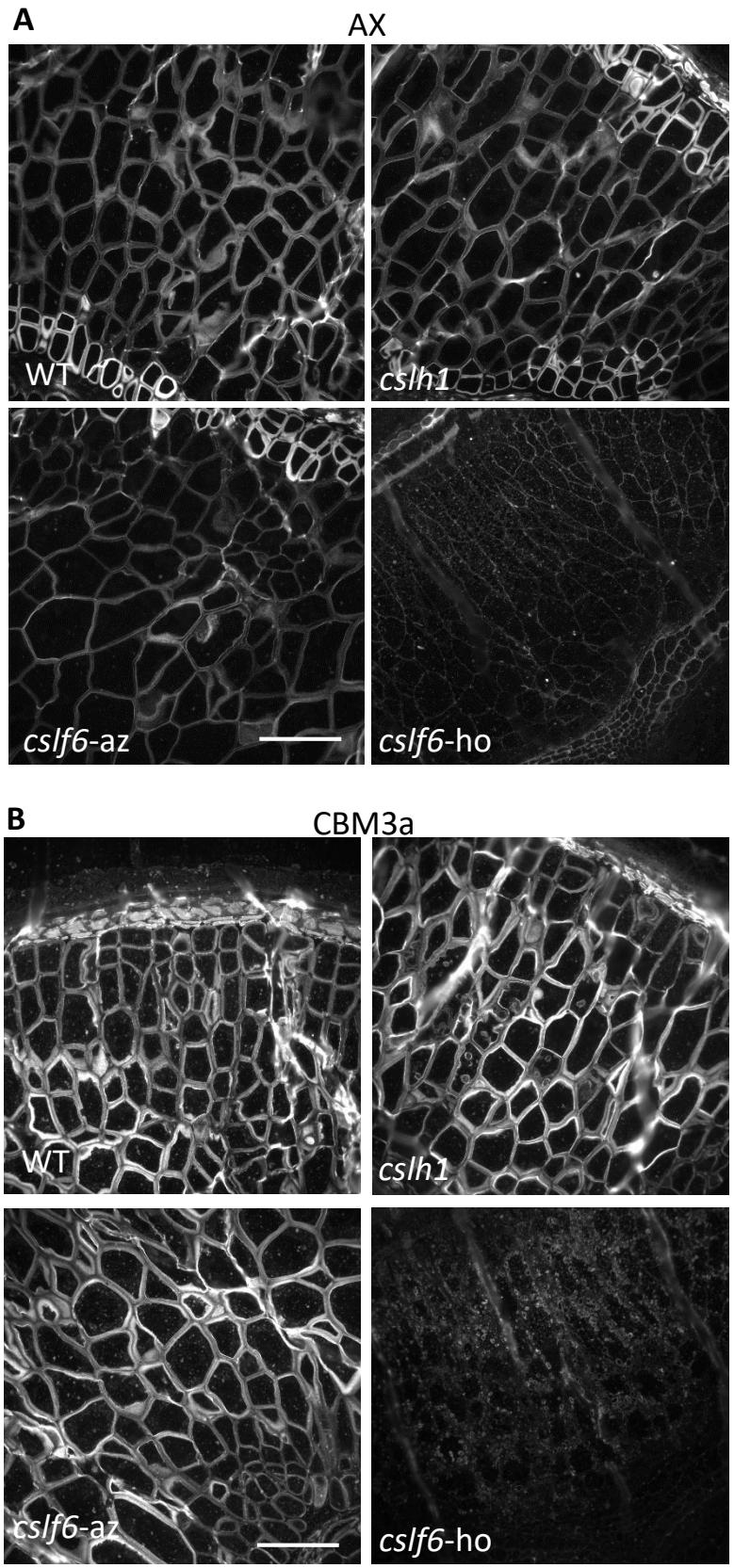

**Lichenase information : phylogenetic tree, multiple alignment, western blot of recombinant lichenases, substrate specificity of Bd2g22222.** **A**. Phylogenetic tree (https://www.ebi.ac.uk). EI : HORVU7Hr1G057680 ; EII : HORVU7Hr1G120450. **B**. Sequence alignment of three lichenases from the GH17 family using Clustal O (1.2.4) multiple sequence. Signal peptides is highlighted in a rectangular box. Amino acids are coloured according to their chemical properties (blue : acidic; red : small and hydrophobic ; magenta : basic ; green: hydroxyl, sulhydryl, amine and G). Asterisk significance : " *" indicates positions which have a single, fully conserved residue; ":" indicates conservation between groups of strongly similar properties; "." indicates conservation between groups of weakly similar properties. **C**. Western blot analysis of recombinant lichenases Bradi2g22222, Bradi2g22224 and Bradi2g22226 produced in *Pichia pastoris*. M : molecular weight marker (kDa). **D**. Enzymatic activity measurement of the recombinant enzyme Bradi2g22222 (MLG : barley mixed-linkage glucan; AX : wheat arabinoxylans; XG : tamarin xyloglucan, CMC : carboxymethylcellulose).

**Supplementary Figure S4**



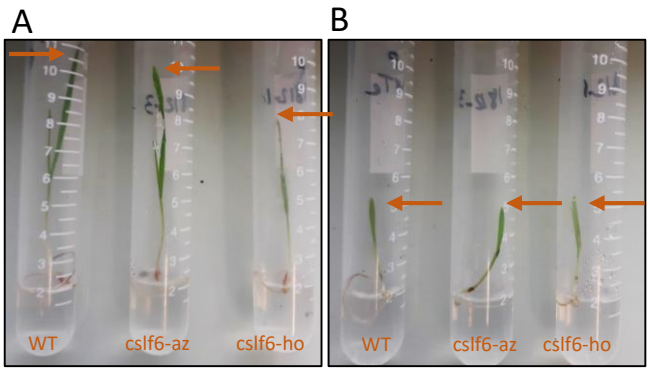

**A.** Schematic of Brachypodium *CSLF6* and *CSLH1* genes. Plain boxes correspond to exon sequences , and lanes correspond to intron sequences. Asterisk indicates the mutation location.
**B**. Brachypodium plants of WT, *cslh1* mutant, azygous and homozygous *cslf6* mutants showing young (top) and advanced growth (bottom), with magnifications (in the middle of the figure) highlighting leaf damage of the homozygous *cslf6* mutant.

**Supplementary Figure S5**




Fluorescence labeling of arabinoxylans and cellulose in grain cross-sections of WT, *cslh1*, homozygous and azygous *cslf6* mutants (15 days after flowering) pre-treated with lichenase, using AX1 antibodies (**A**) and CBM3a (**B**). Scale bar=50µm.

**Supplementary Figure S6**




***In vitro* culture of WT and *cslf6* mutants**. Growth of plants on a phytagel medium from 8 days seedlings with seeds (**A**) or without seeds (**B**) on a phytagel medium (in light, without nutrient) during 6 days. n=15.