Functional characterisation of barley betaglucanless mutants demonstrates a unique role for CslF6 in (1,3;1,4)-ß-D-glucan biosynthesis

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Supplementary material

Figure S1. Molecular mapping of the *bgl* and *HvCslF6* genes on chromosome 7H using 104 F_2 plants from a cross between 'Bowman' and OUM125. The black box indicates the centromere position.

Figure S2. Quantitative RT-PCR analysis of expression of the *HvCslF6* gene in various tissues and developmental stages. DAF: days after flowering. Bars indicate standard deviations.

Figure S3. Alignments of seven barley HvCslF proteins and rice OsCslF6 protein. The mutated amino acids in three *bgl* mutants (positions 253, 638 and 660) are highly conserved as highlighted in colour. The amino acids at position 590, which were mutated in some barley germplasm accessions, are not conserved.

Figure S4. Difference in appearance of leaf tip between wild type barley ('Akashinriki') and *HvCslF6* mutant OUM125. Images were taken from the upper leaves of plants which were heading.

Figure S5. FACE analysis of lichenase digests of cell wall extracts from various barley seedling tissues of 'Akashinriki' (shown in blue line) and OUM125 (shown in red line) demonstrating the absence DP3 and DP4 peaks characteristic of (1,3;1,4)-ß-D-glucan in all tissues of the mutant.

Figure S6. Content of (1,3;1,4)- ß-D-glucan in leaves of three sets of isogenic lines. Error bars represent the standard errors in three replications. **: significant at the 1% level by *t*-test.

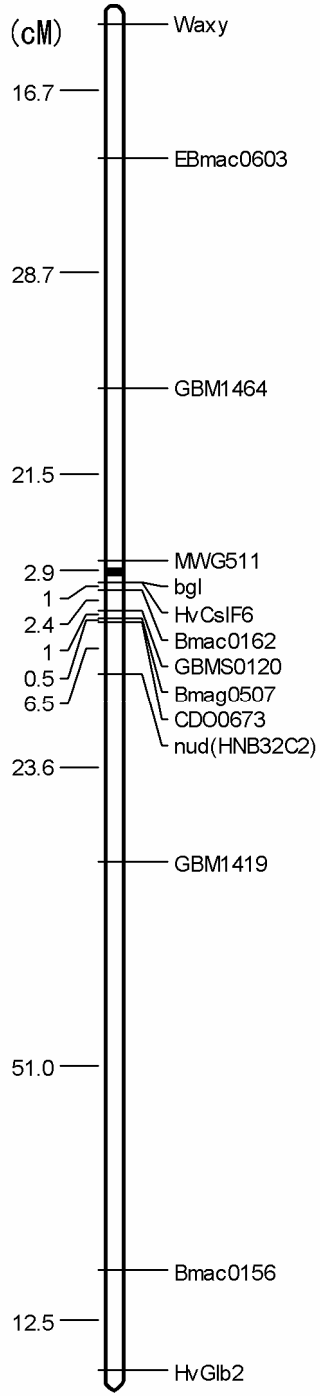

(cM)
Waxy
16.7
EBmac0603
28.7
GBM1464
21.5
MWG511
2.9
bgl
1
HvCslF6
2.4
Bmac0162
1
GBMS0120
0.5
Bmag0507
6.5
CDO673
nud(HNB32C2)
23.6
GBM1419
51.0
Bmac0156
12.5
HvGlb2

Fig. S1

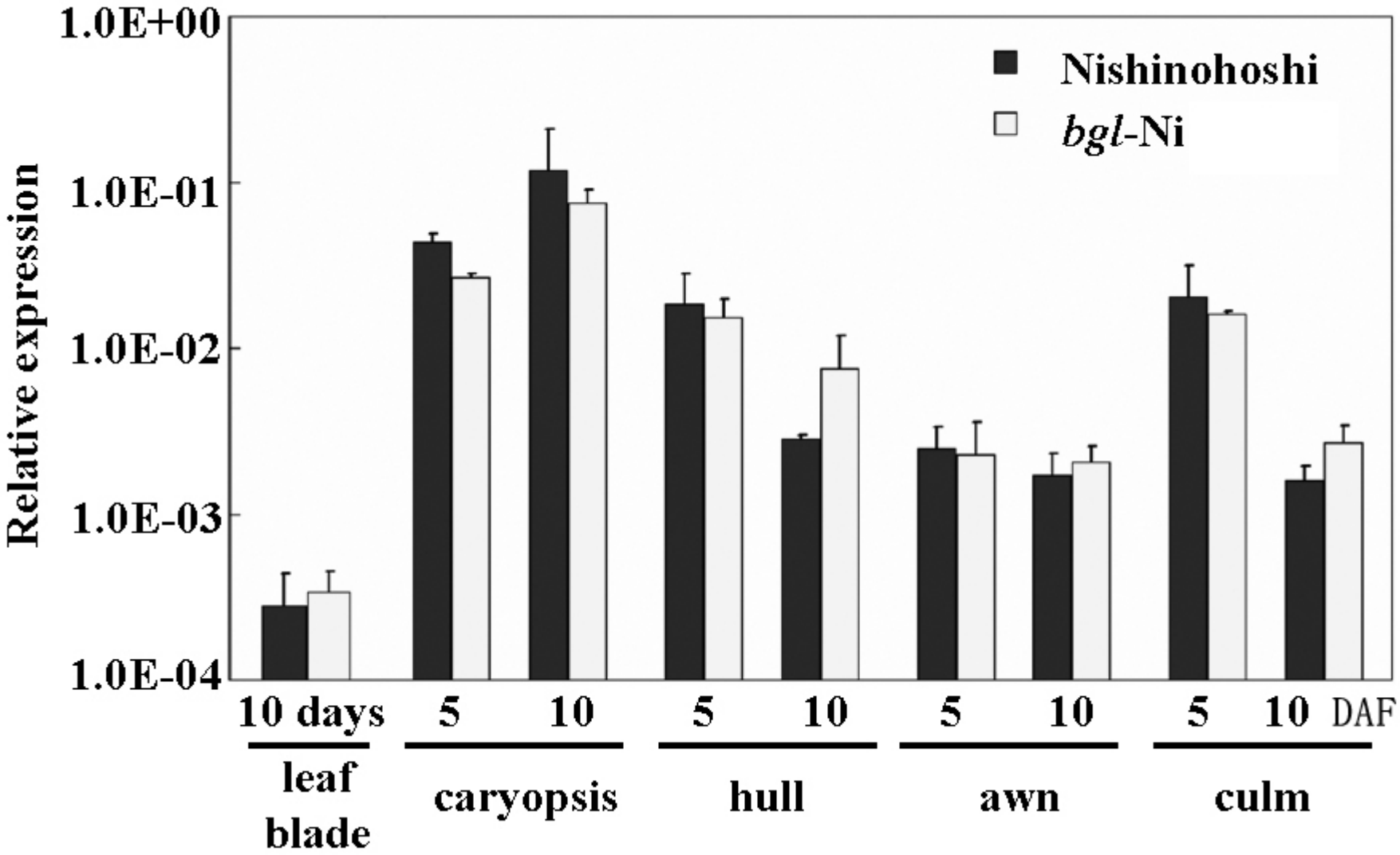

1.0E+00
1.0E-01
1.0E-02
1.0E-03
1.0E-04
Relative expression
Nishinohoshi
bgl-Ni
10 days
5
10
5
10
5
10
5
10
DAF
leaf blade
caryopsis
hull
awn
culm

Fig. S2

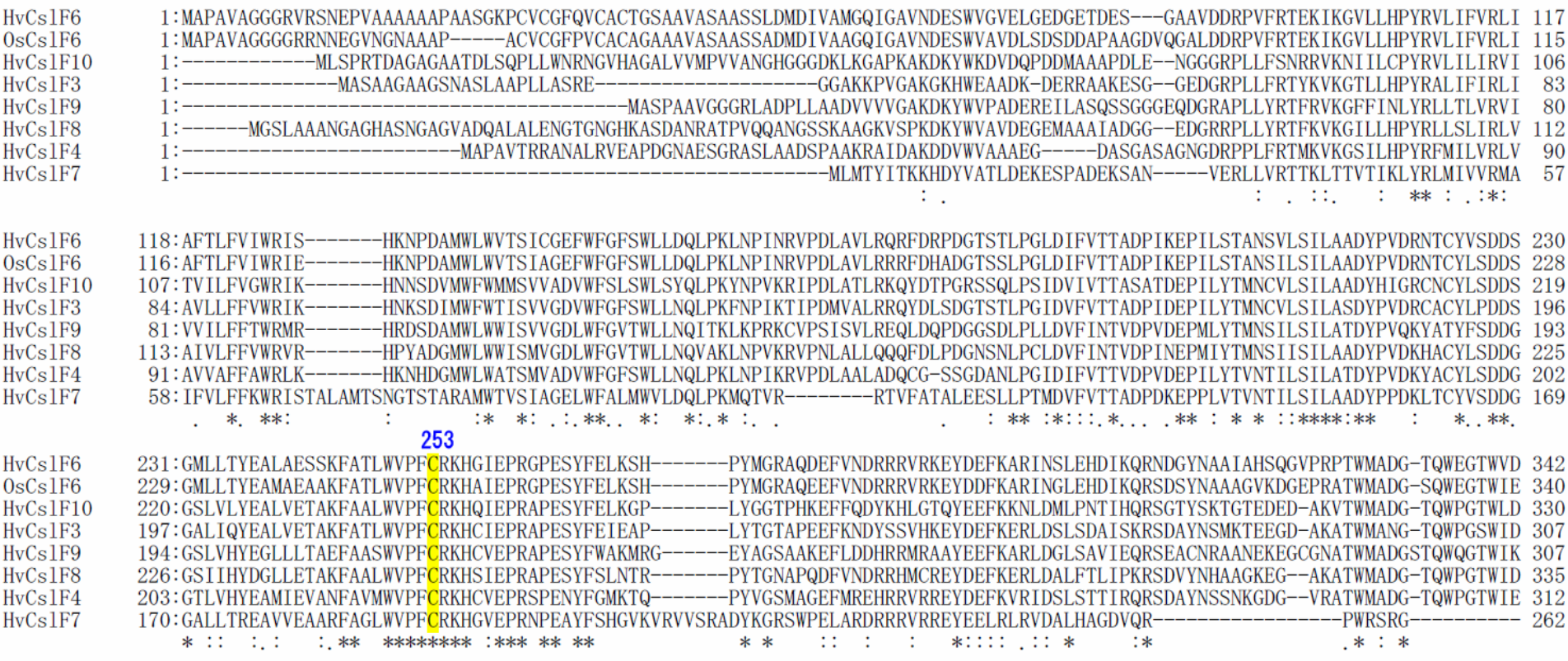


Fig. S3

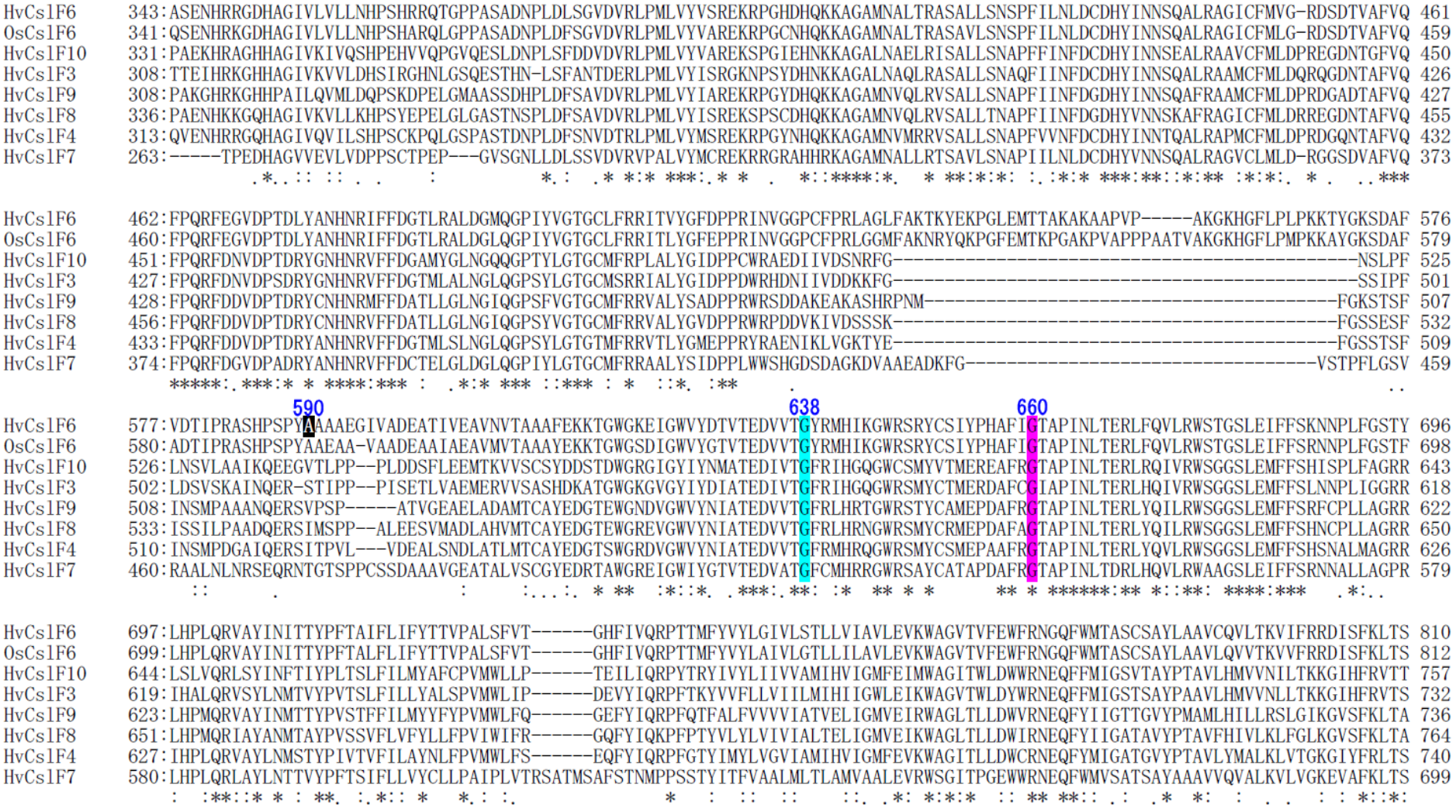


Fig. S3 continued.

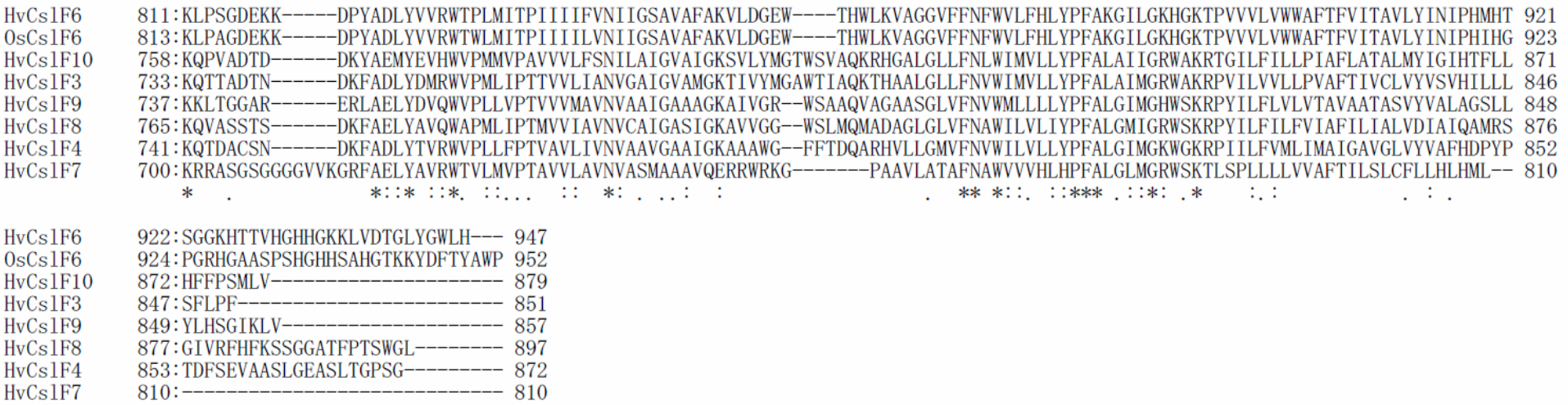
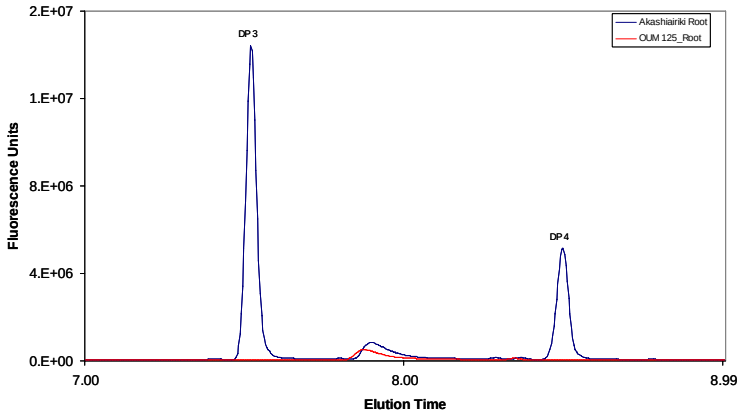


Fig. S3 concluded.

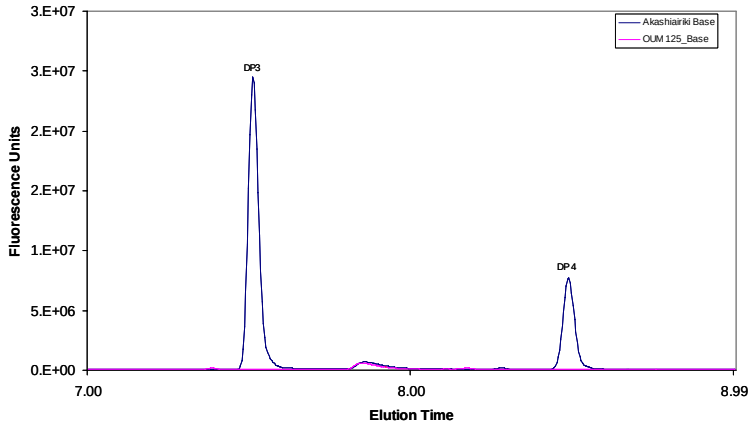
Akashinriki OUM125

Fig. S4

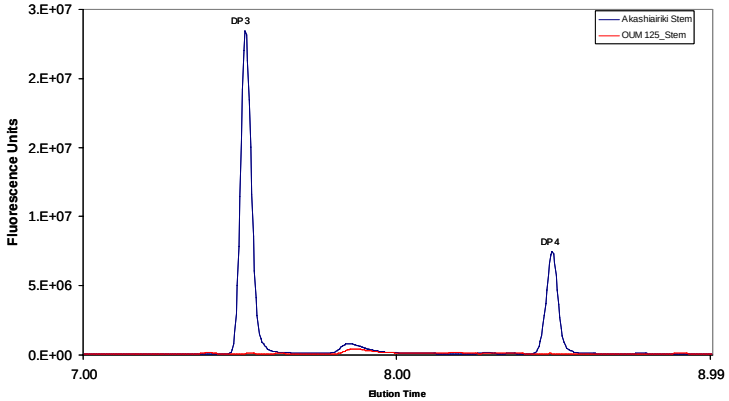
Seedling tissue - Akashiniriki vs OUM 125



ROOT

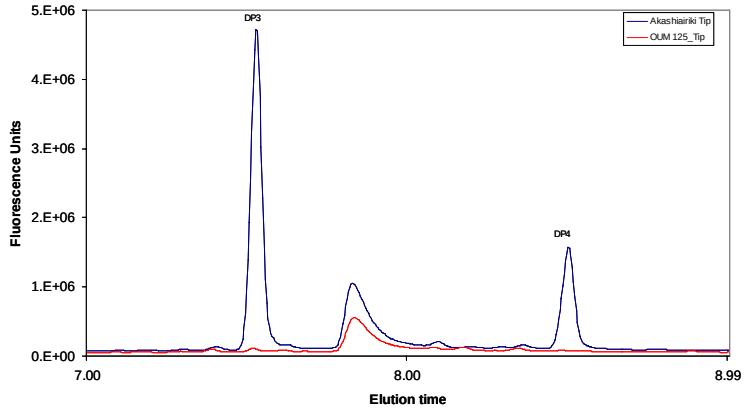


LEAF BASE



STEM

DP3 DP4



LEAF TIP

DP3 DP4

Fig. S5

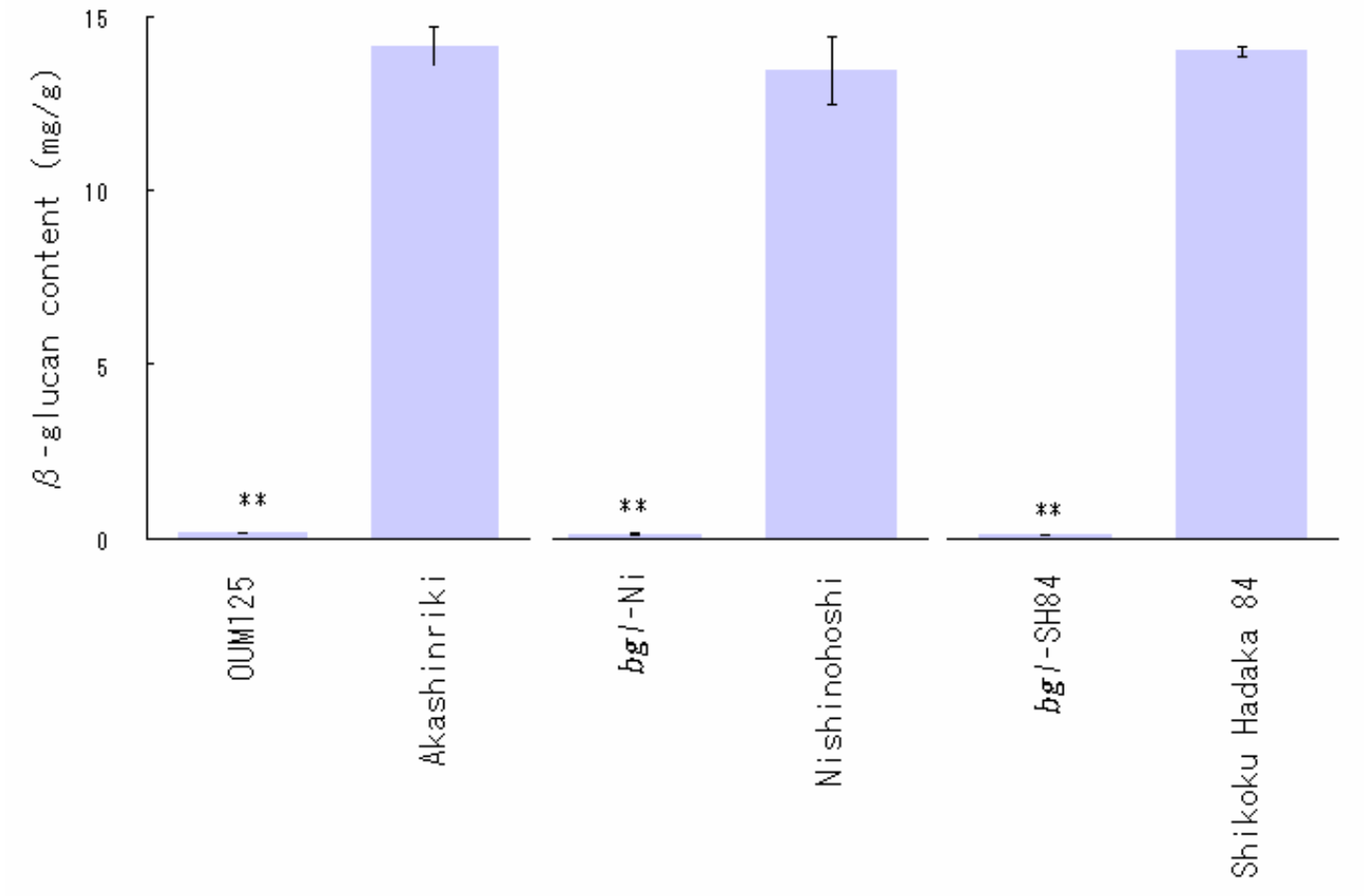

β-glucan content (mg/g)
15
10
5
0
**
**
**
OUM125
Akashinriki
bgl-Ni
Nishinohoshi
bgl-SH84
Shikoku Hadaka 84

Fig. S6

Supplementary Table S1. Primer sequences used in this study.

Primer name	Primer sequence (5'–3')
Mapping	
Waxy (F)	TGGATCCAAATCCTGCATGT
Waxy (R)	CATGGTTTTGTCCGGTTTTC
HvGlb2 (F)	CTCTTGAGAATCCTCACCTTTCAT
HvGlb2 (R)	CTCAATAAAAGTATGCCCAAAATG

Genomic sequencing of *HvCslF6*	
For amplification of the entire gene	
HvCSLF6_CP1-1F	AGAGTGAGTGCGTGCATTGAG
HvCSLF6_CP1-2R	TGTTGAGTGGATGAGTTGTTTCTT
For internal sequencing	
N8.B10-M13-RV(2)	CAATTAACCCAACAAGCACC
N8.B10-M13-RV(3)	AGTTTTTGTTGAGCGTTTTATG
N8.B10-M13-RV(4)	GACTTGCCAGCTCTCTCCTT
N8.B10-M13-RV(5)	AACACATGCTACGTCTCCGA
N8.B10-M13-RV(6)	GTGGTGATAGTCGAGAGTGC
N8.B10-M13-RV(7)	GCTGCTCTCCAACTCCCCCT
N8.B10-M13-RV(8)	AGGACGTGGTCACCGGCTAC
N8.B10-M13-RV(9)	TCACATCCAAGCTACCCTCG
N8.B10-M13-20(2)	GAATATCACCTTGGTCAGCA
N8.B10-M13-20(3)	GCCACGATCCCCTCAGCCGC
N8.B10-M13-20(4)	CACGTACACCAGCATGGGGA
N8.B10-M13-20(5)	GAGGCCCTAACTAACCAAGA
N8.B10-M13-20(6)	AGGATGGAGAGCACCGAGTT
N8.B10-M13-20(7)	CGAGCTGGCACACTTGGC
N8.B10-M13-20(8)	AAACTCGCCGTACATAGGAT
N8.B10-M13-20(9)	TGCTTGTTGGGTTAATTGTAGT

cDNA sequencing of *HvCslF6*	
For amplification of the entire cDNA	
HvCSLF6_CP1-1F	AGAGTGAGTGCGTGCATTGAG
HvCSLF6_CP1-1R	TTGAGTGGATGAGTTGTTTCTTGT
For internal sequencing	
HvCslF6 F2	GTATTCCGCACCGAGAAGAT
HvCslF6 F4	CTACGTCTCCGACGACAGTG
HvCslF6 F7	CCTCGACTGCGATCATTACA
HvCslF6 F9	AAGGGTAAGCACGGCTTCTTG
HvCslF6 F11	CTACCTGGGCATCGTGCTAT
HvCslF6 R1	GAGAGGATGGGCTCCTTGAT
HvCslF6 R3	ATGAAGGGGGAGTTGGAGAG
HvCslF6 R4	GTCGAAGAAGATGCGGTTGT
HvCslF6 R6	ATGAAGGCGTGTGGGTAGAT
HvCSLF6_P28R	GCACCCAGAAGTTGAAGAAGAC

bgl.b mutant allele detection in KM27: Restriction enzyme *Bsr*FI	
HvCSLF6_MPP2-1F	ATCAAGGAGCCCATCCTCTC
HvCSLF6_MPP2-1R	TTGATCCTGGCCTTGAACTC

bgl.c mutant allele detection in KM30: Restriction enzyme *Fnu*4HI	
HvCSLF6-MPP- L	TGGGTGTACGACACCGTCA
HvCSLF6-MPP-R	GCGGAGCACCTGGAAGAG

Supplementary Table S2. Allelic variation in genomic sequences of the *HvCSLF6* gene.

Polymorphism	exon 1	intron 1																	intron 2										exon 3	
nt position	174	333	412	558	620	749	752	988	1064	1137	1162	1188	1212	1398	1491	1530	1656	1737	2768	2958	3070	3133	3164	3173	3175	3262	3317	3347	4064	4801
nt change	G → A	C → T	C → A	A → G	G → C	T→ A	ins. 31	T → G	C → T	Δ 14	T → G	T → C	A → G	G → A	Δ 2	C → G	T → C	C → T	T → C	C → T	G → A	C → T	C → T	ins. 338	(CA TT)n	A → G	(T)n	T → C	G → A	T → C
aa change	no	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	A → T	no
aa position	58																												590	835
	SNP1	SNP2	SNP3	SNP4	SNP5	SNP6	indel 1	SNP7	SNP8	indel 2	SNP9	SNP10	SNP11	SNP12	indel 3	SNP13	SNP14	SNP15	SNP16	SNP17	SNP18	SNP19	SNP20	indel 4	SSR1	SNP21	indel 5	SNP22	SNP23	SNP24
OUH625	G	C	C	G	G	T	no	T	C	no	T	T	A	G	no	C	T	C	T	C	G	C	C	no	3	A	9	T	G	T
Beka	G	C	C	A	G	T	no	T	C	no	T	T	A	G	no	C	T	C	T	C	G	C	C	no	3	A	9	T	G	T
Sachiho Golden	G	C	C	A	G	T	no	T	C	no	T	T	A	G	no	C	T	C	T	C	G	C	C	no	3	A	9	T	G	T
Derkado	G	C	C	A	G	T	no	T	C	no	T	T	A	G	no	C	T	C	T	C	G	C	C	no	3	A	9	T	G	T
Haruna Nijo	G	C	C	A	G	T	no	T	C	no	T	T	A	G	no	C	T	C	T	C	G	C	C	no	3	A	9	T	G	T
OUI444	G	C	C	A	G	T	no	T	C	no	T	T	A	G	no	C	T	T	T	C	G	C	C	no	3	A	9	T	G	T
OUJ066	G	C	C	A	G	T	no	T	C	no	T	T	A	G	no	C	T	C	T	C	G	C	C	no	3	A	9	T	G	T
OUM382	G	C	C	A	G	T	no	T	C	no	T	T	A	G	no	C	T	C	T	C	G	C	C	no	3	A	9	T	G	T
M-737	G	C	C	A	G	T	no	T	C	no	T	T	A	A	no	C	T	C	T	C	G	C	C	no	3	A	9	T	G	T
CDC-Fibar	G	C	C	A	G	T	no	T	C	no	T	T	A	A	no	C	T	C	T	C	G	C	C	no	3	A	10	T	G	T
Azhul	G	C	C	A	G	T	no	T	C	no	T	T	A	A	no	C	T	C	T	C	G	C	C	no	2	A	9	T	G	T
B83-12/21/5	G	C	C	A	G	T	no	T	C	no	T	T	A	A	no	C	T	C	T	C	G	C	C	no	3	A	9	T	G	T
Minerva	G	C	C	A	G	T	no	T	C	no	T	T	A	A	no	C	T	C	T	C	G	C	C	no	3	A	9	T	G	T
OUH725	G	C	C	G	G	T	no	T	T	no	G	T	G	G	no	C	T	C	T	C	A	C	C	+	3	A	9	T	G	T
OUH737	G	C	C	G	G	T	no	T	T	no	G	T	G	G	no	C	T	C	T	C	A	C	C	+	3	A	9	T	G	T
OUH602	C	C	C	G	G	T	no	T	T	no	G	T	G	G	no	C	T	C	T	C	A	C	C	+	4	A	9	T	G	T
Nishinohoshi	G	T	C	G	G	T	no	T	T	no	G	T	G	G	no	G	T	C	T	C	A	C	C	no	3	A	9	C	G	T
OUH639	G	C	C	G	G	T	no	T	T	no	G	T	G	G	no	G	T	C	T	C	A	C	T	no	3	G	9	T	G	T
Shikoku Hadaka 84	G	C	C	G	G	T	no	T	T	no	G	T	G	G	no	G	T	C	T	C	A	C	T	no	3	G	9	T	G	T
SH 84 (lys5h)	G	C	C	G	G	T	no	T	T	no	G	T	G	G	no	G	T	C	T	C	A	C	T	no	3	G	9	T	G	T
Akashinriki	G	C	C	G	G	T	no	T	T	no	G	T	G	G	no	G	T	C	T	C	A	C	T	no	3	G	9	T	G	T
CDC-Bold	G	C	C	G	G	T	no	T	T	no	G	T	G	G	no	G	T	C	T	C	A	C	T	no	3	G	9	T	G	T
Logan	G	C	C	G	G	T	no	T	T	no	G	T	G	G	no	G	T	C	T	C	A	C	T	no	3	G	9	T	G	T
Himalaya	G	C	C	G	G	T	no	T	T	no	G	T	G	G	no	G	T	C	T	C	A	C	T	no	3	G	9	T	G	T
Bowman	G	C	C	G	G	T	no	T	T	no	G	T	G	G	no	G	T	C	T	C	A	C	T	no	3	G	9	T	G	T
Morex	G	C	C	G	G	T	no	T	T	no	G	T	G	G	no	G	C	C	T	C	A	C	T	no	3	G	9	T	G	T
Steptoe	A	C	A	G	C	T	no	G	T	Δ14	G	C	G	G	Δ2	C	T	C	C	T	G	T	C	no	3	A	9	T	A	C
OUT329	A	C	A	G	C	A	+	G	T	Δ14	G	C	G	G	Δ2	C	T	C	C	T	G	T	C	no	3	A	9	T	A	C
TR251	A	C	A	G	C	T	no	G	T	Δ14	G	C	G	G	Δ2	C	T	C	C	T	G	T	C	no	3	A	9	T	A	C