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Down-regulation of the CSLF6 gene results in decreased (1,3;1,4)-beta-D-glucan in endosperm of wheat

Csilla Nemeth, Jackie Freeman, Huw D. Jones, Caroline Sparks, Till K. Pellny, Mark D. Wilkinson, Jim Dunwell, Annica A.M. Andersson, Fabienne Guillon, Luc Saulnier, et al.

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Table S1. Transcript abundance for all *CSL* genes represented on wheat Affymetrix GeneChip®. Wheat *CSL* contigs were identified using the WhETS tool (Mitchell et al., 2007) for all the 33 rice loci in the *CSL* family identified by Hazen et al. (2002). These were matched to the Affymetrix 25mer probe sequences by blastn; the number of probes which match 100% to the contigs are indicated. Mean transcript abundance for whole grain samples through development from wheat cv. Hereward (n=2) and endosperm enriched at 14-21 dpa cv. Cadenza (n=4) are given.

wheat probeset	match quality	Os locus	Os gene	whole grain dpa										endosperm enriched
				6	8	10	12	14	17	21	28	35	42	
Ta.9118.1.S1	250 CJ922175 11	Os06g02180	OsCslD2	206	243	241	243	276	213	170	191	145	141	285
TaAffx.95239.1.A1	113 BE515912 11	Os07g43710	OsCslA7	52	93	127	165	176	158	176	165	160	132	254
Ta.28315.1.S1	216 BT009013 11	Os09g39920	OsCslA12	17	60	90	100	79	55	21	13	12	12	208
TaAffx.30043.1.S1	69 CA629887 11	Os07g43710	OsCslA7	28	48	83	101	110	108	106	91	98	71	183
Ta.7592.1.S1	753 BQ578610 11	Os08g06380	OsCslF6	125	126	85	82	78	52	43	35	36	34	114
TaAffx.11185.3.S1	137 CA629645 11	Os01g56130	OsCslC1	57	56	60	87	82	78	73	50	51	49	55
TaAffx.11185.1.S1_s	250 BE403684 11	Os01g56130	OsCslC1	33	24	27	32	25	34	33	27	29	25	26
TaAffx.9510.1.S1	89 CA596520 11	Os06g12460	OsCslA3	8	9	9	10	10	9	9	9	9	9	25
TaAffx.87259.1.S1	250 CA684999 10	Os09g30130	OsCslE6	17	15	18	19	20	18	19	18	17	15	24
Ta.10065.1.S1	323 CJ625222 11	Os02g09930	OsCslA1	151	71	48	43	41	35	37	60	45	38	18
Ta.7564.1.A1	291 CJ962344 11	Os02g49332	OsCslE2	24	20	16	20	17	20	26	36	27	38	16
TaAffx.42425.1.S1	418 CJ834099 8	Os02g09930	OsCslA1	220	129	71	54	30	27	43	71	48	39	14
Ta.16584.1.A1	141 CJ562262 11	Os08g15420	OsCslC3	13	11	12	11	12	10	11	12	9	11	13
Ta.12382.2.S1	111 BJ250055 11	Os10g20090	OsCslH1	17	14	15	13	13	12	12	12	13	11	13
TaAffx.31533.1.S1_x	87 CA613786 11	Os08g15420	OsCslC3	9	9	9	10	10	10	10	11	10	10	12
TaAffx.118567.1.A1	202 BJ318706 11	Os12g36890	OsCslD4	27	17	11	10	11	14	13	12	10	10	11
TaAffx.83038.1.S1	226 CA666319 10	Os02g49332	OsCslE2	13	11	12	11	11	12	16	16	12	16	10
TaAffx.78802.1.S1	95 CA727078 8	Os09g39920	OsCslA12	9	8	11	10	8	8	8	8	9	8	9
TaAffx.56252.1.S1	81 CA655146 11	Os06g42020	OsCslA9	11	10	9	9	9	9	9	9	8	9	9
Ta.4084.1.S1	87 BJ264002 11	Os09g30130	OsCslE6	9	11	13	15	15	22	19	24	18	72	9
TaAffx.83038.2.S1	127 CA606571 11	Os02g49332	OsCslE2	8	8	8	9	9	9	9	9	9	9	8
Ta.15893.1.S1	121 CA500074 11	Os06g12460	OsCslA3	8	8	8	8	8	9	8	10	8	9	8
TaAffx.97760.1.A1	353 CA722045 11	Os08g06380	OsCslF6	7	7	7	8	10	7	9	7	9	7	8
TaAffx.66064.1.S1	246 BJ209690 11	Os02g49332	OsCslE2	6	6	6	6	6	6	7	6	7	6	6
TaAffx.97003.1.S1	133 CA709225 11	Os07g43710	OsCslA7	5	5	5	5	5	5	5	5	5	5	6
TaAffx.12506.1.S1	127 BJ264735 11	Os08g33740	OsCslA11	6	6	6	6	6	7	6	6	6	7	6
TaAffx.105670.1.S1	220 CA731273 11	Os06g02180	OsCslD2	6	6	6	6	6	6	6	6	6	6	6
TaAffx.78510.1.S1	95 CA731549 11	Os10g20090	OsCslH1	6	6	6	6	6	6	6	6	6	6	6
TaAffx.84431.1.S1	52 CA637007 11	Os07g36750	OsCslF3	5	5	6	5	5	5	5	5	5	5	6
Ta.26183.1.A1	226 CD452591 11	Os08g15420	OsCslC3	5	5	5	5	5	6	5	5	6	6	5
TaAffx.107485.1.S1	186 CA699183 11	Os06g42020	OsCslA9	4	4	4	5	5	5	4	5	5	5	4

Table S2. Wheat EST counts for sequences mapped to rice *CSL* genes. Wheat contigs were identified using the WhETS tool (Mitchell et al., 2007) for all the 33 rice loci in the *CSL* family. The number of ESTs in these contigs categorised into broad tissue types are shown.

rice ID(s)	rice gene	n total	n grain	n leaf	n root
Os09g39920	OsCslA12	11	10	0	0
Os08g06380	OsCslF6	72	8	3	37
Os10g42750	OsCslD1	27	8	1	7
Os01g56130	OsCslC1	36	6	1	16
Os07g43710	OsCslA7	15	6	1	6
Os06g02180	OsCslD2	41	5	0	9
Os12g36890	OsCslD4	18	5	1	5
Os05g43530	OsCslC7	12	5	0	2
Os02g09930	OsCslA1	16	4	1	6
Os08g15420	OsCslC3	27	3	0	15
Os06g12460	OsCslA3	5	3	0	0
Os09g30120	OsCslE1	10	2	1	1
Os09g30130	OsCslE6	43	1	6	2
Os02g49332	OsCslE2	14	1	0	7
Os07g03260	OsCslC10	9	1	1	2
Os06g42020	OsCslA9	4	1	1	2
Os03g26044	OsCslA5	3	1	1	0
Os07g36750	OsCslF3	20	0	1	15
Os08g33740	OsCslA11	15	0	1	12
Os10g20090	OsCslH1	12	0	3	0
Os07g36690	OsCslF2	11	0	0	6
Os07g36700	OsCslF1	5	0	2	2
Os08g25710	OsCslD3	3	0	0	1
Os04g35020	OsCslH2	2	0	0	0
Os07g36610	OsCslF9	2	0	0	1
Os07g36630	OsCslF8	2	0	1	0
Os03g07350	OsCslA4	2	0	0	1
Os07g36740	OsCslF4	1	0	0	0
Os10g26630	OsCslA2	1	0	0	0

Table S3. Enzyme mapping of cell wall polysaccharides in mature grain, determined by HPAEC. Peak areas are given for arabinoxylan oligosaccharides (AXOS) (X, XX, XA³X, XA³XX, XA²⁺³XX, XA³A³XX, XA³XA³XX, XA³A²⁺³XX XA³XA²⁺³XX), β -glucan oligosaccharides (G3, G4) that were used for principal component analysis (Fig 4).

	Treatment	Rep	X	XX	G3	G4	XA ³ X	XA ³ XX	XA ²⁺³ XX	XA ³ A ³ XX	XA ³ XA ³ XX	XA ³ A ²⁺³ XX	XA ³ XA ²⁺³ XX
L9T	T	1	71239	199654	37644	14193	10154	92248	25731	32922	13749	20950	16405
L7T	T	1	83733	250790	54027	20513	10949	97435	31072	31600	13721	21354	20248
L21T	T	1	96629	252801	54178	19576	11391	115616	43471	39291	16340	25766	26439
L4T	T	1	93383	268833	59362	20899	11068	96930	36721	34590	13186	21666	22687
L15T	T	1	95132	289535	66511	24627	6207	95638	35633	30440	9902	21094	21786
L20N	C	1	104233	307093	112668	46661	7009	97750	41611	33253	12613	20914	24637
L23WT	C	1	76460	230928	82827	34121	10684	81987	33900	24394	8090	18187	20134
L15N	C	1	98102	299943	98059	43389	16114	109609	40880	34710	10057	23236	23328
L9T	T	2	86124	248506	49083	18735	13636	109788	32702	38417	14523	22803	24092
L7T	T	2	85611	269374	51312	19643	13882	104833	35489	36653	11561	21320	18913
L21T	T	2	77670	206670	43366	17158	11064	90448	32317	29476	10053	18088	17032
L4T	T	2	66492	198070	45255	17339	9753	79233	27248	29921	9562	15760	15163
L15T	T	2	70803	211946	53807	21260	7808	82033	29200	28248	9811	17983	14322
L20N	C	2	62674	189149	86795	38006	10539	70381	25734	24014	8039	11858	13539
L23WT	C	2	67393	194874	85720	34818	8478	73446	30224	26129	9286	15944	17303
L15N	C	2	63213	196559	70835	31644	11273	84200	27948	30072	9924	17555	16742
L9T	T	3	63784	180970	37580	16104	13070	90402	23229	35525	11400	17948	16547
L7T	T	3	65685	212016	42797	16348	7693	84094	26797	27105	9534	14894	16246
L21T	T	3	61353	180683	33473	13453	9468	77117	26424	24370	7924	16628	15128
L4T	T	3	66062	201998	43556	18028	10557	78843	27883	27840	10113	15521	19258
L15T	T	3	67623	208578	50498	22042	9531	91004	29734	28870	11698	18770	19017
L20N	C	3	76555	232573	94171	39708	7703	81537	33150	28967	11026	17431	22255
L23WT	C	3	66560	205831	75548	32991	11261	74929	32943	23275	8552	15484	16957
L15N	C	3	75943	242444	71502	31650	8718	85392	33392	29526	9606	16379	19294

For nomenclature of AXOS see Fauré et al. (2009):

X: D-Xylp (xylose residue of the β -(1,4)- linked backbone)

A²: α -L-Araf-(1 \rightarrow 2)- β -D-Xylp

A³: α -L-Araf-(1 \rightarrow 2)- β -D-Xylp

A²⁺³: α -L-Araf-(1 \rightarrow 2)-[α -L-Araf-(1 \rightarrow 3)]- β -D-Xylp

G3: 3-O- β -cellobiosyl-D-glucose; G4: 3-O- β -cellotriosyl-D-glucose; G5: 3-O- β -cellotetraosyl-D-glucose

Table S4. DNA sequences and targets for PCR primers used in this work.

	Sequence (5' to 3')	Target
SP for CK201603	AGTGAGTGCGTGATTGAGG	Sense in <i>CSLF6</i> gene 5' UTR
ASP1forRACE/F6longer	GTAGTACCCTGCGTCGACGAACTTCTTGCC	Antisense in <i>CSLF6</i> gene 3' UTR
SP_CSLF6	GGGCATTCACCTTCGTCATC	Sense for <i>CSLF6</i> partial cDNA
ASP_CSLF6	GGCCAGTTGTAGTACCCTGC	Antisense for <i>CSLF6</i> partial cDNA
SP_CSLD4	GGGCATTCACCTTCGTCATC	Sense for <i>CSLD4</i> partial cDNA
ASP_CSLD4	GGCCAGTTGTAGTACCCTGC	Antisense for <i>CSLD4</i> partial cDNA
SP_CSLF6_BglII	GCCAGATCTGGGCATTCACCTTCGTCATC	Sense for <i>CSLF6</i> RNAi fragment
ASP_CSLF6_BamHI	GCCGGATCCGGCCAGTTGTAGTACCCTGC	Antisense for <i>CSLF6</i> RNAi fragment
SP_CSLD4_BglII	GCCAGATCTGCTGGAGATCAAGTGGTCSGG	Sense for <i>CSLD4</i> RNAi fragment
ASP_CSLD4_BamHI	GCCGGATCCCAGCACCCAGAAGCTGAAGAAG	Antisense for <i>CSLD4</i> RNAi fragment
SP_CSLF6_RNAi_2739	CATCCCCACATGCATAGCT	Sense for <i>CSLF6</i> qRT inside RNAi fragment
ASP_CSLF6_RNAi_2828	CAGTTGTAGTACCCTGCGTCGA	Antisense for <i>CSLF6</i> qRT inside RNAi fragment
SP_CSLF6_RNAi_2669	GCAAGACGCCAGTCGTGGT	Sense for <i>CSLF6</i> qRT outside RNAi fragment
prTYW19_Ta2526_qPCR_F	CGAGATCGACCAAGAATGG	Sense for Reference gene Ta2526 qRT
prTYW20_Ta2526_qPCR_R	TGAGTGTTGCCTCCCTCC	Antisense for Reference gene Ta2526 qRT
Dx5P fwd	GTCCCTATAAAAGCCTAGCC	Sense near 5' end of HMW 1Dx5 promoter
Adh5' fwd	GAATCGATCTGGGAGGCCAAGG	Sense near 5' end of maize Adh2 intron
Adh3' rev	TGGAAAACACGGGAGTCTGCC	Antisense near 3' end of maize Adh2 intron
Nos5' rev	ATCGCAAGACCGGCAACAGG	Antisense near 5' end of NOS terminator
Neo1	GAGGCTATTCGGCTATGACTG	Sense in <i>NptII</i> gene
Neo2	ATCGGGAGCGGCGATACCGTA	Antisense in <i>NptII</i> gene

Table S5. Segregation and copy number analysis of 16 transgenic wheat lines

Line #	Transgene(s) in T0 plant	Segregation ratio in T1 plants ¹ (+ : -)	χ^2 (df.) and p-value	class of segregation where p-value of >0.05 allows acceptance of Ho	Transgene copy number estimated by TaqMan PCR
3T	CSLF6	22:7	0.1 (1) 0.914	3:1	nd
4T	CSLF6	41:4	0.53 (1) 0.467	15:1	up to 10copies
5T	CSLF6	24:15	3.43 (1) 0.064	3:1	nd
7T	CSLF6	27:7	0.37 (1) 0.554	3:1	up to 5 copies
8T	CSLD2/4	8:5	1.14 (1) 0.285	3:1	nd
9T	CSLF6	25:9	0.04 (1) 0.844	3:1	2 copies
10T	CSLF6	9:17	18.77(1)<0.001	nd	nd
11T	CSLD2/4	31:5	2.69 (1) 0.101	3:1	nd
12T	CSLF6	26:4	2.49 (1) 0.115	3:1	nd
13T	CSLD2/4	21:4	1.19 (1) 0.275	3:1	nd
15T	CSLF6 & CSLD2/4	23:0:0:7	0.05 (1) 0.832	3:1 ²	1 copy ⁴
16T	CSLF6	28:4	3.09 (1) 0.079	3:1	up to 5 copies
18T	CSLF6	28:3	0.62 (1) 0.431	15:1	nd
19T	CSLD2/4	24:5	1.01 (1) 0.315	3:1	nd
21T	CSLF6	26:6	0.71 (1) 0.399	3:1	up to 14 copies
22T	CSLF6 & CSLD2/4	16:7:5:2	0.95 (3) 0.812	9:3:3:1 ³	nd

¹ All ratios are for presence/absence of *CSLF6* or *CSLD2/4* RNAi cassettes except for lines 15T and 22T where ratios are for D4+F6+ : D4-F6+ : D4+F6- : D4-F6-.

² 3:1 test on D4+F6+ : D4-F6-.

³ 9:3:3:1 test on D4+F6+ : D4-F6+ : D4+F6- : D4-F6-.

⁴ Only *CSLF6* gene tested by TaqMan. *CSLD2/4* not determined.

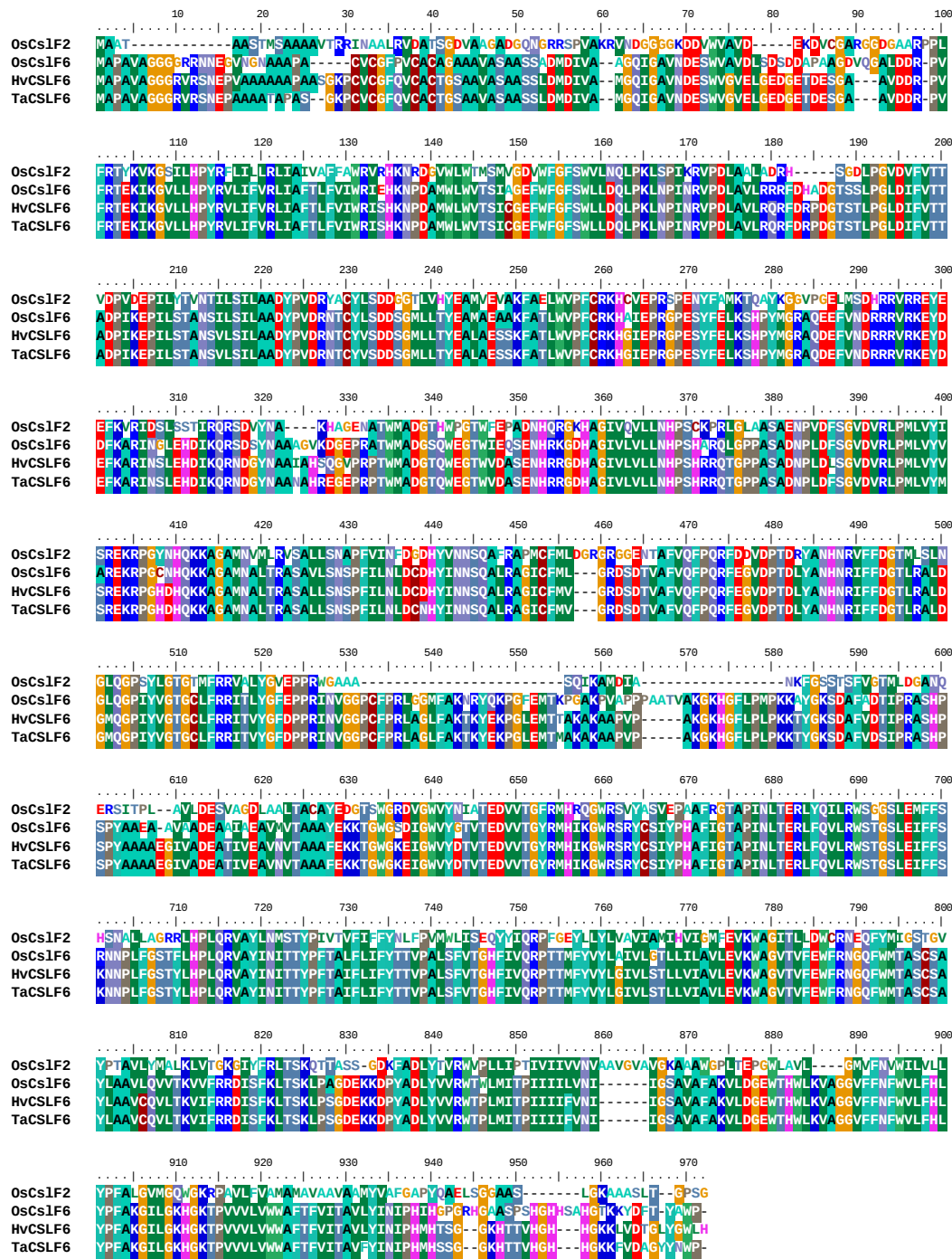


Figure S1. Alignment of wheat, barley and rice CSLF6 protein sequences, with that encoded by the rice *OsCSLF2* gene which was used to transform Arabidopsis resulting in synthesis of β -glucan (Burton et al., 2006). Wheat sequence TaCSLF6 (accession CAN84874) has 97% identity with barley *HvCSLF6* (accession ABZ01578), 85% identity with rice *OsCSLF6* (TIGR locus Os08g06380) and 46% identity with *OsCSLF2* (TIGR locus Os07g36690).



Figure S2. Sequence of 3' end of *TaCSLF6* gene coding region (accession AM743080.2). SNPs corresponding to two putative homoeologues of the gene, variant1 and variant2, also found in Cadenza cDNA are shown. Red line indicates fragment included in the RNAi construct.

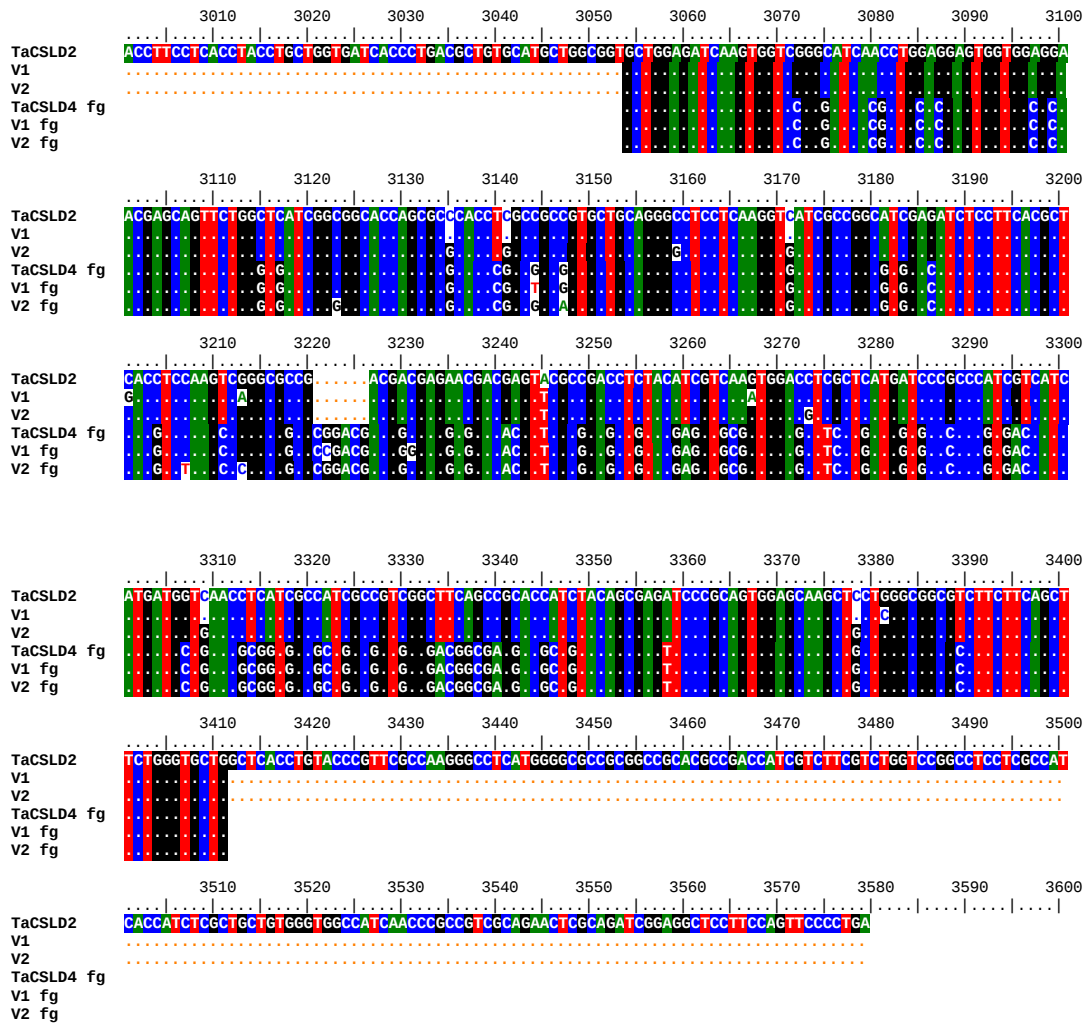


Figure S3. Sequence of the 3' end of *TaCSLD2* gene coding region aligned with the partial cDNA sequence of wheat *TaCSLD4* gene used for the *CSLD2/4* RNAi construct (TaCSLD4 fg). SNPs corresponding to two variants (probable homoeologues) of the *CSLD2* gene (V1 and V2) and of the *CSLD4* fragment (V1 fg and V2 fg), also present in Cadenza cDNA are shown.

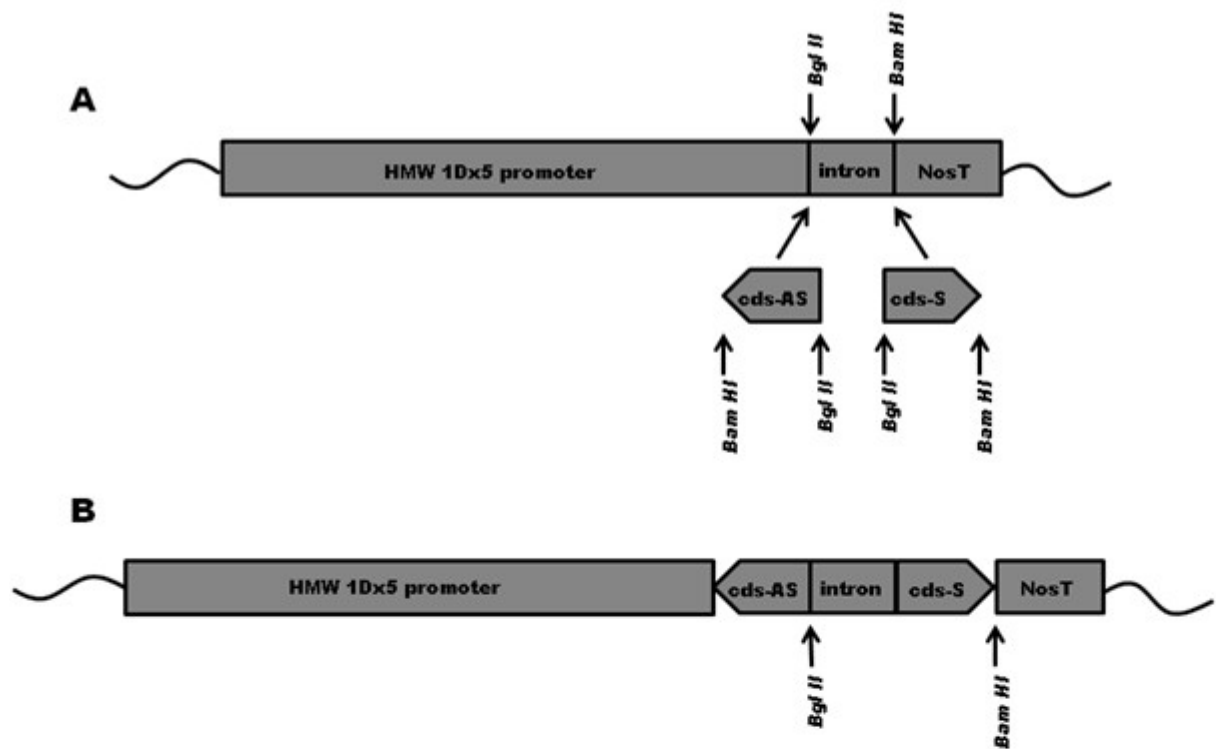


Figure S4. Schematic representation of construction of RNAi expression vectors. (A) pHMW-Adh-Nos, an RNAi expression cassette in pGEM3Zf(+), was digested with *Bgl II* and short regions of coding sequence (cds) from *CSLF6* (133 bp) or *CSLD4* (358 bp) flanked by *Bgl II* and *Bam HI* restriction sites were inserted in the antisense (AS) orientation. The same regions of coding sequence were then inserted in the sense orientation (S) into the *Bam HI* site. (B) The final RNAi constructs therefore contain inverted repeats of either the *CSLF6* gene (pHMW-Adh-Nos-f6ri/ri) or the *CSLD4* gene (pHMW-Adh-Nos-d4ri/ri) flanking the maize Adh2 intron (intron), under the control of the high molecular glutenin 1Dx5 promoter from wheat (HMW 1Dx5 promoter) and followed by the nopaline synthase terminator from *Agrobacterium tumefaciens* (NosT).

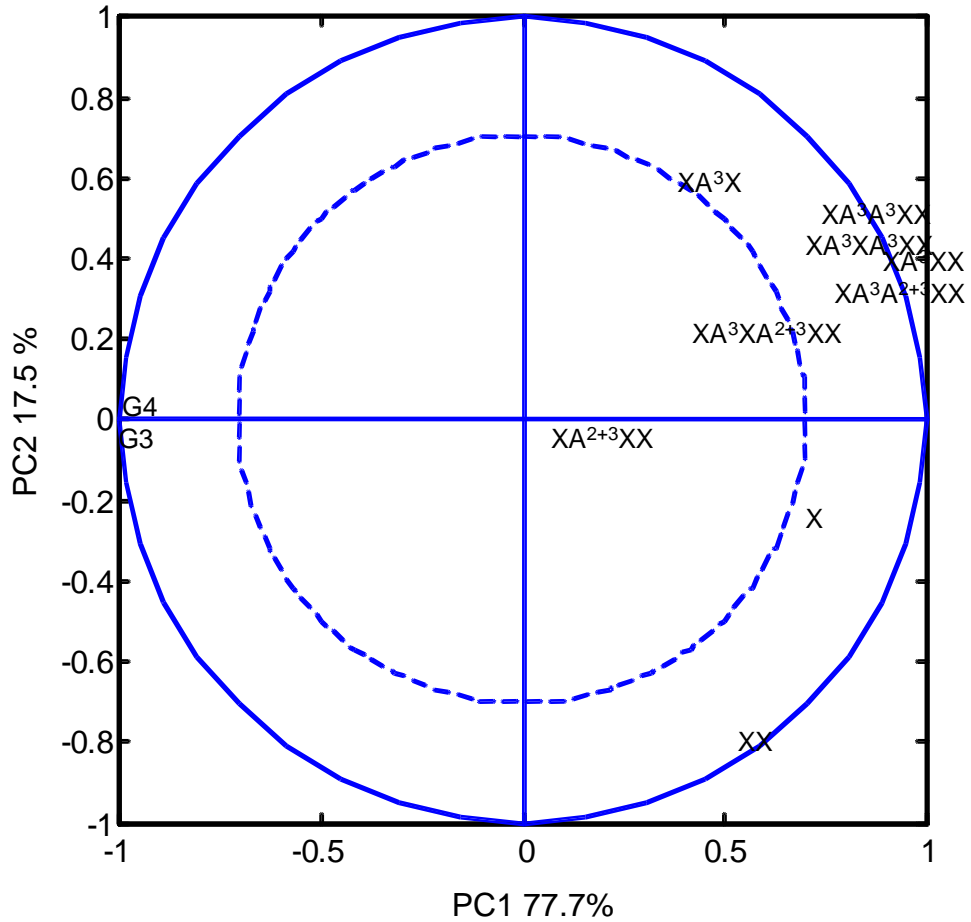


Figure S5. Loading plot for principal component analysis (PCA: Fig. 3) of the enzyme fingerprints of mature seeds of five control and three transgenic line. X, XX, XA³X, XA³XX, XA²⁺³XX, XA³A³XX, XA³XA³XX, XA³A²⁺³XX, XA³XA²⁺³XX, : are AXOS peaks separated by HPAEC (see Fig. 2, Table S3 and Fauré et al. 2009 for nomenclature). G3 and G4 are β -glucan oligosaccharides separated by HPAEC (see Fig. 3 and Table S3)