Cloning and developing markers for genes associated with content of β-glucan



Bin Wu Zongwen Zhang

Institute of Crop Sciences Chinese Academy of Agricultural Sciences

Introduction

Oat β -glucan is a soluble fiber that is found in the cell wall of whole oats.

It is comprised of long chains of glucose and is a polysaccharide. This compound has been shown to help lower the levels of cholesterol in the blood.

Synthesis Pathway of B-glucan



UDP-glucose + (1,3-1,4-b-D-glucosyl)n = UDP + (1,3-1,4-b-D-glucosyl)n+1

Background

- Glucan is an important health-promoting ingredient o f the oats.
- Previous studies have shown that barley glucan synth esis by CSLF, CSLH mediate.(Burton 2006 Science; Doblin 2009,PNAS)

Oat β -glucan synthase gene cloning

	R	S	ML	IS
AsCs/H		asoniaji	-	ada di se
AsCsIF3	-	mant	-	-
AsCsIF4	-	-		S.S.S.
AsCsIF6		_		
AsCsIF8		-	No.	Second 1
AsCsIF9				-
Actin	-	-		

R: Root; S: Stem; ML: Mature Leaf; IS: Immature Seed Oat β-glucan synthase gene expression profile in different organs

Expression profile of gulcan synthase genes in d ifferent oat varieties

0.40



Gene Expression 400 350 300 250 200 Raw RO (Relative Qua 150 100 50

Detectors



Alexandra I.

Glucan synthase genes expression level in different oat varieties

No	Content	CsIF3	CsIF4	CsILF6	CsIF8	CsIF9	CsIH	Glucanase
1	2.82	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
2	3.46	1.3727	8.8397	0.9434	0.8742	1.3287	167.9630	1.6644
3	3.54	3.3519	0.0778	0.9363	0.9917	1.2614	139.0057	1.0253
4	3.57	1.3463	0.4371	0.8932	2.3685	1.7925	90.1965	0.5987
5	4.69	3.0844	0.1866	0.9126	1.3140	2.0662	27.4360	1.5779
6	4.69	2.5036	0.7042	0.9593	1.3623	2.0936	140.6533	0.8975
7	4.71	1.7076	0.1405	1.0260	0.5775	1.2025	117.0515	0.7066
8	4.74	2.3867	3.5801	0.8515	0.8503	2.1053	66.9030	0.5921
9	6.09	2.6208	4.1670	0.7895	1.0246	0.9952	363.7996	1.0000
10	6.15	1.2906	0.8845	0.3069	0.4500	1.0966	251.7765	1.7691
11	6.18	2,7001	0.5987	0.7994	0.8345	0.7443	276.8575	1.8557
12	6.47	1.8674	3.3058	0.8760	0.8151	0.8265	115.5201	1.2492

Correlation Analysis of glucan synthase genes expression profile and glucan content

			(Correlation	n					
		Content	Cs1F3	CslF4	Cs1F6	Cs1F8	Cs1F9	CslH	Glucanase	
Content	Correlation coefficient	1	0.211	0.004	-0.536	-0.564	-0.428	.596 *	0.424	
CallE2	Significant (both sides)		0.511	0.991	0.072	0.056	0.165	0.041	0.169	
	N	12	12	12	12	12	12	12	12	
Cs1LF3	Correlation coefficient	0.211	1	-0.241	0.157	0.007	0.236	0.178	0.053	
	Significant (both sides)	0.511		0.450	0.626	0.983	0.461	0.580	0.870	
	N	12	12	12	12	12	12	12	12	
Cs1F4	Correlation coefficient	0.004	-0.241	1	0.048	-0.200	-0.099	0.224	0.184	
	Significant (both sides)	0.991	0.450		0.883	0.534	0.760	0.483	0.568	
_	N	12	12	12	12	12	12	12	12	
Cs1F6	Correlation coefficient	-0.536	0.157	0.048	1	0.323	0.222	-0.523	-0.479	
	Significant (both sides)	0.072	0.626	0.883		0.306	0.488	0.081	0.115	
	N	12	12	12	12	12	12	12	12	
Cs1F8	Correlation coefficient	-0.564	0.007	-0.200	0.323	1	0.500	-0.286	-0.402	
	Significant (both sides)	0.056	0.983	0.534	0.306		0.098	0.368	0.196	
	N	12	12	12	12	12	12	12	12	
Cs1F9	Correlation coefficient	-0.428	0.236	-0.099	0.222	0.500	1	-0.487	-0.402	
	Significant (both sides)	0.165	0.461	0.760	0.488	0.098		0.108	0.195	
	N	12	12	12	12	12	12	12	12	
CslH	Correlation coefficient	.596 *	0.178	0.224	-0.523	-0.286	-0.487	1	0.368	
	Significant (both sides)	0.041	0.580	0.483	0.081	0.368	0.108		0.239	
_	N	12	12	12	12	12	12	12	12	
Glucanase	Correlation coefficient	0.424	0.053	0.184	-0.479	-0.402	-0.402	0.368	1	
	Significant (both sides)	0.169	0.870	0.568	0.115	0.196	0.195	0.239		
	N	12	12	12	12	12	12	12	12	

Isolation and analysis of AsCsl H



Isolation of *AsCs*/H gene and genomic locus



Nucleotide sequence of AsCslH gene and its deduced amino acid sequence

1	GTATATATTCCTCAGCTAGCGCTTGCTGCTTGCAAGCTCGTGGAGTCGTGGTGGTGGTGGTGGTGGT GTGCCCTCTCTCTCTC	GAGAA	
	CF MASAKKLQERVALP	R P1	15
141	cgccgtggatgctggccgacttcgtcatcctcctcctcctcctcctcctcccccccc	AGGCG	
	TÀ MML À DEVILELLE, À LLÀ RRÀ À SLMEEHÀ STSTSTNI, PÀ LÀ [†] LVCI	F à	62
281		GAGCC	01
		F D	10
421	454,593,593,645,593,597,597,597,597,597,597,597,597,597,597	GTTCG	
	P V T T V N T V L S L L & V D V P D & G G K L & C V V S D D G C S P V T C V & L R F & & F	F	15
561	A DETATT TA SOBOTA A SOBOTA SOBOLO SOBOL	AGAGC	
	à à L. M. V. P. F. C. V. P. H. G. V. G. V. P. S. M. V. F. S. S. À. P. T. F. V. À. T. G. À. G. H. F. F. S. F. N. M. À. F. T.	K S	20
701	GAGTATGAGAAGCTGGTCACCGAATGAGAAGGCGACGAGGGGGGGG	AAGTC	
	E Y E K L V T R T E K & D E G S T L R D G E F & E F T D & E R R N H P T T V K V L M D N S	K S	24
	P5R P5R		24
341	CAAAACAGGGGAAGGTTTCCCACATCTGGTGTACGTCTCCAGAGAGAG	GCTGA	
	KTGEGFPHLVYVSREKSPEHYHNFKÀGÀMNVLTRVSGVMSNÀPIM	L	29
981	acgtggactgcgacatgttcgccaacaacaaccacgcaggtcgccctgcacgcgatgtgccctgctgggggttcggcgacgagacgcaggggttcgtcgtcagggggttcgcccgcagaagttctacggtgctctcaagg	ATGAC	
	N V D C D M F À N N P Q V À L H À M C L L G F G G D E T Q S G F V Q À P Q K F Y G À L K	DD	34
1121	ccctttggcaaccagttggaggttttatacaagaaagttggaggtggagtcgcgggatccaaggaattttatggcgggactggctgttttcaccgcaggaaagtcatttacggcgtgccgccaccagacgttttatgcgcgggatttatacggcgtgtgccgccaccagacgttttatgcgggatttatgacggaaagttggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagtggagga	GTCAA	
	P F G N Q L E V L Y K K V G G G V À G I Q G I F Y G G T G C F H R R K V I Y G V P P D V	V K	38
1261	acacgagagagcaggatcaccatctttcaaggaactccaaatctaggttgggagctcaaaggaactgatagaatcatctccaggagacgtcctcgctagaccagccgtagatatgtcgagtcgagtcgagatcgatc	CGTGG	
	H E R À G S P S F K E L Q I K F G S S K E L I E S S R G I I S G D V L À R P À V D M S S R	v	43
1401	$\lambda\lambda$ gtggc $\lambda\lambda\lambda\lambda$ ctagtggg cgc ctgc λ gc cat gtggg gt cagga gattgg ctgg gt tagga gat gac at a ctg λ cg c cg	CG CTG	
	E V A K L V G A C S Y E A G T C W G Q E I G W V Y G S M T E D I L T G Q R I H A T G W K S	A L	48
	P3R		
1541	TTGGACACCCACCAGCGTTCCTGGGATGTGCTCCGACAGGGGGGGCCAGCCA	TTCAG	50
	L D T T P P A F L G C A P T G G P A S L T Q F K R W A T G L L E I L I S G N S P I L G A I	FR	52
1681	GCGTETECERGETECECGGERGTGCETEGGECGETGEGGEGGEGGEGGEGGEGGEGGEGGEGETGTGGEGGE	AGACG	57
	RLQLRQCLAYLIIDV@PVRAPFELCCALLGPFCLLTNQSFLPKVS	D	57
1821	2 2 T	GGCTC	
		ы т.	62
1961		GACTC	02
	L À F L T V L L K T V G L S E T V F E V T R K E S S S T S D G G À T T D E À D P G L F T F	D S	66
2101	TO DE	TGTCT	
	S P V F T P V T À L S T L N T V À T V V G À H R À L F G T À T À V R G G P G M G E F V C C	v	71
2241	GGATGGTGCTGTGCTGTGGCCATTCGTGAGAGGGACTTGTTAGCAGGGGAAGGTACGGGATCCCGTGGAGTGTCAAGGTGAAAGCCGGCTTGATCGTGTCTGTGTTTGTACACTTATGGACAAGGAACTAACATG	TGGGG	• •
	W M V L C L W P F V R G L V S R G R Y G I P W S V K V K À G L I V S V F V H L W T R N *		75
2381	GCATCGAAATGTTAGAAGCTTGATTCAACAATGTAAGAAACATACGTATACGAATACGAAGCGTGACCGAATGCAGTTCAACAACTAAACTTGGAATAAAGAACTGGTTAGATCTTTGAAAAAAAA	AAAAA	
2521	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA		

Comparison of amino acid sequences of AsCsIH and other published CsIH proteins

Avena sativa	1	MASARRIO <mark>BRVAUPRTAMMLADFVILFILLALLARRAASLWEEHA</mark> STSTSTWIPALALVCEAMFTFVWILLMMNCRWSPVRFDTYPENLPDEELP	94
Hordeum vulgare	1	MACGRRIOBRVAUARTAMMLADFAILFILLAIVARRAASLRERGGTWIAALVCEAMFAFVWILMMNGRWSPVRFDTYPDMLANRMEELP	89
Oryza sativa	1	MEAAAR <mark>C</mark> NKRIOERVPIRRTAWRIADIAILFILLAILFRVIHDSGAPWR-RAALA <mark>CEAWFTFMWILMVNA</mark> RWSPVRFDTFPENLAERIDELP	92
Avena sativa	95	AVDMFVTTADFALEPEVITVNTVLSLLAVDYPDACCKLACYVSDDGCSEVTCYALREAAEFAALWVEFCKRHGVGVRAPSMYFSSAETEVATCAAGHEFS	194
Hordeum vulgare	90	AVDMFVTTADFALEPELITVNTVLSLLALDYPDVC-KLACYVSDDGCSEVTCYALREAAKFAGLWVEFCKRHDVAVRAPFMYFSSTE-EVGTGTADHEFL	187
Oryza sativa	93	AVDMFVTTADFVLEPELVTVNTVLSLLALDYPAGEKLACYVSDDGCSEVTCYALREAAKFAGLWVEFCKRHDVAVRAPFMYFSSTEEFGPADHEFL	189
Avena sativa	195	ENWAFIKSEYEKLVTRIE <mark>KADEG</mark> SILRDGEFAEFIDAERRNHPTIVKVLWDNSKSKTGEGFPHLVYVSREKSPEHYHNFKAGAMNVLTRVSGVMSNAP	292
Hordeum vulgare	188	ESW <mark>AL</mark> MKSEYERLASRIENADEGSIMRDSGDEFAEFIDAERGNHPTIVKVLWDNSKSKVGEGFPHLVYLSREKSPRHRHNFQAGAMNVLTRVSGVMSNAP	287
Oryza sativa	190	EDWTFMKSEYEKLV <mark>HRIED</mark> ADEPSLLRHGGGEFAEFIDVERGNHPTIIKVLWDNNRSRTGDGFPRLIYVSREKSPNL <mark>HHHYKAGAMNA</mark> LTRVSALMTNAP	289
Avena sativa	293	IMLNVDCDMFANNPQVALHAMCLLLGFGGDETQSGFVQAPQKFYGALKDDPFGNQLEVLYKKVGGGVAGIQGIFYGGTGCFHRRKVIYGVPPPDVVKHER	392
Hordeum vulgare	288	IMLNVDCDMFANNPQVALHAMCLLLGFD-DETHSGFVQVPQKFYG <mark>C</mark> LKDDPFGNQMQVITKKIGGGIAGIQGMFYGGTGCFHRRKVIYGMPPPDTLKHET	386
Oryza sativa	290	FMLNLDCDMFVNNPRVVLHAMCLLLGFD-DEISCAFVQTPQKFYGALKDDPFGNQLEVSLMKVGRGIAGIQGIFYCGTGCFHRRKVIYGMRTGREGT	385
Avena sativa	393	AGSPSFKELQIKFGSSKELIESSRGIISGDVLARFAVDMSSRVEVAKLVGACSYEAGTCWGQEIGWVYGSMTEDILTGQRIHATGWKSALLDTTPPAFLG	492
Hordeum vulgare	387	RGSPSYKELQVRFGSSKVLIESSRNIISGDLLARFTVDVSSRIEMAKQVGDCNYEAGTCWGKEIGWVYGSMTEDILTGQRIHAAGWKSALLDTNPPAFLG	486
Oryza sativa	386	TGYSSNKELHSKFGSSNNFRESARDVIYGNLSTEPIVDISS <mark>CVDVAKEVAACNYEIGTCWGQEVGWVYGSLTEDVLTGQRIHAA</mark> GWKSALLDTNPPAFLG	485
Avena sativa	493	CAPTGGPASITQFKRWATGLLEILISGNSPILCAIFRRLQLRQCLAYLIIDVWPVRAFFELCCALLGPFCLLTNQSFLPKVSDEGFRIFLALFLAYNMYN	592
Hordeum vulgare	487	CAPTGGPASITQFKRWATGVLEILISRNSPILGTIFQRLQLRQCLGYLIVEAWPVRAFFELCYALLGPFCLLTNQSFLPKVSDEGFRIFVALFLSYHIYH	586
Oryza sativa	486	CAPNGGPACLTQLKRWASGFLEILISRNNPILTTF <mark>FR</mark> SLQFRQCLAYLHSYVWPVRAFFELCYALLGPYCLLSNQSFLP <mark>R</mark> TSEDGF <mark>YTALALFIAYNTYM</mark>	585
Avena sativa	593	LMEYKDCGLS <mark>ARAWWNNHRMORITSASAWLLAFLTVLLKTVGLSETVFEVTRKE</mark> SSSTSDGGATTDEADPGLFTFDSSEVFIEVTALSILNIVAIVVGAW	692
Hordeum vulgare	587	LMEYKECGLSARAWWNNHRMORITSASAWLLAFLTVILKTLGLSETVFEVTRKESS-TSDGGAGTDEADPGLFTFDSAPVFIEVTALSVLNIVALAVGAW	685
Oryza sativa	586	FMEFIECGCSARACWNNHRMORITSASAWLLAFLTVILKTLGFSETVFEVTRKDKS-TSDGGDSNTDE <mark>EEPGRFTFD</mark> ESTVFIEVTATAMLSVLAIAVGAW	684
Avena sativa	693	RALF <mark>GTATAVRGGPGMGEFVCCVWMVICIWPFVRG</mark> IVSRGRYGIPWSVKVKAGLIVSVFVHI <mark>W</mark> TRN 758	
Hordeum vulgare	686	R <mark>A</mark> VIGTAAVVHGGPGIGEFVCCGWMVICFWPFVRGIVSRGKHGIPWSVKVKAGLIVAAFVHICTRN 751	
Oryza sativa	685	R <mark>VVIVTTEGIPGGPGISEFISCGWIVICFM</mark> PIIRGIVGSGRYGIPWSIKMKA <mark>C</mark> LIVAIFII <mark>FC</mark> KRN 750	

Relationship between glucan content and AsCslH gene diversity in China Oat Core Collections



Frequency distribution of China Oat Core Collections glucan content

Relationship between glucan content and AsCslH gene diversity in China Oat Core Collections

	430	440 450	2 460	470	480 49	500	510	520	530 540	550	560 -
AGA Aga	A A A	AT AG GGAT AGATTO AT AG GGAT AGATTO	GAAGGGG GAGGGGGG	G A AG GG G A G A AG GG G A	I GG GG IG IG I GG GG IG IG	GA AAGTAAAGA Ga aagtaaaga	AA AA G	G GA G GA	CGGAGA G GG CGGAGA G GG	G A G A A G G A G A A G	GA A G A GA A G A
AGA AGA	A A A	AT AG GGAT AGAT O AT AG GGAT AGAT O	G AG G GG G G G AG G G G G G	G A AG GG G A G A AG GG G A	A GG GG G G A GG GG G GG	GA AAGTAAAGA Ga aagtaaaga	AA G	G GA G GA	CGGAGA G GG CGGAGA G GG	G A G A A G G A G A A G	GAAGA GAAGA
AGA AGA	A A A	AT AG GGAT AGAT O	A G GG G G	G A AG GG G A G A AG GG G A	I GG GGG G IG I GG GG IG IG	GA AAG AAAGA GA AAG AAAGA	AA AA	GTGA GTGA	GGGAGA G GG GGGAGA G GG	G A G A A G G A G A A G	GA A G A GA A G A
AGA ACA	Ă ĂT T	AT AG GGAT AGATTO			I ĞĞ ĞĞ TĞ TĞ I ÇÇ ÇÇ TÇ TÇ	GA AAGTAAAGA GA TAAGTAAAAGA	AA G	T TĞTĞA T	IĞĞĞAĞA ĞĞĞ	GTATĞ A ATĞ	TGA ATG A
AĞA	A A	AT AG GGAT AGATTO	A ĞĞĞĞĞ	ĞÂĂĞĞĞĞ			AA G	T C C A	CCCCCCC CCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	G A G A A G	GA A G A
AĞA	ÂÂ	AT AG GGAT AGATTO		Ğ A AĞ ĞĞ Č			AA Ç	T TÇTÇA	CCCACA C CC	ĞĂĞĂĂĞ	ĞĂĂĞĂ

SNP	CC(78%)	CG(5%)	GG(17%)	A2	D3	P value
Glucan content	4.88±0.61	4.44±0.47	4.33±0.68	0.28	-0.17	0.003

Mapping of β -glucan content loci in Oats



Schematic diagram of SSR marker developing

Mapping of β -glucan content loci in Oats



The separation of SSR markers in the mapping population (AM1357&1424)

Mapping of β**-glucan content loci in Oats**

Chr03



QTLs for β -glucan contents of oats in the genetic linkage group

QTLs identified for glucan content by Icimapping

Linkage Group	Position	Left Marker	Right Marker	LOD	PVE(%)	Est A	Est D	
3	57	AM0344	AM0569	7.15	30.78	0.8324	-0.9941	Γ
3	107	AM1789	AM1988	2.71	19.81	1.0291	-1.1032	Γ
3	146	AM1988	AM0641	6.65	18.29	0.3623	-0.3276	
5	15	AM0365	AM0337	3.36	2.07	0.1069	-0.1205	
5	104	AM1382	AM1511	2.76	1.35	0.1032	-0.0532	Γ
6	16	AM0858	AM1084	2.52	1.32	-0.0506	-0.1618	
7	1	AM0610	AM0824	3.04	18.06	0.8119	-0.7783	
7	10	AM1101	AM1605	4.84	21.23	0.7516	-0.7956	
8	78	AM1213	AM2060	4.28	1.85	0.1187	-0.0799	Γ
9	23	AM0374	AM1262	4.72	2.01	-0.0079	-0.2301	Γ
9	108	AM1302	AM1736	3.09	3.18	-0.0619	-0.272	
12	34	AM886-2	AM0983	2.91	7.83	0.1419	0.4023	
12	65	AM0983	AM1738	3.79	13.08	0.0836	0.5329	Γ
13	42	AM1956	AM0086	3.15	24.87	-0.8369	-0.84	
17	26	AM1046	AM1718	2.92	5.84	0.1032	0.3287	
18	19	AM0457	AM635	3.02	10.13	0.2819	-0.4259	
20	19	AM0591	AM1424	4.16	9.55	-0.2162	-0.2925	Γ
20	58	AM1424	AM0789	3.66	6.16	-0.1748	-0.2478	
21	26	AM0164	AM0655	3.91	1.58	0.0332	0.1928	
								T

