

Table 1 Suppl. Plants and their origin.

Species	Names/accessions	Origin
<i>Triticum aestivum</i>	Chinese Spring	China
	Shinchunaga	Japan
<i>Psathyrostachys juncea</i>	W6 19786	Mongolia
	W6 19868; PI 531828; PI 549118	USA
	PI 434241; PI 434242	Canada
	PI 531827	Estonia
	PI 75737; PI 314082; PI 314521; PI 315080	Russia
	PI 222050	Afghanistan
	PI 272136	Kazakhstan
<i>Psathyrostachys fragilis</i>	PI 401393; PI 401397	Iran

Table 2 Suppl. PCR primers used for isolation and bacterial expression of D-hordein ORFs. Tm - melting temperature.

Name/sequence	Tm
D-hordein ORFs	
PF1: 5'-ATGGCTAAGCAGCTGGTCCTCTTTG-3'	67 °C
PR1: 5'-GTGCATGCATGCCCAAGCACCA-3'	
PF2: 5'-CTCA/TTCATCACCGAGAACACC-3'	67 °C
PR2: 5'-GGCTGACAATGAA/GCTGAGACATG-3'	
N-terminal for smaller type	
PF2: 5'-CTCA/TTCATCACCGAGAACACC-3'	55 °C
N-PR1: 5'-CTTGTCTGGCTGC/TTGTGAAGAG-3'	
N-terminal for larger type	
PF2: 5'-CTCA/TTCATCACCGAGAACACC-3'	55 °C
N-PR2: 5'-GGATAGTACCATTGTACTACATG-3'	
C-terminal for smaller type	
C-PF1: 5'-TTCCCT/CGGCCAGCAAATCAC-3'	55 °C
C-PR1: 5'-CTACTACTC/TGTTGGCCAACATGG-3'	
C-terminal for larger type	
C-PF2: 5'-TTCCACGGTGGGTTAACGAC-3'	55 °C
C-PR2: 5'-CTACTACTGGCTGGCA/GGACATGG-3'	
Sequencing primer for <i>Ns 2.6</i>	
pJET 1.2F: 5'-CGACTCACTATAGGGAGAGCGGC-3';	
<i>Ns 2.6-F1</i> : 5'-CTTCACAGCATGTAGTACAATGG-3';	
<i>Ns 2.6-F2</i> : 5'-ACCCGAGCGCAACTTCCCCACGA-3';	
<i>Ns 2.6-R1</i> : 5'-GGAGAAGTTCGACTAGGCAACCA-3';	
pJET 1.2R: 5'-AAGAACATCGATTTTCCATGGCAG-3';	
Sequencing primer for <i>Ns 2.9</i>	
pJET 1.2F: 5'-CGACTCACTATAGGGAGAGCGGC-3';	
<i>Ns 2.9-F1</i> : 5'-CGAGCGCAACTTCCTTCAAACA-3';	
<i>Ns 2.9-R1</i> : 5'-GGAGAAGTTCGACTAGGCAACCA-3';	
<i>Ns 2.9-R2</i> : 5'-CCTTGTCCCAGCTGCTGCAT-3';	
pJET 1.2R: 5'-AAGAACATCGATTTTCCATGGCAG-3';	
Bacterial expression of <i>Ns 2.6</i>	
PET 2.6F: 5'-CTCGCC <u>ATAT</u> TGGAAAGCAAGGCCACAGGGAACAACC-3' ( <i>NdeI</i> site is underlined)	60 °C
PET 2.6R: 5'-GCTGCG <u>GAATTC</u> CTACTACTGGCTGGCAGACAGGGCG-3' ( <i>EcoRI</i> site is underlined)	
Bacterial expression of <i>Ns 2.9</i>	
PET 2.9F: 5'-CTCGCAC <u>ATAT</u> TGGAAAGCAAGGCCATAGGGAACAACC-3' ( <i>NdeI</i> site is underlined)	60 °C
PET 2.9R: 5'-CCTGCG <u>GAATTC</u> CCTACTACTGGCTGGCGGACAGGGCG-3' ( <i>EcoRI</i> site is underlined)	
PCR primer for re-amplified the CDS of <i>Ns 1.3</i> to verify the in-frame stop codon	
<i>Ns 1.3-F</i> : 5'-ATGGCTAAGCGGTTGGTCCTCTTCG-3'	60 °C
<i>Ns 1.3-R</i> : 5'-CTACTACTCGTTGGCCAACATGGC-3'	

Table 3 Suppl. Comparison between D-hordeins from *Ps. juncea*, two *Hordeum* species and HMM-GSs from wheat. The putative pseudogene sequences are marked with a superscript "a".

Genes	GenBank accessions	Numbers of amino acid residues				Numbers of cysteine residues			
		N-terminal	C-terminal	Repetitive domain	Total	N-terminal	C-terminal	Repetitive domain	Total
<i>Ps. juncea</i>									
<i>Ns1.3<sup>a</sup></i>	KY 587711	120	33	263	416	5	1	2	8
<i>Ns2.6</i>	KY 587712	118	42	693	853	5	2	2	9
<i>Ns2.9</i>	KY 587713	118	42	784	944	5	2	2	9
<i>Glu-Ns-1<sup>a</sup></i>	KT 878870	120	33	245	398	5	1	3	9
<i>Glu-Ns-2<sup>a</sup></i>	KT 878871	120	33	245	398	5	1	2	8
<i>Glu-Ns-3<sup>a</sup></i>	KT 878872	120	33	260	413	5	1	2	8
<i>Glu-Ns-4<sup>a</sup></i>	KT 878873	120	33	273	426	4	1	2	7
<i>Glu-Ns-5<sup>a</sup></i>	KT 878874	120	33	263	416	4	1	2	7
<i>Glu-Ns-6<sup>a</sup></i>	KT 878875	120	33	263	416	4	1	2	7
<i>Glu-Ns1</i>	KF 631404	104	42	491	637	4	1	1	6
<i>Glu-Ns2<sup>a</sup></i>	KF 631405	101	42	390	533	5	1	0	6
<i>Glu-Ns3<sup>a</sup></i>	KF 631406	104	42	272	418	5	1	1	7
<i>Glu-Ns4<sup>a</sup></i>	KF 631407	104	42	272	418	5	1	0	6
<i>H. chilense</i>									
<i>D-hordein</i>	EF 417988	110	42	723	875	5	2	2	9
<i>D-hordein</i>	EF 417989	110	42	697	849	5	2	2	9
<i>H. vulgare</i>									
<i>D-hordein</i>	AK 373042	110	42	574	726	5	1	4	10
<i>D-hordein</i>	JQ 867076	110	42	574	726	5	1	4	10
<i>D-hordein</i>	JQ 867077	110	42	554	706	5	1	4	10
<i>D-hordein</i>	JQ 867091	110	42	554	706	5	1	4	10
<i>D-hordein</i>	AY 268139	110	42	584	736	5	1	4	10
<i>D-hordein</i>	D 82941	110	42	534	686	5	1	4	10
<i>T. aestivum</i>									
<i>Glu-Ax1</i>	X 61009	86	42	681	809	3	1	0	4
<i>Glu-Ax2*</i>	M 22208	86	42	666	794	3	1	0	4
<i>Glu-Bx7</i>	X 13927	81	42	645	768	3	1	0	4
<i>Glu-By9</i>	X 61026	104	42	538	684	5	1	1	7
<i>Glu-Dx2</i>	X 03346	88	42	687	817	3	1	0	4
<i>Glu-Dy12</i>	X 03041	104	42	493	639	5	1	1	7
<i>Glu-Dx5</i>	X 12928	89	42	687	818	3	1	1	5
<i>Glu-Dy10</i>	AB 281268	104	42	481	627	5	1	1	7

Table 4 Suppl. Additional genes used in this study.

Species	Loci	Gene	GenBank accessions
<i>Triticum aestivum</i>	<i>Glu-A1</i>	<i>Ay</i>	X 03042
<i>Aegilops markgrafii</i>	<i>Glu-C1</i>	<i>Cx</i>	AF 476959
		<i>Cy</i>	AF 476960
<i>Lophopyrum elongatum</i>	<i>Glu-E1</i>	<i>Ee2.1x</i>	AY 299519
		<i>Ee1.8y</i>	AY 298724
<i>Eremopyrum distans</i>	<i>Glu-F1</i>	<i>Fy</i>	FJ 481573
<i>Crithopsis delileana</i>	<i>Glu-K1</i>	<i>Kx</i>	AY 804128
		<i>Ky</i>	AY 834230
<i>Henrardia persica</i>	<i>Glu-O1</i>	<i>Oy</i>	FJ 481569
<i>Heteranthelium piliferum</i>	<i>Glu-Q1</i>	<i>Qy</i>	FJ 481571
<i>Secale cereale</i>	<i>Glu-R1</i>	<i>Rx</i>	AJ 314782
		<i>Ry</i>	AJ 314781
<i>Pseudoroegneria stipifolia</i>	<i>Glu-St1</i>	<i>St1</i>	DQ 344028
<i>Taeniatherum crinitum</i>	<i>Glu-Ta1</i>	<i>Tax</i>	AY 299654
		<i>Tay</i>	AY 303125
<i>Aegilops umbellulata</i>	<i>Glu-U1</i>	<i>Ux</i>	AF 476961
		<i>Uy</i>	AF 476962
<i>Australopyrum retrofractum</i>	<i>Glu-W1</i>	<i>W1.3</i>	JN 591654
		<i>W2.5</i>	JN 591653
<i>Eremopyrum triticeum</i>	<i>Glu-Xe1</i>	<i>Xex</i>	FJ 481575
		<i>Xey</i>	FJ 481574