

A novel family of γ -gliadin genes are highly regulated by nitrogen supply in developing wheat grain

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Supplementary Table S1. Primers used for PCR sequencing and real time RT-PCR.

gene	primer name	primer sequence	PCR
He-1	He-1F	5-AAC AAT CAA GAG TAA CCA CAA-3	sequencing
	He-1R	5- TAG TAC AAC TAT TGT GAT TTC-3	
He-2	He-2F	5'-ACAGAGTAACCACAAATCCAT-3'	sequencing
	He-2R	5'-TTTCATTTCACTGTCCACCGTTCAT-3'	
He-1	He-FQ	5'-ACAGCTGCCAGTTGAAGCG-3'	Real-time RT-PCR
	He-RQ	5'-GACGATGGCATGCACAATGT-3'	
Ta.2526.1.S1_at	Ta.2526-F	5'-AGAATGGGATGACAAGGAAGA-3'	Real-time RT-PCR
	Ta.2526-R	5'-TCCTCCCATTGCTGGACAG-3'	

Supplementary Table S2. Nitrogen contents of grains grown at Rothamsted in 2009 and 2010 (all means of three replicate plots, with standard deviation of the mean in parenthesis).

2009	Cordiale	Hereward	Istabraq	Malacca	Marksman	Xi-19
N100	1.58 (0.12)	1.65 (0.06)	1.40 (0.06)	1.49 (0.06)	1.52 (0.05)	1.54 (0.08)
N200	1.96 (0.14)	2.16 (0.16)	1.85 (0.07)	1.93 (0.07)	2.04 (0.08)	1.94 (0.11)
N350	2.26 (0.03)	2.44 (0.01)	2.06 (0.04)	2.25 (0.06)	2.28 (0.05)	2.23 (0.08)

2010	Cordiale	Hereward	Istabraq	Malacca	Marksman	Xi-19
N100	1.54 (0.02)	1.64 (0.01)	1.44 (0.04)	1.58 (0.05)	1.52 (0.07)	1.55 (0.05)
N200	2.21 (0.05)	2.31 (0.03)	1.93 (0.04)	2.15 (0.07)	2.11 (0.03)	2.13 (0.05)
N350	2.30 (0.02)	2.44 (0.03)	1.99 (0.04)	2.24 (0.02)	2.28 (0.04)	2.26 (0.08)

Supplementary Table S3. Probesets for γ -gliadin transcripts on the Affymetrix GeneChip® Wheat Genome Array.

Typical gamma-gliadin probe sets	
Ta.24114.8.S1_x_at	TaAffx.128418.2.S1_x_at
Ta.30782.2.S1_x_at	Ta.30782.9.S1_x_at
Ta.23142.4.S1_s_at	Ta.24114.14.S1_x_at
Ta.30782.4.S1_at	Ta.23142.7.S1_x_at
Ta.160.3.S1_x_at	Ta.23142.11.S1_x_at
Ta.28632.1.S1_x_at	TaAffx.79378.1.S1_at
Ta.24114.8.S1_at	Ta.28792.1.S1_x_at

Novel gamma-gliadin probe sets	
Ta.6175.1.S1_at	Ta.9940.1.A1_at
Ta.6175.1.S1_x_at	Ta.6175.2.A1_x_at

Supplementary Table S4. Assembled DNA sequences of three novel γ -gliadin genes from Chinese Spring. Start and stop codons are boxed.

CS-1: ACGAGTGC GTTACACTTGTAAAGTAGCGCCACCGAACAAAACATACCAAAG
GATCAGTTTGATAAGAGTAGAGGAACTTTAACAAGAAAAGACAAATAGTGA
AGACGAAAAGAAATACATTTTCATGGCAGCTATAAATAGCCATACGCCATG
AAGACCCCTTCCATCATCCATCCTTCAGAAATTTGGAGCACAAGCATCC
ATAATAAACAATCAAGAGTAACCACAAATTCACCAATGAGATCTTCATGG
TCTTTGCCCTCCTCGTTGCATCAACGACCATCACCACCGCGACCGCACAG
CTCGACCTCGCATCCATGACCAAGAAAAGGCCACAACAATCGTTTCTGCA
ACAGCAACCCTTATCCAGCAACAACCATAACCGCTCAAGAGCCACAAC
AACCCTATTCCCGCAAAAAGAGCCACAACAACCATTTCGCTGCAGCAG
CCACAATACCAGCAACAACAACCGTATCCACAACAACCCTTCCCAAGA
ACAACCTTCCCGCAGCAACATTTATTTCCGCGAGCAGCCGCCACAACAAT
TTCCACAACAGATGCCACTTCCGCATCAACAACAACATTCCCGCAACAA
CAACAACAACAAGAACAACCTCCCAACAACAACCACAATTCCCGCAACA
ACAACCATTTTCCCAATATCAACAACCATTAACACAACAACCATACTCGC
AAGAGCAACCATTGCCACAACAACAACCTTCTGTAGAGGAAAAACAACAA
TTGAACTTGTGCAAGGAGTTCTCCTGCAGCAGTGTAACCCAGAGGAGAA
ACTGTGCTTACTCCAGTCAGTGATCCCGTTCTCCGACCAAGACCTCGC
AACAGAACAGCTGCCAGTTGAAGCGACTACAATGTTGTCGACAACCTGCA
CATATCAGTGAACCGTCCCGATGCCCGGCCATCCACAACATTTGTGCATGC
CATCGTCATGCAACAACAACATGTGGATAGAGGTTTCGGCCAGCCTCAAC
CACAACAGTTGGGCCAGGAAATGCCCATGCAGCCTCAACATCAATTGGGC
CAGCACTCTATCCTACCTCAACAACCTAGCCAGTACCAGTTGGTTAGGTT
ACTTGTGATTTCAGACCCTTCTATGTTATGCAACGTGCATGTCCCGTCTG
ATTGCTACACCATTACTGCACCATTGGTAGCATGACTGCCTACAATGGT
GGACAAATGAATCACAATAGTTGTACTACTAGATAGATGGACCTTCATTG
TTTACTCGATTGGACGATCGATGTAGCAATGAAATAAAAATAAAATGCCGT
GCACCATCATATGCGTGAACCAACAGTACAAGTTCAAACCTGGGAATA
AAGGCAAAACAATAATTCACGTCATGCACATCTACTTTGATAGGAACTG
CAATCCATCGATTATGTATCATTTTTTTTCATAGGTATATCAACCCAGTCC
CACCATGTGATGTCAAAAAAGGAACCTCCTTATCCGAAAATTTAGTTCA
G

CS-2: AATATGATGAGTCATTACGATTATCAAGCTTTCTGACACTCCATGGATG
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TAGCCCTGCGAAGATAGTTTGATAAGAGTATCAGAACTTTACAAGAAAAGA
AAATATGAAGACGAAAAGAAATCATTTCATGGCATCTATAAATAGCCATA
CACCGTGAAGACCCCTTCCATGATCCATCTTTCACACATTTAGAGCACA
AACATTCAAAATAACAATAACAGAGTAACCACAAATCCATCATGAAGATC
CTCTTGGTCTTTGCCCTCCTCGTTGTATCAACGACCATCACCACCGCGAT
CGTGCAGCTCGACCTAGTGTCCATGTCCAAGAAAGGCCACAACAATCAT
TTCTGCAGCAGCAACCCTTACCCAGCAACAACCATTGCCGCTGCAAGAA
CCACAACAACCCTATTCCCGCAAAAAGAGCCACAACAACCATTTTCGCT
GCAGCAACCACAACCCAGGAACAACAACCATATCCACAACAGCCACTTC
TCCAACAACAACCTTCCCGCAACATCTATTCCCGCGAGCAGCCACCACAA
CAACAATTTCCGCGAGCAGATGCCACTTCCATATGAACAACAACATTTCCC
GCAACAACAGCAACAGCAACCACAATTCCCGCAACAACAACCATTTCCC
AATATCAACAACCATTAACACAACAACCATAACCCGCAAGAGCAACCATTG
CCACAACAACAACCTTCTGTGGAGGAAAAACAACAATTTGAACCTTGTGCAA
GGAGTTCTCCTGCAACAGTGCAACCCGGAGGAGAACTGTCATTAATCC
AGTCAGTGATCCCGTTCTCCGACCAAGACCTCGCAACAGAAATAGCTGC
CAGTTGAAGCGTCTACAATGTTGTCGACAACCTTGACGTATCAATGAACC
GTCCCGATGCCCGGCCATCCACAACATTTGTGCATGCCATCGTCATGCAAC
AACAACATGTGGATAGAGGTTTCGGCCAGCCTCAACCACAACAGTTGGGC
CAGGGAATGCCATGCAGCCTCAATATCAATTTGGGCCAGGGCTTTATCCT
ACCTCAACAACATGCCCAGTTCAAGTTGGTTAGGTTACTTGTGATTCAGA
CCCTGCCTATGTTATGCAACGTGCATGTCCCATCTGACTGCTACACCCT
AGTGCACCATTTGGTAGCATGACTGCCATGAACGGTGGACAGTGAATGA
AAACAGTTGTACTACTAGATAGATGGACCTTCATTGTTTACTCGATTGGA

CGATCGATGTAGCAATAAAAAAAAAATATAAAATGTCATGCACCATCATATG
TGTGACTCCAACCAA

CS-3: TCCTGACACTCCATGGATGTGTGCTACCAGAAAGCAACTTTGGCGATCAA

TCCAAAAATTACACTTGTAAGTAGCGCCACCGAACAAAACATACCAAAGA
ATCAGTTTTGATACGAGTAGAGGAACTTTACAAGAAAGCAAATGTGAAGAC
GAAGAGAAATCATTTTCATGGCCGTATAAATAGCCATACACCATGAAGAC
CCCCTCCCATCCTCCATCCTTCACACATTTGGAGCACAAAATTCACACT
AAACAATCAAGAGTAACCAAAAAATCCACCATGAAGATCTTCTTGGTCTTT
GCCCTCCTCGTTGTATCAACGATCATCACCACCGCGACCGCGCAGCTCGA
CCCTAGCATCCATGTACAAGAAAGGCCACAACAATCGTTTCCGCAGCAGC
AACCATTACCCAACAACAACCATTCCCCGCTGCAAGAGCCACAACAACCA
CTATTCCAGCAACAACAACCATTCCACAACAGTCACTTCGCCAACAACA
ACTTCCCCAGCAACATTTATTTCCGCAGCAACCGCCGAACAACAATTTTC
CATAGTAGATGCCACTTCCGCATCAACAACAATAATTCCCCGAACAACA
CAACCCCAACAACAACAACCATTTTACCAATATCAACAACCATTAACACA
ACAACCATACACGCGAGAGCAACCATTGCCACAACAACAACCTTCTGTGG
AGGAAAACCAACAATTGAACTTGTGCAAGGAGTTCTGCTGCAGCAGTGC
AACCCGGAGGAGAAAGTGCATTACTGCAGTCAATGATCCCGTTCCTCCG
ACCAAAGACCTCGCAACAGAACAACACTGCCAGTTGAAGCGGCAACAATGTT
GTCGACAACCTTGACATATCAGCGAGCCGTCCCGATGTCCGGCCATCCAC
AACATTGTGCACGCCATCATCATGCAACAACAACAACAACATGTGGATAG
AGGTTTTCGTCCAGCCTCAACCACAACAGTTGGGCCAGGGAATGCCCATGC
AGCCTCAACATCAATTGGGCCAGGGCTTAAGCCTACCTCAACAACCTAGCC
CAGTTCAAGTTGGTTAGGTTACTTGTGATTTCAGACCTTGCCTATGTTATG
CAATGTGCATGTCCCATCTGATTGCTACACCATTAGTGCACCATTTGGTG
GCATCACTGCCTACAACGGTGGACAAATGAATCACAAGAGTTGTACTACT
AT

Supplementary Table S5. Numbers of amino acid residues and calculated molecular weights of the mature γ -gliadin proteins.

			B genome		D genome			A genome	
	CS- γ	HC	CS-2	He-2	Ae	CS-1	He-1	CS-3	Tm
amino acid residues	276	264	298	298	305	305	308	280	284
molecular weight (kD)	31.6	30.1	34.6	34.3	35.5	35.6	36	32.2	32.9

Supplementary Table S6. Sequence similarity of the mature γ -gliadin proteins.

	CS- γ	HC	B genome		D genome			A genome	
			CS-2	He-2	Ae	CS-1	He-1	CS-3	Tm
CS- γ	100.00%	41.30%	46.00%	45.30%	45.50%	44.90%	44.40%	44.70%	45.00%
HC	41.30%	100.00%	65.50%	64.50%	62.10%	62.40%	61.80%	66.70%	68.30%
CS-2	46.00%	65.50%	100.00%	94.60%	87.90%	87.90%	87.10%	81.10%	81.30%
He-2	45.30%	64.50%	94.60%	100.00%	85.60%	85.00%	84.10%	79.40%	79.70%
Ae	45.50%	62.10%	87.90%	85.60%	100.00%	98.70%	97.70%	80.20%	80.80%
CS-1	44.90%	62.40%	87.90%	85.00%	98.70%	100.00%	98.40%	80.20%	80.80%
He-1	44.40%	61.80%	87.10%	84.10%	97.70%	98.40%	100.00%	79.70%	80.30%
CS-3	44.70%	66.70%	81.10%	79.40%	80.20%	80.20%	79.70%	100.00%	95.10%
Tm	45.00%	68.30%	81.30%	79.70%	80.80%	80.80%	80.30%	95.10%	100.00%

Supplementary Table S4. Assembled DNA sequences of three novel γ -gliadin genes from Chinese Spring. Start and stop codons are boxed.

CS-1: ACGAGTGC GTTACACTTGTAAAGTAGCGCCACCGAACAAAACATACCAAAG
GATCAGTTTGATAAGAGTAGAGGAACTTTAACAAGAAAAGACAAATAGTGA
AGACGAAAAGAAATACATTTTCATGGCAGCTATAAATAGCCATACGCCATG
AAGACCCCCTTCCATCATCCATCCTTCAGAAATTTGGAGCACAAGCATCC
ATAATAAACAATCAAGAGTAACCACAAATTCACCAATGAGATCTTCATGG
TCTTTGCCCTCCTCGTTGCATCAACGACCATCACCACCGCGACCGCACAG
CTCGACCCTCGCATCCATGACCAAGAAAAGGCCACAACAATCGTTTCTGCA
ACAGCAACCCTTATCCAGCAACAACCATAACCGCTCAAGAGCCACAAC
AACCCTATTCCCGCAAAAAGAGCCACAACAACCATTTCCGCTGCAGCAG
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CAACAACAACAAGAACAACCTCCCAACAACAACCACAATTTCCCGCAACA
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AAGAGCAACCATTGCCACAACAACAACCTTCTGTAGAGGAAAAACAACAA
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ACTGTGCTTACTCCAGTCAGTGATCCCGTTCTCCGACCAAGACCTCGC
AACAGAACAGCTGCCAGTTGAAGCGACTACAATGTTGTGCAACTTGCA
CATATCAGTGAACCGTCCCGATGCCCGGCCATCCACAACATTTGTGCATGC
CATCGTCATGCAACAACAACATGTGGATAGAGGTTTCGGCCAGCCTCAAC
CACAACAGTTGGGCCAGGAAATGCCCATGCAGCCTCAACATCAATTTGGGC
CAGCACTCTATCCTACCTCAACAACCTAGCCAGTACCAGTTGGTTAGGTT
ACTTGTGATTTCAGACCCTTCCATGTTATGCAACGTGCATGTCCCGTCTG
ATTGCTACACCATTACTGCACCATTTGGTAGCATGACTGCCTACAATGGT
GGACAAATGAATCACAATAGTTGTACTACTAGATAGATGGACCTTCATTG
TTTACTCGATTGGACGATCGATGTAGCAATGAAATAAAAATAAAATGCCGT
GCACCATCATATGCGTGAACCAACCAGTACAAGTTCAAACCTTGGGAATA
AAGGCAAAACAAAATATTCCACGTCATGCACATCTACTTTGATAGGAACTG
CAATCCATCGATTATGTATCATTTTTTTTCATAGGTATATCAACCCAGTCC
CACCATGTGATGTCAAAAAAGGAACCTCCTTATCCGAAAAATTTAGTTCA
G

CS-2: AATATGATGAGTCATTACGATTATCAAGCTTTCTGACACTCCATGGATG
TGTGCTACAAGAAAGCAACTTTGGCGATTAATCCAAATTACACTTGCAAG
TAGCCCTGCGAAGATAGTTTGATAAGAGTATCAGAACTTTACAAGAAAAGA
AAATATGAAGACGAAAAGAAATCATTTCATGGCATCTATAAATAGCCATA
CACCGTGAAGACCCCCTTCCATGATCCATCTTTCACACATTTAGAGCACA
AACATTCAAAATAACAATAACAGAGTAACCACAAATCCATCATGAAGATC
CTCTTGGTCTTTGCCCTCCTCCTGTATCAACGACCATCACCACCGCGAT
CGTGCAGCTCGACCTAGTGTCCATGTCCAAGAAAGGCCACAACAATCAT
TTCTGCAGCAGCAACCCTTACCCAGCAACAACCATTGCCGCTGCAAGAA
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TCCAACAACAACCTTCCCGCAACATCTATTTCCCGCAGCAGCCACCACAA
CAACAATTTCCGCGAGCAGATGCCACTTCCATATGAACAACAACATTTCCC
GCAACAACAGCAACAGCAACCACAATTTCCGCAACAACAACCATTTCCC
AATATCAACAACCATTAACACAACAACCATAACCCGCAAGAGCAACCATTG
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AGTCAGTGATCCCGTTCTCCGACCAAGACCTCGCAACAGAAATAGCTGC
CAGTTGAAGCGTCTACAATGTTGTGCAACTTGCACGTATCAATGAACC
GTCCCGATGCCCGGCCATCCACAACATTTGTGCATGCCATCGTCATGCAAC
AACAACATGTGGATAGAGGTTTCGGCCAGCCTCAACCACAACAGTTGGGC
CAGGGAATGCCATGCAGCCTCAATATCAATTTGGGCCAGGGCTTTATCCT
ACCTCAACAACATGCCCAGTTCAAGTTGGTTAGGTTACTTGTGATTCAGA
CCCTGCCTATGTTATGCAACGTGCATGTCCCATCTGACTGCTACACCCT
AGTGCACCATTTGGTAGCATGACTGCCATGAACGGTGGACAGTGAATGA
AAACAGTTGTACTACTAGATAGATGGACCTTCATTGTTTACTCGATTGGA

CGATCGATGTAGCAATAAAAAAAAAATATAAAATGTCATGCACCATCATATG
TGTGACTCCAACCAA

CS-3: TCCTGACACTCCATGGATGTGTGCTACCAGAAAGCAACTTTGGCGATCAA
TCCAAAAATTACACTTGTAAAGTAGCGCCACCGAACAAAACATACCAAAGA
ATCAGTTTTGATACGAGTAGAGGAACTTTACAAGAAAGCAAATGTGAAGAC
GAAGAGAAATCATTTTCATGGCCGTATAAATAGCCATACACCATGAAGAC
CCCCTCCCATCCTCCATCCTTCACACATTTGGAGCACAAAATTCACACT
AAACAATCAAGAGTAACCAAAAAATCCACCATGAAGATCTTCTTGGTCTTT
GCCCTCCTCGTTGTATCAACGATCATCACCACCGCGACCGCGCAGCTCGA
CCCTAGCATCCATGTACAAGAAAGGCCACAACAATCGTTTCCGCAGCAGC
AACCATTACCCAACAACAACCATTCCCCGCTGCAAGAGCCACAACAACCA
CTATTCCAGCAACAACAACCGTATCCACAACAGTCACTTCGCCAACAACA
ACTTCCCCAGCAACATTTATTTCCGCAGCAACCGCCGAACAACAATTTTC
CATAGTAGATGCCACTTCCGCATCAACAACAATAATTCCCCGAACAACA
CAACCCCAACAACAACAACCATTTTACCAATATCAACAACCATTAACACA
ACAACCATAACGCGAGAGCAACCATTGCCACAACAACAACCTTCTGTGG
AGGAAAACCAACAATTGAACTTGTGCAAGGAGTTCTGCTGCAGCAGTGC
AACCCGGAGGAGAAAGTGCATTACTGCAGTCAATGATCCCGTTCTCTCCG
ACCAAAGACCTCGCAACAGAACAACCTGCCAGTTGAAGCGGCAACAATGTT
GTCGACAACCTTGACATATCAGCGAGCCGTCCCGATGTCCGGCCATCCAC
AACATTGTGCACGCCATCATCATGCAACAACAACAACAACATGTGGATAG
AGGTTTTCGTCCAGCCTCAACCACAACAGTTGGGCCAGGGAATGCCCATGC
AGCCTCAACATCAATTGGGCCAGGGCTTAAGCCTACCTCAACAACCTAGCC
CAGTTCAAGTTGGTTAGGTTACTTGTGATTTCAGACCTTGCCTATGTTATG
CAATGTGCATGTCCCATCTGATTGCTACACCATTAGTGCACCATTTGGTG
GCATCACTGCCTACAACGGTGGACAAATGAATCACAAGAGTTGTACTACT
AT

Supplementary Figure S1. Sequence of novel γ -gliadin He-1 and probesets positions. The primer sequences for PCR are indicated with arrow. The start and stop codon are boxed.

Probeset (Ta.9940.1.A1_at) sequence cover from 10 to 169bp in square bracket. Probeset (Ta.6175.2.A1_x_at) sequences cover from 492 to 611bp, and probesets (Ta.6175.1.S1_at, and Ta.6175.1.S1_x_at) cover from 588 to 1007bp)

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1         10         20         30         40         50
|         |         |         |         |         |
AACAAATCAAAGAGTAACCACAAATTCACCATGAAGATCTTCATGGTCTTTG
CCCTCCTCGTTGCATCAACGACCATCACCACCGCGACCGCACAGCTCGAC 100
CCTCGCATCCATGACCAAGAAAGGCCACAACAATCGTTTCTGCAACAGCA
ACCACTTATCCAGCAACA]CCATACCCGCCTCAAGAGCCACAACAACCAC 200
TATTCCCGCAAAAAGAGCCACAACAACCATTTCCGCTGCAGCAGCCACAA
TACCAGCAACAACAACCGTATCCACAACAACCACTTCCCCAAGAACAAC 300
TCCCCAGCAACATTTATTTCCGCGAGCAGCCGCCACAACAACAATTTCCAC
AACAGATGCCACTTCCGCATCAACAACAACATTTCCCGCAACAACAACAA 400
CAACAACAACAACAAGAACAACACTCCCACAACAACACTCCACAATTTCCGCA
ACAACAACCATTTTCCCAATATCAACAACCATTAACACAAC[AACCATACT 500
CGCAAGAGCAACCATTGCCACAACAACAACCTTCTGTAGAGGAAAAACAA
CAATTGAACTTGTGCAAGGAGTTCCCTCCTGCAGCAGT]GTAACCCAGAGGA 600
GAAACTGTCGT]TACTCCAGTCAGTGATCCCGTTCCTCCGACCAAAGACCT
CGCAACAGAACAGCTGCCAGTTGAAGCGACTACAATGTTGTGACAAC 700
GCACATATCAGTGAACCGTCCCGATGCCCGGCCATCCACAACATTGTGCA
TGCCATCGTCATGCAACAACAACATGTGGATAGAGGTTTCGGCCAGCCTC 800
AACCACAACAGTTGGGCCAGGAAATGCCCATGCAGCCTCAACATCAATTG
GGCCAGCACTCTATCCTACCTCAACAACACTAGCCAGTACAAGTTGGTTAG 900
GTTACTTGTGATTGAGACCCTTCCCTATGTTATGCAACGTGCATGTCCCGT
CTGATTGCTACACCATTACTGCACCATTTGGTAGCATGACTGCCTACAAT 1000
GGTGGACAA]TGAATCACAATAGTTGTACTACTA 1034

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Supplementary Figure S2. Neighbour-joining tree of 129 γ -gliadins from *Triticum*, *Aegilops* species and *Hordeum chilense* (based on full length protein sequences).

