Supporting Information

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SI Materials and Methods

PCR and Sequencing. All PCR amplifications were performed using Platinum Taq polymerase (Invitrogen). PCR product purity and quantity was determined by gel electrophoresis. PCR products were purified by ethanol precipitation. Sequencing reactions were performed using ABI BigDye Terminator version 3.1 (Applied Biosystems) in forward and reverse directions using the appropriate volume of purified PCR product as determined by gel quantification postpurification. Purification of sequencing products was by ethanol precipitation. Samples were sequenced using an Applied Biosystems 3730 genetic analyzer. Genomic regions containing exons coding for functionally important regions of the respective proteins were sequenced (Table S3). *Adh1* exon DNA sequence coding for the catalytic and coenzyme binding domains was obtained along with surrounding intron sequence. Exon sequence coding for the catalytic domain and surrounding intron sequence was obtained for *betaine aldehyde dehydrogenase 1* (*BADH1*), *BADH2*, ABC1037, and *Rpg1*.

Correction of Proportion of Samples Possessing Predominant Sequence. Consider a population of B samples, in which A_x individuals possess the predominant (DNA or encoded amino acid) sequence within a sequence length of x bp. The expected number of samples (A_y) possessing the predominant sequence in a sequence length of y that is double the length of the initial region x was calculated using the following method, where total sample number = B, length of sequence assessed in base pairs = x, samples possessing predominant sequence from x length of sequence = A_x, proportion of samples possessing predominant sequence from x length of sequence = A_x/B, corrected length of sequence in base pairs = y (in this example, $y = 2x$), samples possessing predominant sequence from y length of sequence = A_y, and proportion of samples possessing predominant sequence from y length of sequence = A_y/B.

In correcting for sequence length, it is assumed that the likelihood of discovery of samples with predominant sequence is the same per base pair in the original length of sequence (x) and the corrected length of sequence (y). If an additional length of sequence in the subset of samples (A_x) possessing the predominant sequence is assessed, one will discover that a proportion of this subset of samples possesses the predominant sequence in this additional region of sequence, whereas a proportion does not. If the additional region is equal in length to the region initially assessed (i.e., additional sequence length = x), one will find that $A_x/B \times A_x$ samples possess the predominant sequence over the complete length of sequence ($y = 2x$) or (Eq. **S1**)

$$\mathrm{A}_y = \mathrm{A}_x(\mathrm{A}_x/\mathrm{B}). \qquad \textbf{[S1]}$$

This equation can be extended to $y = 3x$ (Eq. **S2**),

$$\mathrm{A}_y = \mathrm{A}_x(\mathrm{A}/\mathrm{B}) \times \mathrm{A}_x/\mathrm{B} = \mathrm{A}_x(\mathrm{A}_x/\mathrm{B})^2, \qquad \textbf{[S2]}$$

or $y = 4x$ (Eq. **S3**),

$$\mathrm{A}_y = \mathrm{A}_x(\mathrm{A}_x/\mathrm{B})^2 \times \mathrm{A}_x/\mathrm{B} = \mathrm{A}_x(\mathrm{A}_x/\mathrm{B})^3. \qquad \textbf{[S3]}$$

Conceptually, this equation is easiest to understand when one thinks in terms of increasing lengths of y, which are integer multiples of x; however, a generalized equation can be derived for any value of $y \geq 0$, where (Eq. **S4**)

$$\mathrm{A}_y = \mathrm{A}_x(\mathrm{A}_x/\mathrm{B})^{(y/x-1)}. \qquad \textbf{[S4]}$$

This equation yields values of A_y, which decrease as y/x increases, approaching zero as y/x approaches infinity, and increase as y/x decreases, approaching B as y/x approaches zero.

Weeping Ricegrass Chloroplast Genome Sequencing. DNA samples from the two most environmentally differentiated sites, site 29 (site A) and site 37 (site B), were selected for massively parallel sequencing. Key demographic characteristics of sites A and B were similar: both were small populations in terms of area (~20 m^2) and number of individuals (~100), and both were 0.5–1 km from the nearest discrete population. Ongoing gene flow within the (maternally inherited) cp-genome, facilitated by seed dispersal, from other populations is unlikely at either site A or site B because of the small area encompassing these populations and their geographic isolation.

Eleven individuals were sampled per site from which DNA was extracted and quantified by nanodrop spectroscopy (NanoDrop) and gel visualization. Two pools of DNA corresponding to each site were created by mixing equimolar amounts of DNA derived each individual at each site; each sample contributed 270 ng DNA, giving a total of 2.7 μg/pool. Each of these pooled samples was processed as described in the work by Nock et al. (1) and sequenced on an Illumina GAII Platform (Illumina).

Sequence data were trimmed on CLC genomics workbench (www.clcbio.com), where reads with a quality score of less than 0.01 were discarded; paired-end reads were trimmed to a minimum of 30 bp, and single-end reads were trimmed to a minimum of 20 bp in length. Reads from the two sites were then assembled to a *Microlaena stipoides* chloroplast sequence (GenBank accession no. GU592211). Reference assembly was completed with the following parameters: mismatch cost of two, insertion and deletion costs of three, length fraction of 0.8, and similarity of 0.8. Minimum distance for paired-end reads was set at 180, with a maximum distance of 340, and criteria were set to ignore nonspecific matches and vote for ambiguous calls. SNP detection parameters were assigned: window length of 21, maximum number of gaps or mismatches of two, and minimum quality score of 30 for SNP site and surrounding bases. Minimum coverage was initially set as 1×, and minimum variant frequency was 1%. Intraindividual diversity was estimated by analysis of whole-chloroplast genome sequence data derived from an individual plant.

Secondary analysis of the CLC output was conducted using Microsoft Excel 2007, where SNPs at any reference position were eliminated from the analysis if the number of variants equaled one. A minimum sequence coverage of 88× and a minimum frequency of 5% were required for an SNP to be considered real and incorporated into analysis.

DNA Extraction. Leaf tissue was collected from individuals, and DNA extraction was performed using an MWG Theonyx Liquid Performer robot (MWG Biotech) with a modified MagAttract 96 DNA Plant Protocol (Qiagen) that included two Buffer RPW washes and three ethanol washes at steps 6 and 8, respectively.

Identification of SNPs and Genotypes. Forward and reverse sequences were aligned and trimmed using Sequencher (Gene Codes). Polymorphic sites were identified in Sequencher by comparison of sequence chromatograms. Heterozygous individuals were identified from either mismatches to the consensus or ambiguous base calls and were confirmed by viewing chromatogram peaks. Arlequin version 3.0 software (1) was used to assist with grouping sequences for each locus into genotypes.

Calculation of Genotypic Diversity (G_e). Genotype frequencies and the genetic diversity index by Nei (2) were calculated for all samples and samples within individual populations for all loci. The index by Nei (2) is defined as haplotype diversity (H_e) (Eq. **S5**):

$$H_e = 1 - \sum_{i=1}^{k} x_i^2, \qquad \textbf{[S5]}$$

where x_i is allele frequency and k is the number of alleles. The index by Nei (2) may be used to calculate genotypic diversity at a single locus (G_e). Values for G_e based on total polymorphism (G_eT) and nonsynonymous exon polymorphism (G_eA) were calculated.

Frequency of Synonymous vs. Nonsynonymous Polymorphism. A random exon base substitution has a 76% chance of resulting in a change in encoded amino acid sequence in a codon (3). For the loci in which exon polymorphisms were detected, a Fisher exact test was conducted to determine whether the ratio of nonsynonymous to synonymous SNPs was different from the ratio expected by chance, which would be indicative of selection (4).

1. Nock CJ, et al. (2011) Chloroplast genome sequences from total DNA for plant identification. *Plant Biotechnol J* 9:328–333.
2. Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): An integrated software package for population genetics data analysis. *Evol Bioinform Online* 1:47–50.
3. Nei M (1973) Analysis of gene diversity in subdivided populations. *Proc Natl Acad Sci USA* 70:3321–3323.
4. Bundock PC, Henry RJ (2004) Single nucleotide polymorphism, haplotype diversity and recombination in the *Isa* gene of barley. *Theor Appl Genet* 109:543–551.
5. Cronin JK, Bundock PC, Henry RJ, Nevo E (2007) Adaptive climatic molecular evolution in wild barley at the *Isa* defense locus. *Proc Natl Acad Sci USA* 104:2773–2778.

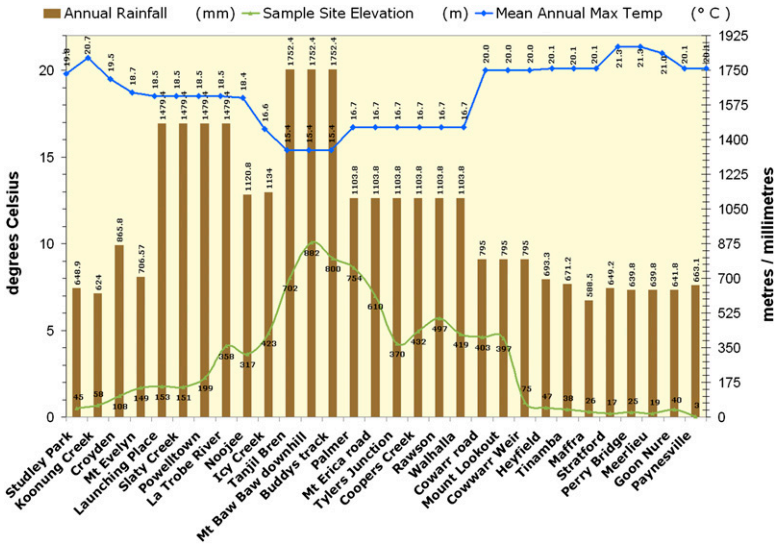

Fig. S1. Climatic data indicating elevation, maximum temperature, and rainfall of collection transect for weeping ricegrass samples.

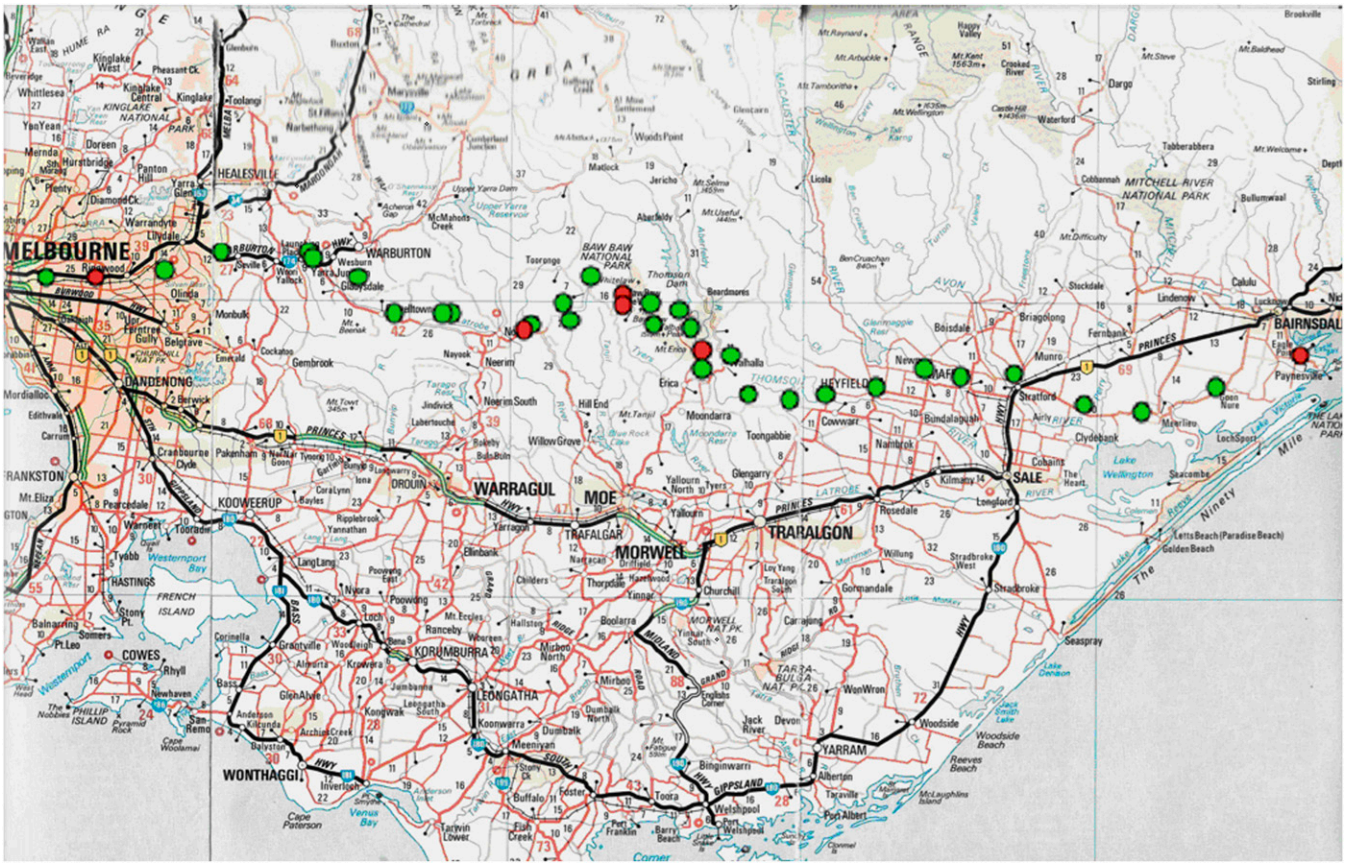

Fig. S2. Sample site transect with two whole-plant samples from each site; an additional 10 leaf samples were collected from sites indicated in red.

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CLUSTAL 2.1 multiple sequence alignment


Isa             GGCCAACCGCGCCCACGGCGGGGGGCTGACGATGGCGCCCGGCCACGGGCGCCACTGCCC 60
MsIsa           GGCCATCCGCGGGCGCGGTGGCGGGCTCACGATGGCCCCC---CACGTGTYCCCCTGCCC 57
                ***** *****  * *** ** ***** ******** ***   **** *  ** ******

Isa             GCTCTTCGTGTCGCAGGACCCCAACGGGCAGCACGACGGGTTCCCCGTGCGCATCACCCC 120
MsIsa           GCTCTTGGTCGCGCAGGAGGCCAACGAGCTCCGCAAGGGCTTCCCCGTGCGCTTCATCCM 117
                ****** **  *******  ****** **  * * * ** ************ *** **

Isa             GTACGGCGTCG-CGCCGTCCGACAAGATCATCCGGCTGTCGACCGACGTCCGCATCTCCT 179
MsIsa           GCGGGAGGACGGCGGCGCC-------ACCGTCCRCCKCTCGACCGACGTCCGCATCGGAT 170
                *   *  * ** ** ** *       * * ***  *  ******************   *

Isa             TCCGCGCCTACACGACGTGTCTGCAGTCCACTGAGTGGCACATCGACAGCGAGCTGGCGG 239
MsIsa           TCAAGGCCGTGACGACGTGCCTGCAGTCCACCGAGTGGCACATCGGYGACGAGCCGTTCT 230
                **   ***   ******** *********** *************    ***** *

Isa             CGGGCCGCCGGCACGTGATCACCGGCCCGGTCAAGGACCCGAGCCCGAGCGGCAGGGAGA 299
MsIsa           CGGGGAGCCGGCGCGTGGTCACCGGACCGGTCGTGGAACTGAGCCCGAGCGGCCGGGAGA 290
                ****  ****** **** ******* ******  *** * ************* ******

Isa             ACGCCTTCCGCATCGAGAAGTACAGCGGCGCCG---AGGTGCACGAGTACAAGC------ 350
MsIsa           ACGCGTTCCGAGTGGAGAAGCACGGCGGCGGCGCGGGGGCGCGCGGGTACAAGCTGGTGT 350
                **** *****  * ****** ** ****** **    ** ** ** ********
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Fig. S3. Alignment of *Isa* and *MsIsa* within the BASI/AMY2 interaction domain (150–500 bp *Isa* cDNA sequence).

Table S1. Distribution of nonsynonymous exon diversity G_eA for wild-barley defense loci within populations

Population	*Rpg1*	ABC1037
Nahal Oren SFS	0.38 (0.25)	0.22
Nahal Oren NFS	0.28 (0.50)	0.48
Mt. Meron	0.00 (0.33)	0.28
Maalot	0.00 (0.08)	0.00
Sede Boqer	0.67 (0.50)	0.29
Wadi Qilt	0.45 (0.25)	0.41
Tabigha TR	0.50 (0.91)	0.62
Tabigha B	0.00 (0.91)	0.48

Values in parentheses in the *Rpg1* column are proportions of samples within a population from which *Rpg1* could be amplified.

Table S2. χ^2 comparison of corrected values (*Materials and Methods*) for predominant genotype (PG) vs. not predominant genotype (NPG) among wild barley defense loci

	Isa	*Rpg1*	ABC1037	*Adh1*	*BADH1*	*BADH2*
Isa	X	14.5*	8.50†	74.8*	63*	11.9*
Rpg1	14.5*	X	2.40	22.5*	12.7*	1.20
ABC1037	8.50†	2.40	X	40.4*	30.4*	0.30
Adh1				X	3.00	34.7*
BADH1				3.00	X	25.1*
BADH2				34.7*	25.1*	X

Red shading indicates comparison among biotic defense loci. Blue shading indicates comparison among abiotic defense loci. Purple shading indicates comparison between biotic and abiotic defense loci. *$P < 0.001$; †$P < 0.01$. X indicates a comparison of a gene to itself (not applicable).

Table S3. χ^2 comparison of corrected values (*Materials and Methods*) for predominant amino acid sequence (PAA) vs. not predominant amino acid sequence (NPAA) among wild barley defense loci

	Isa	*Rpg1*	ABC1037	*Adh1*	*BADH1*	*BADH2*
Isa	X	2.21	2.02	7.49*	7.25*	6.94*
Rpg1	2.21	X	0.07	15.8†	15.3†	14.6†
ABC1037	2.02†	0.07	X	14.3†	13.9†	13.3†
Adh1				X	0.00	0.00
BADH1				0.00	X	0.00
BADH2				0.00	0.00	X

Red shading indicates comparison among biotic defense loci. Blue shading indicates comparison among abiotic defense loci. Purple shading indicates comparison between biotic and abiotic defense loci. *$P < 0.01$; †$P < 0.001$. X indicates a comparison of a gene to itself (not applicable).

Table S4. Origin, sample size (*N*), and ecogeographic variables for each of the eight localities from which wild barley samples were assessed in this study

Locality	*N*	Long (E)	Lat (N)	Alt (m)	Tm (°C)	Rn (mm)
Nahal Oren SFS	12	35.02	32.43	75	19	690
Nahal Oren NFS	12	35.02	32.43	75	19	690
Mt. Meron	12	35.4	33.05	1,150	14	1,010
Maalot	12	35.27	33	500	17	785
Sede Boqer	12	34.78	30.87	450	19	91
Wadi Qilt	12	35.38	31.83	50	23	170
Tabigha TR	11	35.53	32.9	0	24	436
Tabigha B	11	35.53	32.9	0	24	436

Alt, altitude; Lat (N), latitude north; Long (E), longitude east; Rn, mean annual rainfall; Tm, mean annual temperature. Modified from Nevo et al. (1). SFS, south-facing slope; NFS, north-facing slope; TR, terra rosa soil; B, basalt soil.

1. Nevo E, et al. (1985) *Genetica* 67:209–222.

Table S5. Geographic location and description of sample sites from which live weeping ricegrass were collected

Site number	Location name	Sample site elevation (m)	Latitude (S)	Longitude (E)	Degrees east of Melbourne	Land use [natural (N) or agricultural/urban (A/U)]	Soil pH	Soil type	Brief description of sample site location
1	Studley Park	45	37.799	145.01	0.01	A/U	5.5	Loam	River edge, shaded
2	Koonung Creek	58	37.798	145.12	0.12	A/U	5	Loam	Up from creek (4 m), shaded
3	Croyden	108	37.81	145.27	0.27	A/U	5	Clay loam	Partly shaded, not mowed
4	Mt Evelyn	149	37.795	145.38	0.38	A/U	5.5	Loam silt	Shaded, not mowed
5	Launching Place A	153	37.78	145.58	0.58	A/U	5	Clay loam	Heavily shaded, closely mowed
31	Launching Place B	153	37.78	145.58	0.58	A/U	5	Clay	Shaded, not mowed garden verge
6	Slaty Creek	151	37.825	145.66	0.66	A/U	5	Silt	Grazed pasture
7	Powelltown	199	37.864	145.75	0.75	A/U	5	Shallow loam over clay	Mowed ridge in car park
8	La Trobe River Edge	358	37.87	145.8	0.8	N	5.5	Clay loam	Heavily shaded roadside, not mowed
32	La Trobe River Edge	358	37.87	145.8	0.8	N	4.5	Clay loam	Heavily shaded roadside, not mowed
9	Noojee 9	317	37.9	145.98	0.98	N	3.5	Clay	Open, sloping area, not mowed
33	Noojee 33	237	37.897	146.01	1.01	A/U	5.5	Clay loam	Lawn, shaded
10	Icy Creek B	423	37.885	146.08	1.08	A/U	5	Shallow clay over rock	Roadside gutter, not mowed
34	Icy Creek Edge A	501	37.866	146.12	1.12	N	4.5	Clay	Forest verge, not shaded, not mowed
11	Tanjil Bren	702	37.824	146.18	1.18	N	4.5	Loam over clay	Partially shaded, mowed amenity
35	Mt Baw Baw A	882	37.854	146.24	1.24	N	4.5	Clay with organic matter	Roadside, thick bush coverage
37 (B)	Mt Baw Baw B	882	37.852	146.24	1.24	N	4.5	Clay with organic matter	Roadside, thick bush coverage
13	Buddy's Track	800	37.871	146.25	1.25	N	4	Organic layer over clay	Edge of track, full sun
14	Palmer	754	37.911	146.3	1.3	N	4.5	Loam over clay	Track edge, not mowed, thick bush
36	Mt Erica Road	610	37.893	146.38	1.38	N	4	Loam	On side of track
15	Tylers Junction	370	37.925	146.38	1.38	N	4.5	Silt over clay	Rarely mowed near creek edge
17	Coopers Creek	432	37.971	146.4	1.4	N	4.5	Clay	Edge of road, not mowed
18	Rawson	497	37.956	146.4	1.4	A/U	5	Loam with organics	Mowed lawn, under shade
16	Walhalla	419	37.929	146.45	1.45	A/U	3.5	Clay	Roadside, no soil depth on rock
19	Cowwarr Road	403	38.021	146.46	1.46	A/U	5	Clay	Edge of road, not mowed
20	Mt Lookout Area	397	38.003	146.6	1.6	N	4	Clay	Barren, open, not shaded
21	Cowwarr Weir	75	37.999	146.66	1.66	A/U	4	Loam	Passive recreation area, open sun, regular mowing
22	Heyfield	47	37.985	146.78	1.78	A/U	5	Sandy clay	Flood plain, infrequent mowing
23	Tinamba	38	37.96	146.89	1.89	A/U	5.5	Silt over clay	Not mowed passive roadside area
24	Maffra	26	37.967	146.97	1.97	A/U	5	Clay	Not mowed passive amenity area
25	Stratford	17	37.971	147.08	2.08	A/U	5	Sandy loam	Not mowed roadside
26	Perry Bridge	25	38.002	147.23	2.23	A/U	5.5	Loamy sand	Mowed roadside, may be for hay
27	Meerlieu	19	38.001	147.39	2.39	A/U	5	Organic layer over sand	Edge of swamp, mosquitoes
28	Goon Nure	40	37.952	147.52	2.52	A/U	5	Sand	Reserve, no grazing or mowing
29 (A)	Paynesville	3	37.907	147.72	2.72	A/U	5.5	Sand	Rarely mowed sunny amenity

Table S6. Accessions of wild-barley and weeping ricegrass DNA samples as indexed within the Australian Plant DNA Bank (https://www.dnabank.com.au/)

Hordeum spontaneum		*Hordeum spontaneum*		*Microlaena stipoides*		*Microlaena stipoides*	
Sample	Accession	Sample	Accession	Sample	Accession	Sample	Accession
NFS-2	AC05-1005444	TB-55	AC05-1005530	41,670	AC11-1008273	41,717	AC11-1008320
NFS-3	AC05-1005445	TB-56	AC05-1005531	41,671	AC11-1008274	41,718	AC11-1008321
NFS-4	AC05-1005446	TB-57	AC05-1005532	41,672	AC11-1008275	41,719	AC11-1008322
NFS-5	AC05-1005447	TB-58	AC05-1005533	41,673	AC11-1008276	41,720	AC11-1008323
NFS-6	AC05-1005448	TB-60	AC05-1005534	41,674	AC11-1008277	41,721	AC11-1008324
NFS-7	AC05-1005449	TB-61	AC05-1005535	41,675	AC11-1008278	41,722	AC11-1008325
NFS-8	AC05-1005450	TB-62	AC05-1005536	41,676	AC11-1008279	41,723	AC11-1008326
NFS-9	AC05-1005451	TB-63	AC05-1005537	41,677	AC11-1008280	41,724	AC11-1008327
NFS-10	AC05-1005452	TB-67	AC05-1005538	41,678	AC11-1008281	41,725	AC11-1008328
NFS-11	AC05-1005453	TB-70	AC05-1005539	41,679	AC11-1008282	41,726	AC11-1008329
NFS-12	AC05-1005454	TB-76	AC05-1005540	41,680	AC11-1008283	41,727	AC11-1008330
NFS-13	AC05-1005455	SB-1	AC05-1005550	41,681	AC11-1008284	41,728	AC11-1008331
SFS-1	AC05-1005467	SB-2	AC05-1005551	41,682	AC11-1008285	41,729	AC11-1008332
SFS-7	AC05-1005469	SB-3	AC05-1005552	41,683	AC11-1008286	41,730	AC11-1008333
SFS-10	AC05-1005470	SB-4	AC05-1005553	41,684	AC11-1008287	41,731	AC11-1008334
SFS-13	AC05-1005471	SB-7	AC05-1005554	41,685	AC11-1008288	41,732	AC11-1008335
SFS-16	AC05-1005472	SB-13	AC05-1005557	41,686	AC11-1008289	41,733	AC11-1008336
SFS-17	AC05-1005473	SB-36	AC05-1005563	41,687	AC11-1008290	41,734	AC11-1008337
SFS-18	AC05-1005474	SB-37	AC05-1005564	41,688	AC11-1008291	41,735	AC11-1008338
SFS-19	AC05-1005475	SB-38	AC05-1005565	41,689	AC11-1008292	41,736	AC11-1008339
SFS-20	AC05-1005476	SB-39	AC05-1005566	41,690	AC11-1008293	41,737	AC11-1008340
SFS-21	AC05-1005477	SB-40	AC05-1005567	41,691	AC11-1008294	41,738	AC11-1008341
SFS-22	AC05-1005478	SB-45	AC05-1005568	41,692	AC11-1008295	41,739	AC11-1008342
SFS-23	AC05-1005479	Maalot-1	AC05-1005569	41,693	AC11-1008296	41,740	AC11-1008343
WQ-4	AC05-1005492	Maalot-3	AC05-1005570	41,694	AC11-1008297	41,741	AC11-1008344
WQ-8	AC05-1005493	Maalot-4	AC05-1005571	41,695	AC11-1008298	41,742	AC11-1008345
WQ-19	AC05-1005494	Maalot-5	AC05-1005572	41,696	AC11-1008299	41,743	AC11-1008346
WQ-24	AC05-1005495	Maalot-6	AC05-1005573	41,697	AC11-1008300	41,744	AC11-1008347
WQ-41	AC05-1005498	Maalot-8	AC05-1005574	41,698	AC11-1008301	41,745	AC11-1008348
WQ-42	AC05-1005499	Maalot-9	AC05-1005575	41,699	AC11-1008302	41,746	AC11-1008349
WQ-53	AC05-1005500	Maalot-10	AC05-1005576	41,700	AC11-1008303	41,747	AC11-1008350
WQ-58	AC05-1005501	Maalot-11	AC05-1005577	41,701	AC11-1008304	41,748	AC11-1008351
WQ-61	AC05-1005502	Maalot-14	AC05-1005578	41,702	AC11-1008305	41,749	AC11-1008352
WQ-62	AC05-1005503	Maalot-15	AC05-1005579	41,703	AC11-1008306	41,750	AC11-1008353
WQ-64	AC05-1005504	Maalot-18	AC05-1005580	41,704	AC11-1008307	41,751	AC11-1008354
WQ-65	AC05-1005505	Meron-3	AC05-1005595	41,705	AC11-1008308	41,752	AC11-1008355
TTR-18	AC05-1005517	Meron-5	AC05-1005596	41,706	AC11-1008309	41,753	AC11-1008356
TTR-19	AC05-1005518	Meron-9	AC05-1005597	41,707	AC11-1008310	41,754	AC11-1008357
TTR-20	AC05-1005519	Meron-10	AC05-1005598	41,708	AC11-1008311	41,755	AC11-1008358
TTR-23	AC05-1005520	Meron-12	AC05-1005599	41,709	AC11-1008312	41,756	AC11-1008359
TTR-28	AC05-1005521	Meron-16	AC05-1005600	41,710	AC11-1008313	41,757	AC11-1008360
TTR-29	AC05-1005522	Meron-17	AC05-1005601	41,711	AC11-1008314	41,758	AC11-1008361
TTR-33	AC05-1005524	Meron-18	AC05-1005602	41,712	AC11-1008315	41,759	AC11-1008362
TTR-34	AC05-1005525	Meron-19	AC05-1005603	41,713	AC11-1008316	41,760	AC11-1008363
TTR-40	AC05-1005527	Meron-21	AC05-1005604	41,714	AC11-1008317	41,761	AC11-1008364
TTR-41	AC05-1005528	Meron-26	AC05-1005605	41,715	AC11-1008318	41,762	AC11-1008365
TTR-45	AC05-1005529	Meron-27	AC05-1005606	41,716	AC11-1008319	41,763	AC11-1008366
						41,764	AC11-1008367
						41,765	AC11-1008368

Table S7. Details of loci analyzed

Gene	Samples	PCR/sequencing primers	Exon (bp)	Intron (bp)	Highly functionally significant exon region assayed	Ref.
MsIsa	96	**F** TCCCCCTCATACTCCTCTCC; **R** CACGAGTCCCTGCACGACAC	351	0	*MsIsa* region corresponding to 150–500 bp *Isa* cDNA region (coding for BASI/AMY2 interaction domain) (Fig. S3)	1, 2
Rpg1	44*	**F** CATACCGTTGGTTGGTTACTG; **R** GATTGGACTCCACGCATTTT	380	211	Full exon 5, full exon 6 (pk1 catalytic domain)	3
ABC1037	91	**F** ATTCAGTCTGGGTGTCATAATCTTG; **R** AAACAAATCACTCTTTCTATCACAC	289	109	Full exon 7, partial exon 8 (pk1 catalytic domain)	4
Adh1	94	**F CATDOM** CGTGACTGATGTTGCCCCTGGTGAC; **F COENZYME** TATTTGTATGATTTAGGCTGCAGAA; **R CATDOM** GAGATAAGCTGTAGCCATAATAAGC; **R COENZYME** GTCAACTCCGCCATTTGTCATGTCA	304	170	Full exon 4 (catalytic domain); full exon 6, partial exon 7 (coenzyme binding domain)	5
BADH1	91	**F** CCATGTGGATAAGATCGCTTTTACA; **R** CGACAAGTCTATCCAAAAATCGCTC	166	359	Full exon 8, full exon 9 (catalytic domain)	6
BADH2	87	**F** CATCAGGTGTCTTAAACATTGTGAC; **R** GGATAAGAAGACGAGATGTCGCACT	134	467	Full exon 7, full exon 8 (catalytic domain)	7

Gene name, number of samples for which sequence was obtained, primers used for PCR and sequencing reactions, length of exon and intron sequences obtained, and highly functionally significant exon sequences targeted are shown. AMY2, α-amylase 2; BASI, bifunctional amylase/subtilisin inhibitor; *MsIsa*, *Microlaena stipoides Isa*. Boldface letters indicate primer directions (F, forward; R, reverse). For *Adh1*, "CATDOM" indicates primers targeting the catalytic domain and "COENZYME" indicates primers targeting the coenzyme binding domain.
*PCR was performed on 94 samples using the *Rpg1* primers, and product was amplified from 44 of 94 samples (*Results*).

1. Bønsager BC, et al. (2005) Mutational analysis of target enzyme recognition of the β-trefoil fold barley α-amylase/subtilisin inhibitor. *J Biol Chem* 280:14855–14864.
2. Micheelsen PO, et al. (2008) Structural and mutational analyses of the interaction between the barley α-amylase/subtilisin inhibitor and the subtilisin savinase reveal a novel mode of inhibition. *J Mol Biol* 380:681–690.
3. Brueggeman R, et al. (2002) The barley stem rust-resistance gene Rpg1 is a novel disease-resistance gene with homology to receptor kinases. *Proc Natl Acad Sci USA* 99:9328–9333.
4. Brueggeman R, Drader T, Kleinhofs A (2006) The barley serine/threonine kinase gene *Rpg1* providing resistance to stem rust belongs to a gene family with five other members encoding kinase domains. *Theor Appl Genet* 113:1147–1158.
5. Strommer J (2011) The plant ADH gene family. *Plant J* 66:128–142.
6. Tylichová M, et al. (2010) Structural and functional characterization of plant aminoaldehyde dehydrogenase from *Pisum sativum* with a broad specificity for natural and synthetic aminoaldehydes. *J Mol Biol* 396:870–882.
7. Chen S, et al. (2008) Badh2, encoding betaine aldehyde dehydrogenase, inhibits the biosynthesis of 2-acetyl-1-pyrroline, a major component in rice fragrance. *Plant Cell* 20:1850–1861.