Supplementary Figure S1. Genomic sequence of *HvAACT1*.

a, Morex. **b**, Murasakimochi. The UTR region shows blue letters, *HvAACT1* ORF region shows red letters, 1kb insertion in Murasakimochi shows green letters. Alternative splicing sites between 5'-UTR-3,4,5 are indicated as blue triangles.

a) Morex

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b) Murasakimochi

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TGGGTTTTCCTCCGGAAGTGATGGATCAGCTCGCGAGGATGATGGCGGTTCGTCTACAG
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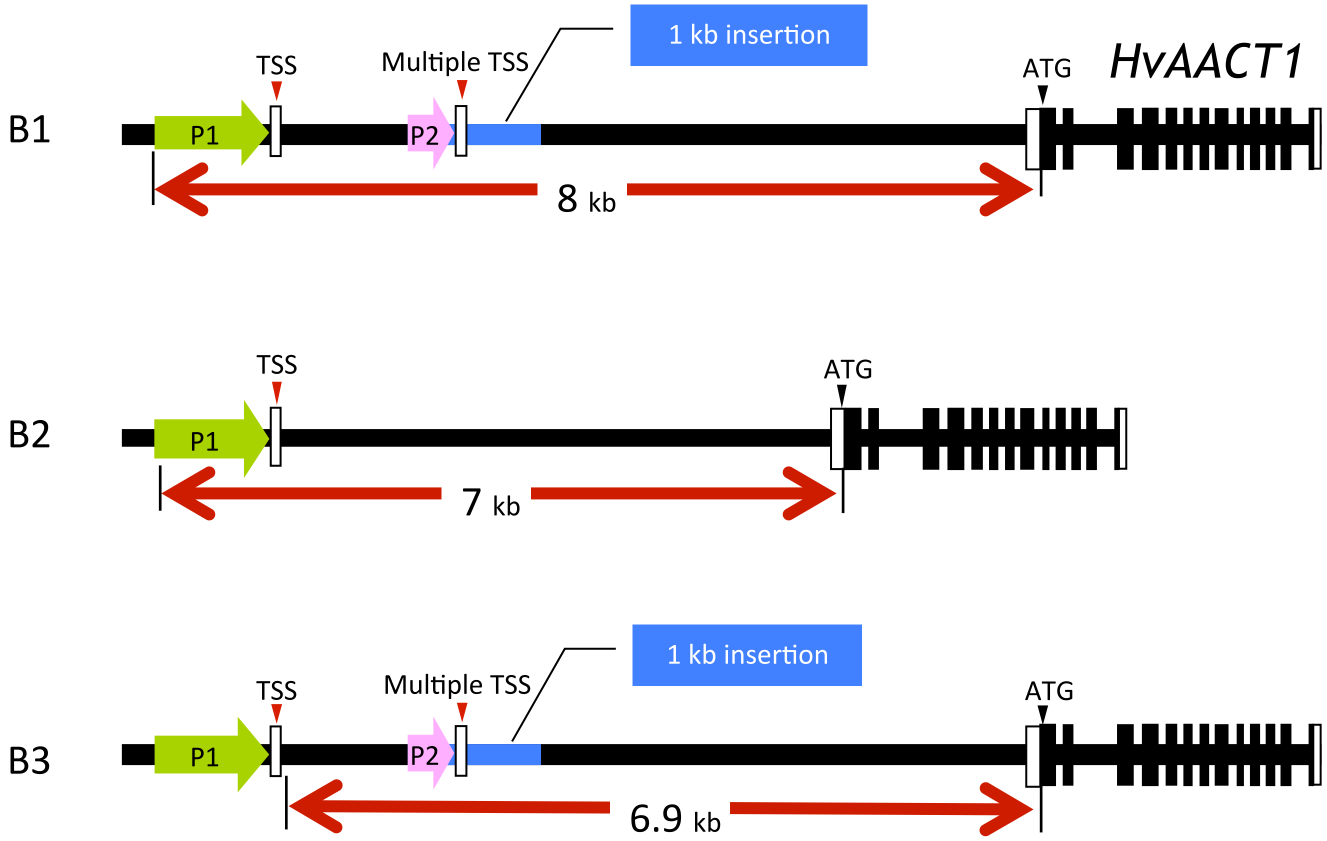
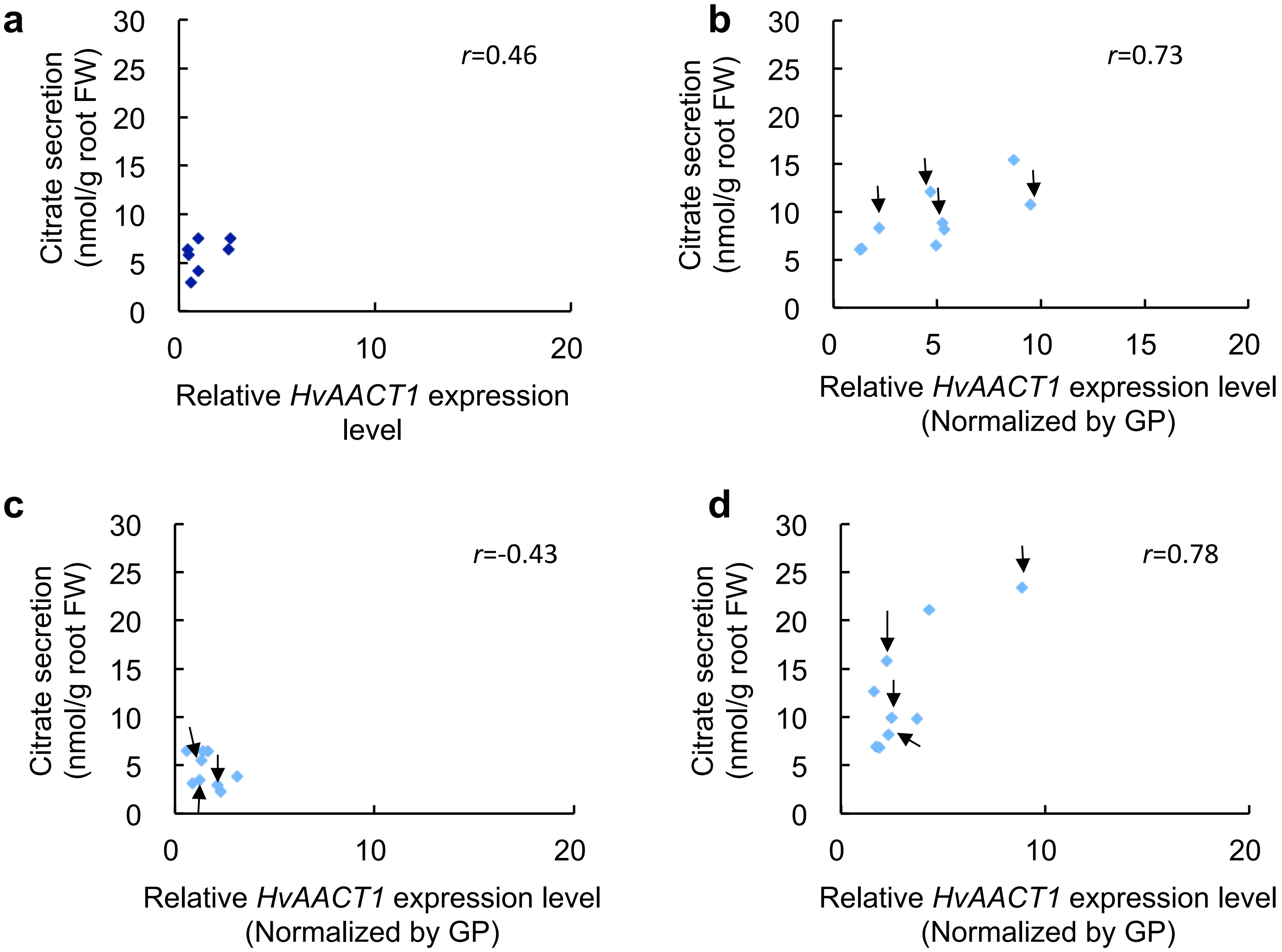
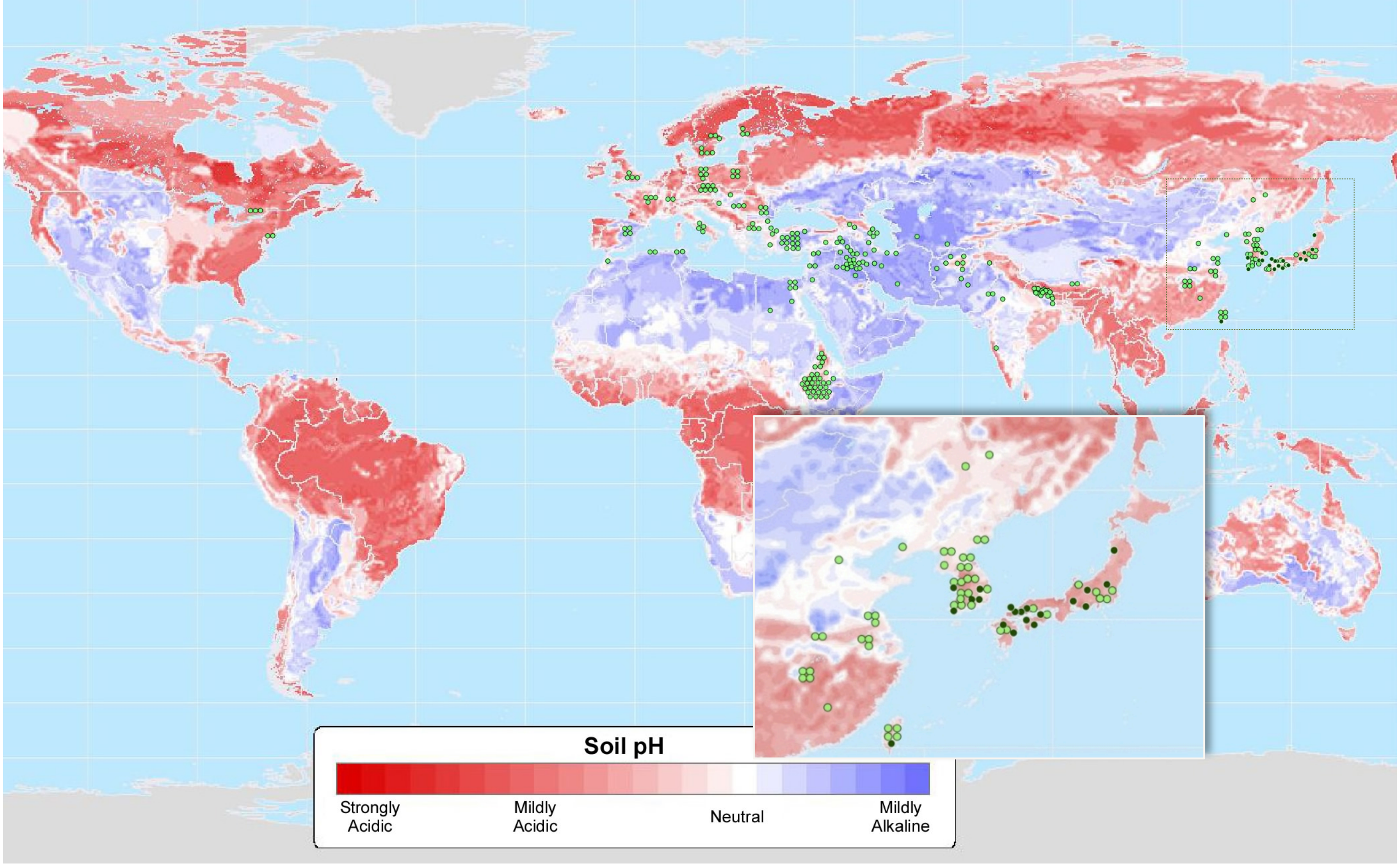


Supplementary Figure S2. Schematic presentation of constructs used for transformation. Promoters of Murasakimochi-type (B1), Morex-type (B2) and insertion-originated promoter (B3) were fused with cDNA of *HvAACT1* from Morex. Red arrows indicate the sequence used for each construction. Black vertical boxes and black lines show exons and introns, respectively, and white box shows the 5’-UTR. P1 means common promoter, P2 means insertion region promoter.



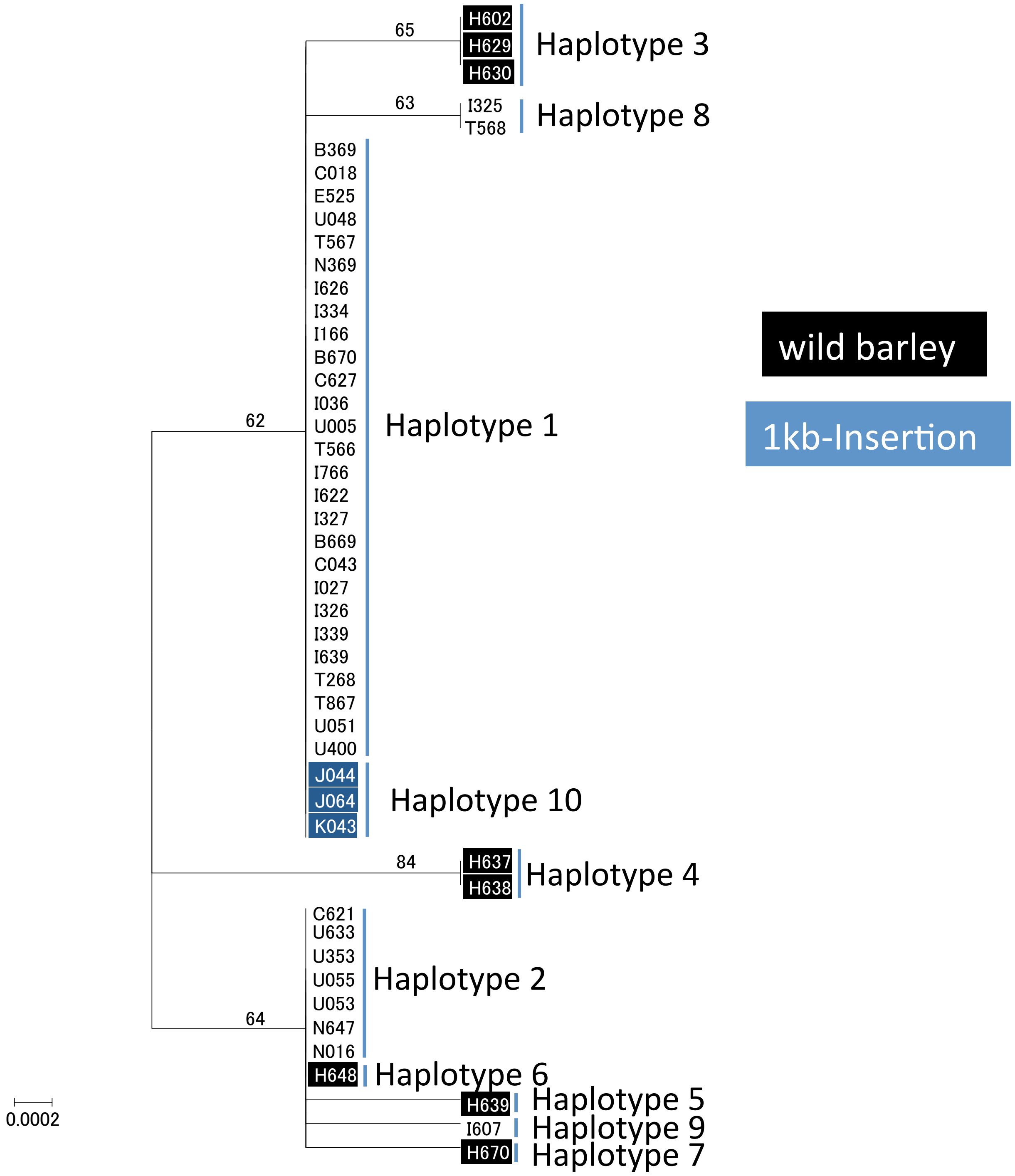
Supplementary Figure S3. Correlation between expression level of *HvAACT1* in the roots and Al-induced secretion of citrate in T0 transgenic barley (GP, B1-B3). The root exudates were collected for 6 h in the presence of 10 µM Al. *r* means correlation coefficient. (**a**) non-transgenic barley (Golden promise), (**b**) *HvAACT1* ORF fused with Murasakimochi promoter (B1), (**c**) Morex promoter (B2) and (**d**) insertion-originated promoter (B3) were transformed to the barley cultivar Golden Promise (GP) by *Agrobacterium* mediated transformation. The arrow showed that lines used for further analysis.



Supplementary Figure S4. Geographic distribution of cultivated barley accessions with (dark green) or without (light green) 1-kb insertion in the upstream of *HvAACT1* gene

A total of 265 cultivated barley accessions collected from the world collection are spotted. Soil pH image is originally provided as the Atlas of the Biosphere by Center for Sustainability and the Global Environment (SAGE), part of the Nelson Institute for Environmental Studies at the University of Wisconsin – Madison (http://sage.wisc.edu/atlas/).

Supplementary Figure S5. Evaluation of Al tolerance in 40 barley cultivars. Seedlings (1-w-old) were exposed to 1.0 mM $CaCl_2$ soltuion (pH 5.0) containing 3 μM Al. After 48 h, the roots were stained by 0.1% Eriochrome cyanine for 15 min. Upper row, cultivars with 1-kb insertion; lower row, cultivars without 1-kb insertion.



Supplementary Figure S6. Neighbor-joining (NJ) tree based on SNPs in the 5'flanking region and 2nd intron of *HvAACT1* .

Wild barley accessions and cultivated accessions with the 1kb-insertion are highlighted by black and blue, respectively. Haplotype numbers indicated in Supplementary Table S3 are shown next to each taxa. The evolutionary distances were computed using the Kimura 2-parameter method. The analysis involved 48 nucleotide sequences, and all positions containing indel mutations were eliminated. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown above to the branches. NJ analyses were conducted in MEGA5.

Supplementary Table S1. List of barley accessions used for sequence analysis of *HvAACT1*

Taxon	Geographic region	n
Wild	Near East	4
	Southwest Asia	2
	Transcaucasus	1
	Unknown	1
	(Total)	8
Domesticated		
	(Western region)	
	Europe	7
	North Africa	3
	Ethiopia	1
	Turkey	5
	Near East	10
	(Eastern region)	
	Southwest Asia	4
	Himalaya	3
	China	4
	Korea	1[a]
	Japan	2[a]
	(Total)	40

a. 1 kb inserted

Supplementary Table S2. Summary of nucleotide variation of *HvAACT1* gene among 48 accessions

	5' flanking region [a]						2nd intron [b]														
	-5348	-5281	-5226	-5217	-4752	-4745	596	597	598	599	600	601	602	618	767	776	869	937	n	wild	cultivated
Hap_1	G	C	T	G	T	-	A	A	G	G	G	G	A	C	C	G	C	-	27		27
Hap_2	.	.	.	T	C	-	.	.	.	.	.	.	.	.	.	.	.	-	7		7
Hap_3	.	T	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	-	3	3	
Hap_4	.	.	.	T	.	-	.	.	.	.	.	.	.	T	.	T	.	-	2	2	
Hap_5	.	.	G	T	C	-	.	.	.	.	.	.	.	.	.	.	.	T	1	1	
Hap_6	.	.	.	T	C	-	-	-	-	-	-	-	-	.	.	.	.	-	1	1	
Hap_7	.	.	.	T	C	-	.	.	.	.	.	.	.	.	T	.	.	-	1	1	
Hap_8	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	A	T	2		2
Hap_9	A	.	.	T	C	-	.	.	.	.	.	.	.	.	.	.	.	-	1		1
Hap_10	.	.	.	.	.	(1kb-Ins)	.	.	.	.	.	.	.	.	.	.	.	-	3		3
																			48	8	40

Deletion mutations are indicated by '-'.

a. positions of 'Morex' 5' flanking sequence are shown (1st base of initiation codon as '1').

b. Positions of 'Haruna Nijo' (AB331641) sequence are shown (1st base of initiation codon as '1').

Supplementary Table S3. Surveys of the 1 kb-insertion in wild barley accessions

No.	OUNo.	Variety	Geographic group	Origin	**HvAACT1 Indel**	sequenced (Haplotype_ID)[a]
1	H601	spont. eu-spont.	Southwest Asia	AFG	0	
2	H602	spont. transcasp.	unknown		0	3
3	H603	spontaneum 2558	Southwest Asia	IRN	0	
4	H604	spontaneum 2294	unknown		0	
5	H605	spontaneum 3325	Southwest Asia	IRN	0	
6	H606	spontaneum 4140	Southwest Asia	AFG	0	
7	H607	spontaneum 4142	Southwest Asia	AFG	0	
8	H608	spontaneum 4163	Southwest Asia	AFG	0	
9	H609	spontaneum 4164	Southwest Asia	AFG	0	
10	H610	spontaneum 5060	Transcaucasus	SSU	0	
11	H611	spontaneum 5101	Transcaucasus	SSU	0	
12	H612	spontaneum 6229	Near East	TKY	0	
13	H613	spontaneum 6586	Southwest Asia	AFG	0	
14	H614	spontaneum 6908	Southwest Asia	IRN	0	
15	H615	spontaneum 6912	Southwest Asia	IRN	0	
16	H616	spontaneum 7111	Southwest Asia	IRN	0	
17	H617	spontaneum 8825	unknown		0	
18	H618	spontaneum 211041	Southwest Asia	AFG	0	
19	H619	spontaneum 212305	Southwest Asia	AFG	0	
20	H620	spontaneum 212306	Southwest Asia	AFG	0	
21	H621	spontaneum 219921	Southwest Asia	AFG	0	
22	H622	spontaneum 220341	Southwest Asia	AFG	0	
23	H623	spontaneum 220523	Southwest Asia	AFG	0	
24	H624	spontaneum 220664	Southwest Asia	AFG	0	
25	H625	spontaneum 227019	Near East	IRN	0	
26	H626	spontaneum 227301	Near East	IRN	0	
27	H627	spontaneum 235639	unknown		0	
28	H628	spontaneum 6(b) (5022)	Southwest Asia	PKS	0	
29	H629	spontaneum 15(b) (5024)	Southwest Asia	PKS	0	3
30	H630	spontaneum 56(b) (5026)	Southwest Asia	AFG	0	3
31	H631	spontaneum 56(c) (5027)	Southwest Asia	AFG	0	
32	H632	spontaneum 372 (5031)	Southwest Asia	IRN	0	
33	H633	spontaneum 389I (5032)	Southwest Asia	IRN	0	
34	H634	spontaneum 625II (5028)	Southwest Asia	AFG	0	
35	H635	spontaneum 625III(5029)	Southwest Asia	AFG	0	
36	H636	spontaneum 657I (5030)	Southwest Asia	AFG	0	
37	H637	spontaneum 168 (5058)	Near East	SAR	0	4
38	H638	spontaneum 332	Near East	JAD	0	4
39	H639	spontaneum 1625	Near East	TKY	0	5
40	H640	spontaneum (Nepal)	Southwest Asia	NPL	0	
41	H642	proskowetzii P.I.247928	Near East	CPS	0	
42	H643	ischnatherum SUM I-a	Southwest Asia	SSU	0	
43	H644	ischnatherum SUM I-b	Southwest Asia	SSU	0	
44	H645	ischnatherum SUM I-c	Southwest Asia	SSU	0	
45	H646	ischnatherum SUM I-d	Southwest Asia	SSU	0	
46	H647	spontaneum SUM II-a	Southwest Asia	SSU	0	
47	H648	spontaneum SUM II-b	Southwest Asia	SSU	0	6
48	H649	spontaneum SUM II-c	Southwest Asia	SSU	0	
49	H650	ischnatherum KON I-a	Southwest Asia	SSU	0	
50	H651	ischnatherum KON I-b	Southwest Asia	SSU	0	

51	H652	ischnatherum KON I-c	Southwest Asia	SSU	0
52	H653	ischnatherum KON I-d	Southwest Asia	SSU	0
53	H654	spontaneum KON II-a	Southwest Asia	SSU	0
54	H655	spontaneum KON II-b	Southwest Asia	SSU	0
55	H656	ischnatherum MAN I-a	Southwest Asia	SSU	0
56	H657	ischnatherum MAN I-b	Southwest Asia	SSU	0
57	H658	ischnatherum MAN II-a	Southwest Asia	SSU	0
58	H659	ischnatherum MAN II-b	Southwest Asia	SSU	0
59	H660	ischnatherum MAN II-c	Southwest Asia	SSU	0
60	H664	ischnatherum GAU I-a	Southwest Asia	SSU	0
61	H665	ischnatherum GAU I-b	Southwest Asia	SSU	0
62	H666	ischnatherum GAU I-c	Southwest Asia	SSU	0
63	H667	spontaneum TAK I-a	Southwest Asia	SSU	0
64	H668	spontaneum TAK I-b	Southwest Asia	SSU	0
65	H669	spontaneum TAK IIA-a	Southwest Asia	SSU	0
66	H670	spontaneum TAK IIA-b	Southwest Asia	SSU	0
67	H671	spontaneum TAK IIB	Southwest Asia	SSU	0
68	H672	ischnatherum TSH I-a	Southwest Asia	SSU	0
69	H673	ischnatherum TSH I-b	Southwest Asia	SSU	0
70	H674	spontaneum 6247	Southwest Asia	SSU	0
71	H675	spontaneum Caucasas 2	Transcaucasus	SSU	0
72	H676	spontaneum Caucasas 4	Transcaucasus	SSU	0
73	H677	spontaneum Caucasas 5	Transcaucasus	SSU	0
74	H678	spontaneum Caucasas 6	Transcaucasus	SSU	0
75	H679	spontaneum Caucasas 7	Transcaucasus	SSU	0
76	H680	spontaneum Caucasas 8	Transcaucasus	SSU	0
77	H681	spontaneum Caucasas 9	Transcaucasus	SSU	0
78	H682	spontaneum Caucasas 10	Transcaucasus	SSU	0
79	H683	spontaneum Caucasas 11	Transcaucasus	SSU	0
80	H684	spontaneum Caucasas 12	Transcaucasus	SSU	0
81	H686	spontaneum Caucasas 14	Transcaucasus	SSU	0
82	H687	spontaneum Caucasas 36	Transcaucasus	SSU	0
83	H688	spontaneum 487-1	unknown		0
84	H689	spontaneum 4969	Near East	TKY	0
85	H690	spontaneum 12148	Near East	TKY	0
86	H691	spontaneum S- 1	Near East	IRQ	0
87	H694	spontaneum S- 4	Near East	IRQ	0
88	H695	spontaneum S- 5	Near East	IRQ	0
89	H696	spontaneum S- 6	Near East	IRQ	0
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104	H712	spontaneum S-22	Near East	IRQ	0
105	H713	spontaneum S-23	Near East	IRQ	0
106	H714	spontaneum S-24	Near East	IRQ	0
107	H715	spontaneum S-25	Near East	IRQ	0
108	H716	spontaneum S-26	Near East	IRQ	0
109	H717	spontaneum S-27	Near East	IRQ	0
110	H718	spontaneum S-28	Near East	IRQ	0
111	H719	spontaneum S-29	Near East	IRQ	0
112	H721	spontaneum S-31	Near East	IRQ	0
113	H722	spontaneum S-32	Near East	IRQ	0
114	H723	spontaneum S-33	Near East	IRQ	0
115	H724	spontaneum S-34	Near East	IRQ	0
116	H725	spontaneum S-35	Near East	TKY	0
117	H726	spontaneum S-36	Near East	TKY	0
118	H727	spontaneum S-37	Near East	TKY	0
119	H728	spontaneum S-38	Near East	IRN	0
120	H729	spontaneum S-39	Near East	IRN	0
121	H730	proskowetzii II	Transcaucasus	SSU	0
122	H731	proskowetzii III	Transcaucasus	SSU	0
123	H732	proskowetzii S- 1	Near East	IRQ	0
124	H733	proskowetzii S- 2	Near East	IRQ	0
125	H734	proskowetzii S- 3	Near East	IRQ	0
126	H735	proskowetzii S- 4	Near East	IRQ	0
127	H736	proskowetzii S- 5	Near East	IRQ	0
128	H737	proskowetzii S- 6	Near East	IRQ	0
129	H738	proskowetzii S- 7	Near East	IRQ	0
130	H739	proskowetzii S- 8	Near East	IRQ	0
131	H740	proskowetzii S- 9	Near East	IRQ	0
132	H741	proskowetzii S-10	Near East	IRQ	0
133	H742	proskowetzii S-11	Near East	IRQ	0
134	H743	proskowetzii S-12	Near East	IRQ	0
135	H744	proskowetzii S-13a	Near East	IRQ	0
136	H745	proskowetzii S-13b	Near East	IRQ	0
137	H746	proskowetzii S-14	Near East	IRQ	0
138	H747	proskowetzii S-15	Near East	IRQ	0
139	H748	proskowetzii S-16a	Near East	IRQ	0
140	H749	proskowetzii S-16b	Near East	IRQ	0
141	H751	proskowetzii S-17	Near East	IRQ	0
142	H752	proskowetzii S-18	Near East	IRQ	0
143	H753	proskowetzii S-19	Near East	IRQ	0
144	H754	proskowetzii S-20	Near East	IRQ	0
145	H755	proskowetzii S-21	Near East	IRQ	0
146	H756	proskowetzii S-22	Near East	IRQ	0
147	H757	proskowetzii S-23	Near East	IRQ	0
148	H758	proskowetzii S-24	Near East	IRQ	0
149	H759	proskowetzii S-25	Near East	IRQ	0
150	H760	proskowetzii S-26	Near East	IRQ	0
151	H761	proskowetzii S-27	Near East	IRQ	0
152	H762	proskowetzii S-28	Near East	IRQ	0
153	H763	proskowetzii S-29	Near East	IRN	0
154	H764	proskowetzii S-30	Near East	IRN	0

a. Haplotype IDs defined in supplementary Table S2 are indicated by numerals.