SUPPLEMENTARY MATERIAL

Genome-wide identification and characterisation of ammonium transporter gene family in barley

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Table S1. Non-synonymous/synonymous mutation rate (*Ka*/*Ks*) of syntenic gene pairs between Morex and B1K-04-12 accessions of barley

Seq_1 (Morex)	Seq_2 (B1K-04-12)	Ka	Ks	Ka_Ks
HORVU.MOREX.r3.1HG0072140.1	Horvu_FT11_1H01G441300.1	0.001400997	0.009409	0.148903979
HORVU.MOREX.r3.1HG0072140.1	Horvu_FT11_3H01G534500.1	0.116259971	0.366509	0.317209343
HORVU.MOREX.r3.2HG0180820.1	Horvu_FT11_2H01G514800.1	0.016259971	0.02987	0.544357909
HORVU.MOREX.r3.2HG0180820.1	Horvu_FT11_6H01G331600.1	0.071908395	0.44944	0.159995585
HORVU.MOREX.r3.3HG0303830.1	Horvu_FT11_1H01G441300.1	0.117877458	0.356158	0.330969324
HORVU.MOREX.r3.3HG0299120.1	Horvu_FT11_3H01G501600.1	0.003454	0.019866	0.173868921
HORVU.MOREX.r3.3HG0303830.1	Horvu_FT11_3H01G534500.1	0.00176438	0.012848	0.137324218
HORVU.MOREX.r3.5HG0530810.1	Horvu_FT11_5H01G685700.1	0.010241252	0.037839	0.270653007
HORVU.MOREX.r3.6HG0595580.1	Horvu_FT11_2H01G514800.1	0.071908395	0.454246	0.158302879
HORVU.MOREX.r3.6HG0595580.1	Horvu_FT11_6H01G331600.1	0.0135425	0.012507	1.082766336

Explanations: Seq_1 = gene ID in Morex accession, Seq_2 = gene ID in B1K-04-12 accession.

Source: own study.

Table S2. Identification of potential miRNA target sites in the coding sequences of barley *AMT* genes

miRNA_Acc.	**Target_Acc.**	**Expectation**	***UPE***	**miRNA_length**	**Target_start**	**Target_end**	**miRNA_aligned_fragment**	**Target_aligned_fragment**	**Inhibition**
hvu-miR6176	HvAMT1.1	4.5	16.189	23	340	363	GAAGCUGUAGU-GCAGCCGGCGUU	CAGACCGGCUUCGACUACAGCUUC	translation
hvu-miR6176	HvAMT1.2	5	13.563	23	337	360	GAAGCUGUAGU-GCAGCCGGCGUU	CAGGUCGGCUUCGACUACAGCUUC	translation
hvu-miR6186	HvAMT3.2	5	22.177	21	1,151	1,171	CGAGGAAGGCGCUGAGAGAGA	ACUCUCGGGGCGCCUUCUAUG	cleavage

Explanations: *UPE* = maximum energy needed to unpair the secondary structure around the target site on target mRNA.

Source: own study.

Table S3. Clustering details of protein-protein interaction network of HvAMT proteins in barley

Clustering method	Cluster number	Cluster colour	Gene count	Protein name	Protein description
k-means	1	red	10	A0A287G4Q6	Ammonium_transp domain-containing protein; Belongs to the ammonium transporter (TC 2.A.49) family. Rh subfamily
k-means	1	red	10	A0A287N574	TPT domain-containing protein
k-means	1	red	10	A0A287PFN6	uncharacterised protein
k-means	1	red	10	A0A287R0X3	uncharacterised protein
k-means	1	red	10	A0A287VGU7	uncharacterised protein
k-means	1	red	10	A0A287W085	G_PROTEIN_RECEP_F2_4 domain-containing protein
k-means	1	red	10	A0A287WZA9	TPT domain-containing protein
k-means	1	red	10	HvAMT1.1	ammonium transporter
k-means	1	red	10	HvAMT3.1a	ammonium transporter
k-means	1	red	10	HvAMT3.1b	ammonium transporter
k-means	2	green	6	A0A287FMF6	proline dehydrogenase; Converts proline to delta-1-pyrroline-5-carboxylate
k-means	2	green	6	A0A287KK40	AA_permease_C domain-containing protein
k-means	2	green	6	A0A287RIH1	glutamate receptor; glutamate-gated receptor that probably acts as non-selective cation channel; belongs to the glutamate-gated ion channel (TC 1.A.10.1) family
k-means	2	green	6	HvAMT2.3	ammonium_transp domain-containing protein; Belongs to the ammonium transporter (TC 2.A.49) family. Rh subfamily
k-means	2	green	6	HvAMT3.2	ammonium transporter

Clustering method	**Cluster number**	**Cluster colour**	**Gene count**	**Protein name**	**Protein description**
k-means	2	green	6	M0VBP8	uncharacterised protein; belongs to the sodium:solute symporter (SSF) (TC 2.A.21) family
k-means	3	blue	9	A0A287J008	protein kinase domain-containing protein; belongs to the protein kinase superfamily; Ser/Thr protein kinase family
k-means	3	blue	9	A0A287K9F9	protein kinase domain-containing protein
k-means	3	blue	9	A0A287KAV2	protein kinase domain-containing protein; belongs to the protein kinase superfamily; Ser/Thr protein kinase family
k-means	3	blue	9	A0A287KJ63	protein kinase domain-containing protein
k-means	3	blue	9	A0A287KLA0	protein kinase domain-containing protein
k-means	3	blue	9	A0A287MTL5	protein kinase domain-containing protein
k-means	3	blue	9	A0A287MYX7	protein kinase domain-containing protein
k-means	3	blue	9	F2DAM6	predicted protein
k-means	3	blue	9	M0W5N3	protein kinase domain-containing protein

Source: own study.

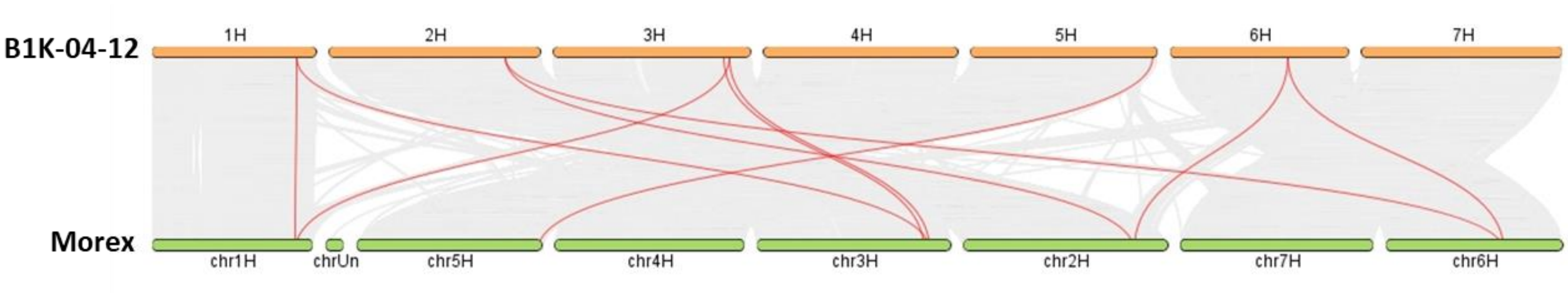


Fig. S1. Synteny analysis of *AMT* genes between the Morex (green chromosomes) and B1K-04-12 (orange chromosomes) accessions of barley; red lines = orthologous *AMT* gene pairs between Morex and B1K-04-12, grey lines = orthologous gene pairs between Morex and B1K-04-12; source: own study

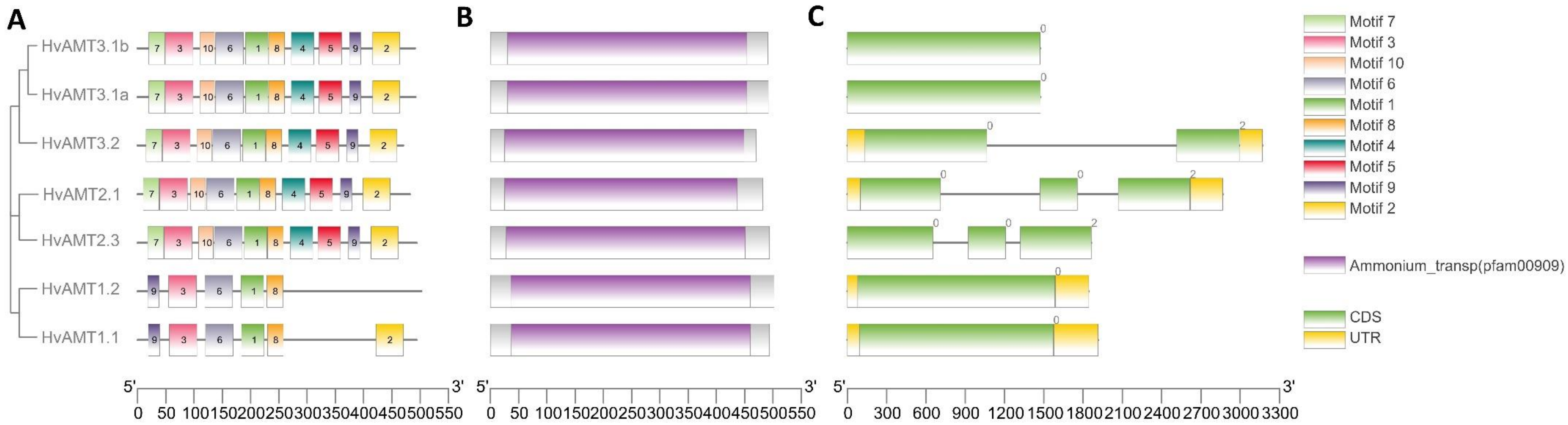


Fig. S2. Sequence structural features and gene structure analysis of *HvAMT* genes: a) conserved ten protein motifs, b) Pfam domain, c) intron-exon organisation; UTR = untranslated region, CDS = coding sequences; source: own study

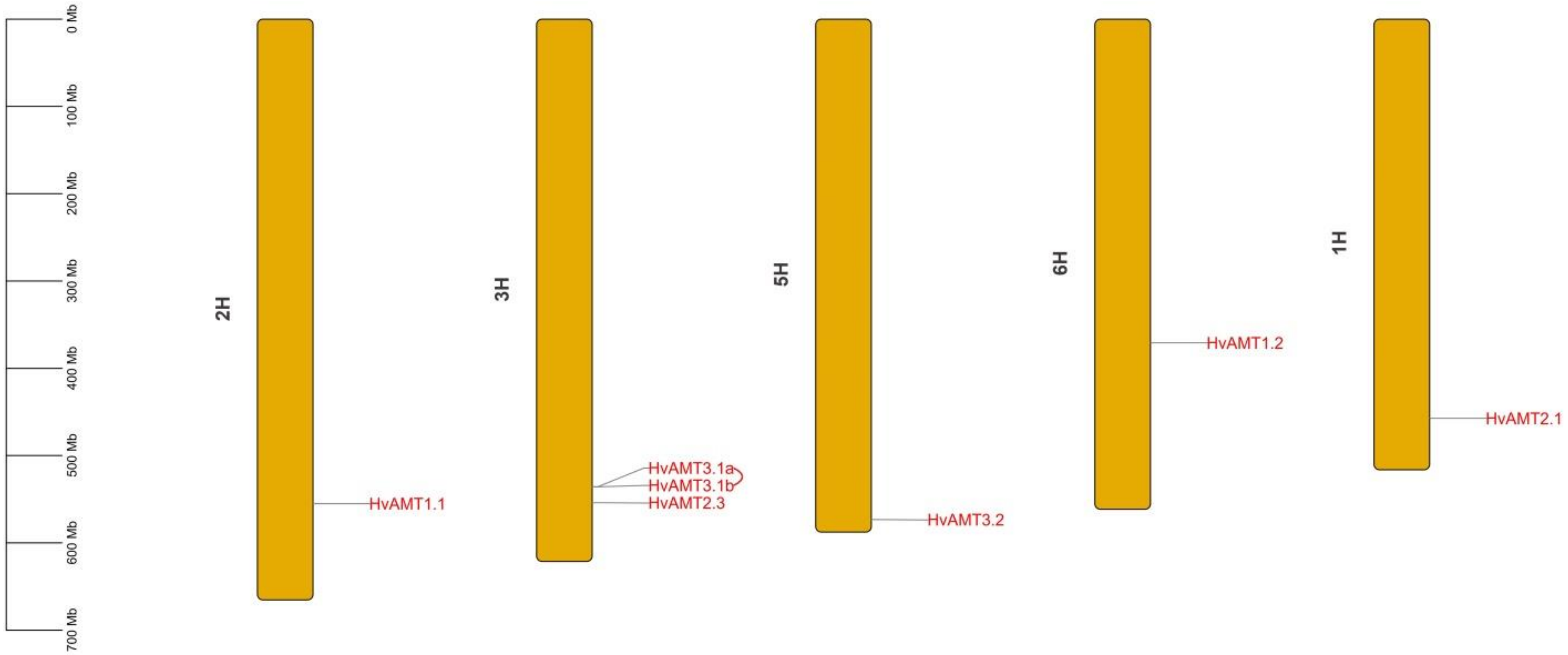
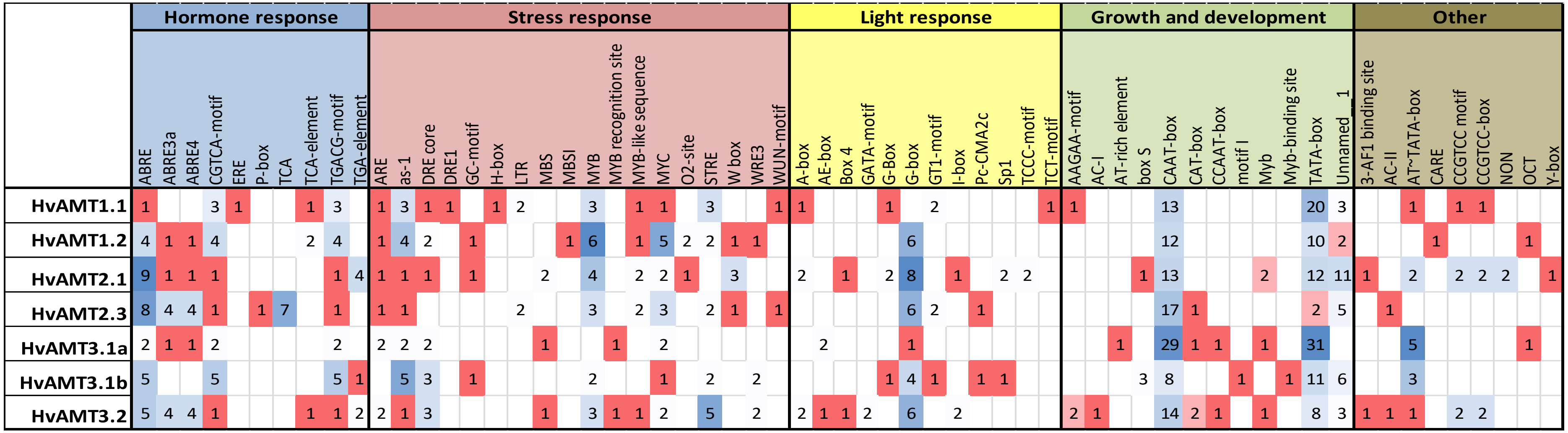


Fig. S3. Chromosomal distribution and gene duplication of *HvAMT* genes; red colour = gene duplication of the gene pair *HvAMT3.1a*/*HvAMT3.1b*; source: own study



	Hormone response										Stress response																		Light response												Growth and development												Other								
	ABRE	ABRE3a	ABRE4	CGTCA-motif	ERE	P-box	TCA	TCA-element	TGACG-motif	TGA-element	ARE	as-1	DRE core	DRE1	GC-motif	H-box	LTR	MBS	MBSI	MYB	MYB recognition site	MYB-like sequence	MYC	O2-site	STRE	W box	WRE3	WUN-motif	A-box	AE-box	Box 4	GATA-motif	G-Box	G-box	GT1-motif	I-box	Pc-CMA2c	Sp1	TCCC-motif	TCT-motif	AAGAA-motif	AC-I	AT-rich element	box S	CAAT-box	CAT-box	CCAAT-box	motif I	Myb	Myb-binding site	TATA-box	Unnamed__1	3-AF1 binding site	AC-II	AT~TATA-box	CARE	CCGTCC motif	CCGTCC-box	NON	OCT	Y-box
HvAMT1.1	1			3	1			1	3		1	3	1	1		1	2			3		1	1		3			1	1				1		2					1	1				13						20	3			1		1	1			
HvAMT1.2	4	1	1	4				2	4		1	4	2		1				1	6		1	5	2	2	1	1							6											12						10	2				1				1	
HvAMT2.1	9	1	1	1					1	4	1	1	1		1			2		4		2	2	1		3			2		1		2	8		1		2	2					1	13				2		12	11	1		2		2	2	2		1
HvAMT2.3	8	4	4	1		1	7		1		1	1					2			3		2	3		2	1		1						6	2		1								17	1					2	5		1							
HvAMT3.1a	2	1	1	2					2		2	2	2					1			1		2							2				1									1		29	1	1		1		31				5					1	
HvAMT3.1b	5			5					5	1		5	3		1					2			1		2		2						1	4	1		1	1						3	8			1		1	11	6			3						
HvAMT3.2	5	4	4	1				1	1	2	2	1	3					1		3	1	1	2		5		2		2	1	1	2		6		2					2	1			14	2	1		1		8	3	1	1	1		2	2			

Fig. S4. Distribution of putative *cis*-acting regulatory elements in 1500 bp upstream regions of *HvAMT* genes in barley; source: own study