	MT1a	MT1b1	MT1b2	MT1b3	
MT1a	100	75.6	75.1	75.6	
MT1b1	75.6	100	91.1	91.1	
MT1b2	75.1	91.1	100	95.5	
MT1b3	75.6	91.1	95.5	100	
	MT2a	MT2b1	MT2b2	MT2c	
MT2a	100	65.4	65.1	66.2	
MT2b1	65.4	100	94	79.6	
MT2b2					
	65.1	94	100	78.4	

Supplemental Table S1. Identity scores (%) among MT1 and MT2 protein subgroups in barley.

Gene/cDNA	Primer ¹	Sequence 5'-3'
Cloning		
MT1a	1a	GGCTTAAUTTCGGGTTCACCAGATCT
	1b	GGTTTAAUCAGATCATCAGACGCTCG
MT1b1	2a	GGCTTAAUGGCAACACAACAGAACCA
	2b	GGTTTAAUCACAAGCGCATCGACTGC
MT1b2/MT1b3	3a	GGCTTAAUAGCAGCACAGGTTACAGC
1 (772)	3b	GGTTTAAUCACCACAATCGCATACACC
MT2a	4a	GGUITAAUATAACUAACICGAGGGAG
MT2b1	40 50	
W11201	Ja 5h	GGTTTAAUAAGCACGCACGGTGCATG
MT2b2	50 5a	as above
	5d	GGTTTAAUATGCAGGCAGGTGCTTACA
MT2c	6a	GGCTTAAUTCGCCACCGCCCACCAAG
	6b	GGTTTAAUCGTGCTCGCCTCTGCCGG
MT3	7a	GGCTTAAUGAGGCACTTCTCCGATCA
	7b	GGTTTAAUGACACGGGTTACTAGTACGGG
MT4	8a	GGCTTAAUACGCCTGAGAAGATCGAG
	8b	GGTTTAAUGGCTCAGCGGCACTTATT
CUPI	9a	
Subalaning into n462	90	GGIIIAAUIIAIICIIGGGGGGACAI
Subcioning into p462		
MTla	10a	GCAGATCTATGTCTTGCAATTGTGGATC
	10b	CICGAGITAACAGITACAGGGGTIG
MTTbT/MTTb2	11a 11b	
MT163	110	
1011105	11b	as above
MT2a	12a	AGATCTATGTCGTGCTGCGGAGGC
11120	12a 12b	CTCGAGTCACTTGCAGGTGCACGG
MT2b1/MT2b2	13a	CGGATCCATGTCTTGCTGCGGAGGA
	13b	CTCGAGCTAGCAACTGCAGCAGGAGC
MT2c	14a	GGATCCATGTCTTGCTGCGGCGGC
	14b	GCTCGAGTCACTTGCAACTGCAGCAGG
MT3	15a	AGATCTATGGCTGACAAGTGCGGCAAC
	15b	CTCGAGTCAGTGTCCGCAGGTGCA
MT4	16a	CAGATCTATGGGCTGCGACGACAAGTGCG
CUDI	16b 17a	
CUPI	17a 17b	
Subcloning into pMal-c2	170	CICOAUTCATTICCCAUAUCAUCA
Subcioning into piviai-e2		
MT3	15c	GAAGGATTTCAATGGCTGACAAGTGCGGCAAC
MT4	150	
1/114	100	
	166	as above
RT-qPCR		
MT3	18a	GACAAG ACC CAG TGC GTG AA
	18b	GGTGTCAACCATGACGATGC
MT4	19a	GCGTGTGTGGAAAGTGTTGTGTATA
	19b	CACTGATGCGAGTAGGTGGTAGG
GAPDH	20a	GCTCAAGGGTATCATGGGTTACG
	20b	GCAATTCCAGCCTTAGCATCAAAG
LTP2	21a	ACCCCAACTACGGGCACT
	21b	AGTGCGGTACGGGTATGC
11K1	22a	
HOP2	220	CCCAAC ACT CCC TCC AAC
110K2	23a 23h	

Supplemental Table S2. Primers used in this study

¹All a and c primers are forward orientated whereas b and d represent reverse primers. Modifications with 2'-deoxyuridine are indicated with a U.



Supplemental Figure S1. Exon-intron gene organization of barley MT genes. Exons and introns are indicated with blue boxes and lines, respectively. MT2b1 and MT2b2 introns are not identical but share intron positions. Genomic DNA encoding MT1b3 was not detected.

AtMT3	1 MSSN <mark>GGS<mark>O</mark>DADKTQ<mark>O</mark>VKKGTSYTFDIVETQESYKEAMIMDVG-AEENNAN<mark>GKO</mark>KGGSS<mark>OS</mark>-VN<mark>GTGO</mark>PN 69</mark>
OsAF001396	1 MSDK <mark>e</mark> gn <mark>e</mark> deadksQ <mark>e</mark> vkkgtsygvviveaeks <mark>h</mark> feeva-ageengg <mark>eke</mark> gts <mark>e</mark> se-td eke -gk 62
OsAF009959	1 MSDK <mark>G</mark> GNODOADKSQOVKKGTSYGVVLVDAEKTTSKWL-RRSA-TKKTTGGOKOTTGOSO-AGONO-GK 65
HvMT3	1 MADK <mark>E</mark> GNEDEADKTOEVKKGDSYGIVMVDTEKSHLEVHE-TAENDDKEKEGTSETE-TNETE-GH 62
BdCCYP9938	1 MSSG <mark>GGN<mark>O</mark>DCADKTQ<mark>O</mark>VKKGNGYGIVMVDTEKS<mark>H</mark>FEVQE-S-AAENDGKOKOGTSOTO-TSOTO-GH 63</mark>
BdCCYP14611	1 MSSG <mark>G</mark> GN <mark>O</mark> DCADKTQ <mark>C</mark> VKKGNGYGIVMVDTEKSHFEVQE-S-AAENDGKCKGGTSCTC-TSCTC-GH 63
PcAF257465	1 MSDK <mark>e</mark> GN <mark>e</mark> DeadksQevkkgnsygvvlvdteks <mark>h</mark> leei-aa-a-gaendgekegssegtdeke-gk 64
OsNM001061420	1 MSDK <mark>e</mark> gnødeadksoevkkgtsygvvivdaeks <mark>h</mark> fema-eevg-yeendgkekettgese-agene-gk 65
VvAJ237990	1 MS-TEGNEDEADKSQEVKKGNSYGIDIVETEKSYVATVVMEVP-AAQHEGSEKEGDSEAE-IDETE-GQ 65
CaDQ124082	1 MSDK <mark>E</mark> GNEDEADKSQEVKKGSSYAADIVETENTFVETFVMMEG-GAQ-NGKEKEGPSEAE-VNETE-DN 65
CpY08322	1 MSDTGGNODOADKTQOVKKGSSYTADIIETEKSIMTV-VMDAP-AAENDGKOKOGPSOSO-TNOTO-GE 65
EgAJ236913	1 MS-TEGNEDEADKSQEVKKGNSYGIEIIETEKSNFNNVIDAPA-AAEHEGNEKEGASEAE-VDEKE-GQ 65
MaAF268393	1 MS-T <mark>G</mark> GNODOVDKSQOVKKGNSYGIDIVETEKSYVDEVIVAAE-AAE <mark>H</mark> DGK <mark>G</mark> KGGAAQAC-TD <mark>GK</mark> G-GN 65
FaAF039002	1 MSST <mark>GGNODOADKNOOVKKSNSFGVVIMETEKSYFDGM-MDVAE<mark>H</mark>DPK<mark>G</mark>KGGSSOSO-TDONO-GK 63</mark>
PbAY594299	1 MSSTEDNEDEADKTOEVKKGSSYTADIVETEKSHVYTGVMEVP-ATENDGKEKEGANETE-TTETE-G 66
TcAY531114	1 MSDKEGSEDCCDKTOCVTKSTSYTLDMVETQESYKEAMNMDVG-AEENGKEMEGSTESO-VNGTESPN 67
BjAB057413	1 MS-S <mark>C</mark> GN <mark>CDC</mark> ADKTQ <mark>C</mark> VKKGTSYTLDIVETQESYKEAMIMEVNGAEENG <mark>COCKC</mark> GSS <mark>C</mark> SC-VNCTCCPN 67
	*: **** **.***.*: .: :::::: :

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AtMT4a	1MADTGKGS	SASAS <mark>C</mark> NDR <mark>C</mark> G <mark>C</mark> PSP <mark>C</mark> PG	GES <mark>C</mark> RCKMMSEASGGDQ	EHNTCPCGEHCGCNPCNCP	• KTQTQTSAKG <mark>C</mark> T <mark>C</mark> GEG <mark>C</mark> T	AT <mark>C</mark> AA	85
AtMT4c	¹ mv <mark>h</mark> sty <mark>h</mark> vsf <mark>c</mark> taq <mark>c</mark> lyise <mark>c</mark> grgtlrlktkmadtgkgs	SVAG <mark>C</mark> NDS <mark>C</mark> GCPSPCPG	GNS <mark>C</mark> RCRMREA-SAGDQ	QGHMVCPCGEHCGCNPCNCP	*KTQTQTSAKG <mark>C</mark> T <mark>C</mark> GEG <mark>C</mark> T	AS <mark>C</mark> AT	115
OsAK102405	1MM	G <mark>C</mark> DDK <mark>C</mark> GCAVPCPG	GTG <mark>C</mark> RCASSARSGGG	dHTTCSCGDHCGCNPCRCG	RESQPTGRENRRAG <mark>C</mark> SCGDSCT	AS <mark>C</mark> GSTTTTAPAATT	87
HvMT4	1MMMM	G <mark>C</mark> DDK <mark>C</mark> GCAVPCPG	GTG <mark>C</mark> RCTSARSGA	AE <mark>H</mark> TT <mark>CAC</mark> GE <mark>HC</mark> GCNP <mark>C</mark> ACG	REGTPSGRENRRSN <mark>C</mark> SCGAACN	AS <mark>C</mark> GSTA	77
TaX68289	1MMMM	G <mark>C</mark> DDK <mark>C</mark> GCAVPCPG	GTG <mark>C</mark> RCTSARSG-AAAG	}E <mark>H</mark> TT <mark>CG</mark> CGEHCGCNPCACG	REGTPSGRANRRAN <mark>C</mark> SCGAACN	CAS <mark>C</mark> GSATA	81
BdMT4	1MG	G <mark>C</mark> DDK <mark>C</mark> GCAVPCPG	GAT <mark>C</mark> RCTRSGAGASAGQ	QHTTCGCGEHCGCNPCACG	REGTPSGRANRTAT <mark>C</mark> SCGAACD	AS <mark>C</mark> RSSSTA	84
ZmU10696	1MM	G <mark>C</mark> DDK <mark>C</mark> GCAVPCPG	GKD <mark>C</mark> RCTSGSGGQF	RE <mark>HTTCGCGEHCEC</mark> SPCTCG	RATMPSGRENRRAN <mark>C</mark> SCGASCN	CAS <mark>C</mark> ASA	77
GmAF004808	1MADTGGGI	avrpvvi <mark>c</mark> dnk <mark>c</mark> gctlpctg	GST <mark>C</mark> RCTSAGTA-TGGG	dhvt <mark>cscgehcgc</mark> np <mark>csc</mark> p	YKIAAAGSG <mark>C</mark> RCGTDCA	CAS <mark>C</mark> RT	85
GmAF010186	1MADTSGGI	avrpvvi <mark>c</mark> dnk <mark>c</mark> gctvpctg	GST <mark>C</mark> RCTSVGMT-TGGG	n <mark>hvtcscgehc</mark> gcnpcscp	°KTAASGTG <mark>C</mark> RCGTDCS	AS <mark>C</mark> RT	85
AhDQ665258	1MADTAMKO	;GTR <mark>C</mark> NDR <mark>C</mark> GCSVP <mark>C</mark> PG	DST <mark>C</mark> RCASGNEG-GGG1	'Q <mark>H</mark> LT <mark>C</mark> P <mark>C</mark> GE <mark>HC</mark> E <mark>C</mark> NP <mark>C</mark> TCP	rtvaagag <mark>c</mark> k <mark>c</mark> gpg <mark>c</mark> t	AS <mark>C</mark> RRA	82
AhDQ665259	1MADKAMKO	;GAR <mark>C</mark> NDR <mark>C</mark> GCSIPCPG	DST <mark>C</mark> RCASGSEG-GGE1	'Q <mark>H</mark> LT <mark>C</mark> P <mark>C</mark> GE <mark>HC</mark> E <mark>C</mark> NP <mark>C</mark> TCP	rtfaagag <mark>c</mark> k <mark>c</mark> gpg <mark>c</mark> s	AS <mark>C</mark> RRA	82
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Supplemental Figure S2. ClustalW alignments of MT3 (A) and MT4 (B) proteins. Alignments were performed in T-COFFE. Putative metal binding amino acid residues such as cysteine and histidine are shown in red and blue, respectively. The synthetic peptides used for the production of anti-MT3 and anti-MT4 antibodies are boxed in gray.



Supplemental Figure S3. Effects of barley MT expression on H_2O_2 tolerance in *skn7* and salt tolerance in wild type yeast. Expression of *MT1a*, *MT1b1*, *MT1b2*, *MT1b3*, *MT2a*, *MT2b1*, *MT2b2*, *MT2c*, *MT3* and *MT4* in *skn7* (A) and in wild type (B). In both (A) and (B) cells were spotted in two concentrations ($OD_{600} = 0.05$ and $OD_{600} = 0.0005$). *skn7* or wild type were transformed with vector p426 or CUP1 as negative and positive controls, respectively.



Supplemental Figure S4. (A-I) Controls of developing grain transversal sections stained by fuchsin and examined under bright field epipolarized light (A) Control section of a 7 dap grain lacking the incubation with the anti-MT antibody. (B) Control sections of 7 dap grain (B) and 14 dap grain (C) treated with MT4 pre-immune serum. Control sections of 21 dap embryo lacking the incubation with the anti-MT antibody (D), treated with MT3 (E) or MT4 (F) pre-immune serum. Control sections of mature grain lacking the incubation with the anti-MT antibody (G), treated with MT3 (H) or

MT4 (I) pre-immune serum. (J-R) Control sections of developing grain transversal sections observed with a transmission electron microscope. View of pericarp cells (J, K) and of endosperm cells (L) of a 7 dap grain sections lacking the incubation with the anti-MT antibody (J) or treated with MT4 (K, L) pre-immune serum. View of embryo cells of a 14 dap grain (M-N) and of a 21 dap grain (O) sections lacking the incubation with the anti-MT antibody (M) or treated with MT3 (N, O). View of embryo cells (P) and of the aleurone layer (Q-R) of 21 dap grain section treated with MT3 pre-immune serum (P, Q) or lacking the incubation with the anti-MT antibody is cells. Abbreviations; AL, aleurone cell layer; cw, cell wall; cy, cytosol; en, endosperm; m, mitochondria; N, nucleus; p, plastid; s, spherosome; SC, seed coat. v, vacuole. Bars = 100 μ m (A-I) and = 0.5 μ m (J-R).