Supplemental Table S1. Identified compounds with statistical significance and their variation tendency for each of the comparisons. RMT (relative migration time *versus* IS). % change ([average concentration in the case group-average concentration in the WT group] *100/ average concentration in the WT group).

Compound	Formula	Mass	RMT	**KD Pap1 1130 vs WT**		**KD Icy2 1399 vs WT**		**OE Pap1 937 vs WT**	
				% Change	p-Value	% Change	p-Value	% Change	p-Value
Alanine	C3 H7 N O2	89.0477	0.67	-84.4	5.42E-04	-73.0	1.05E-03		
Sarcosine	C3 H7 N O2	89.0477	0.79	-77.0	1.39E-03	-36.4	1.64E-02		
Serine	C3 H7 N O3	105.0426	0.86	-86.4	1.55E-03	-73.2	3.03E-03	134.5	9.22E-03
Proline	C5 H9 N O2	115.0633	0.92			144.3	4.31E-02	810.2	3.21E-07
Valine	C5 H11 N O2	117.0790	0.86	-80.8	4.67E-04	-65.2	1.22E-03		
Betaine	C5 H11 N O2	117.0790	0.96	-77.7	6.65E-06	-36.7	2.36E-04	-66.2	1.47E-05
Leucine + Isoleucine	C6 H13 N O2	131.0946	0.88	-84.5	3.30E-04	-72.0	6.82E-04		
Asparagine	C4 H8 N2 O3	132.0535	0.90	-59.0	1.20E-03	-34.7	9.44E-03		
Aspartic acid	C4 H7 N O4	133.0375	0.97	-83.3	1.85E-04	-84.1	1.74E-04		
Glutamine	C5 H10 N2 O3	146.0692	0.92	-89.6	1.94E-02			1758.9	6.43E-04
Lysine	C6 H14 N2 O2	146.1055	0.63	-88.7	5.09E-04	-76.5	9.88E-04		
Glutamic acid	C5 H9 N O4	147.0532	0.93	-82.2	8.85E-04	-66.5	2.10E-03	-37.3	1.39E-02
Methionine	C5 H11 N O2 S	149.0514	0.91	-91.0	5.25E-04	-83.8	7.44E-04		
Histidine	C6 H9 N3 O2	155.0695	0.67	-92.6	3.87E-03	-91.4	4.07E-03	23.2	6.84E-01
Phenylalanine	C9 H11 N O2	165.0795	0.94	-86.1	1.30E-04	-78.4	2.06E-04	175.2	1.29E-02
Arginine	C6 H14 N4 O2	174.1117	0.65	-80.4	6.49E-04	-61.8	1.91E-03		
Tyrosine	C9 H11 N O3	181.0739	0.96	-84.9	3.80E-04	-77.6	5.79E-04		
Tryptophan	C11 H12 N2 O2	204.0899	0.94	-96.3	4.35E-05	-95.6	4.54E-05	-79.5	9.16E-05

Supplemental Table S2. Primer sequences used for RT-qPCR amplification to analyse the copy number in transgenic barley plants. *HvCycl* gene (cyclophilin), *Hv4Hppd* gene (4-hydroxyphenyl-pyruvate dioxygenase), *HvPap-1* gene (cathepsin F-like protease), *HvIcy-2* gene (cystatin) and *miR* (Osa-MIR528 miRNA gene).

Barley genes	**Primers**
HvCycl	forward: 5´-CCTGTCGTGTCGTCGGTCTAAA-3´ reverse: 5´-ACGCAGATCCAGCAGCCTAAAG-3´
Hv4Hppd	forward: 5´-GCTCCAAATCTTCACCAAGC-3´ reverse: 5´-CTCTTCCCCTCTCTCGTCCT-3´
HvPap-1	forward: 5´-TCCTGGAGTCGATCTTGGTTTC-3´ reverse: 5´- CAAGCATACTGTTGCGGCTTC-3´
HvIcy-2	forward: 5´-TCCTGGAGTCGATCTTGGTTTC-3´ reverse: 5´-CAAGCATACTGTTGCGGCTTC-3´
miR	forward: 5´-AGTTATGCGGCATTGATACCGGTCAGGAGATTCAGTTTGA-3´ reverse: 5´-AATTATGCGGCATAGATTCCGGTAGAGAGGCAAAAGTGAA-3´

Supplemental Table S3. Primer sequences used for the amplification of barley genes in RT-qPCR assays. *HvPap-4*, *HvPap-6*, *HvPap-9*, *HvPap-10* and *HvPap-17* genes (*c*athepsin L-like protease), *HvPap-1* and *HvPap-2* genes (cathepsin F-like protease), *HvPap-12* gene (cathepsin H-like protease) *HvPap-19* and *HvPap-20* genes (cathepsin B-like protease) and *HvCycI* (cyclophilin).

Barley genes	**Primers**
HvCycI	forward: 5'-TCCACCGGAGAGGAAGTACAGT-3' reverse: 5'-AATGTGCTCAGAGATGCAAGGA-3'
HvPap-1	forward: 5´-TCCTGGAGTCGATCTTTGGTTTC-3' reverse: 5´-CAAGCATACTGTTGCGGCTTC-3'
HvPap-2	forward: 5'-ATGGCTCGCCTCCGCCTCCGC-3' reverse: 5'-CTATTCCTTCTTAGAGGTATG-3'
HvPap-4	forward: 5´-CCTTGAGAGTCCTTGTTCCCGA-3´ reverse: 5´-CCATGTTCGTCGTTTTAACCGA-3´
HvPap-6	forward: 5´-TGCAATTGACGGCAAGAAGA-3´ reverse: 5´-TGGATCACCAGGTGATCATTTG-3´
HvPap-9	forward: 5'-ACTGCGACAACGTCAACAAC-3' reverse: 5'-TCTTCTGGATGAACTGGAAGGC-3'
HvPap-10	forward: 5'-TCGATCCATGTGCTTATCCGA-3' reverse: 5'-AACACACGCCTAATCCTTGGC-3'
HvPap-12	forward: 5´-ATGTGCGCTATTGCTACCTGC-3´ reverse: 5´-CACCTTATTCATGTCTGGCGAA-3´
HvPap-16	forward: 5´-CTGGATCGGTAAGAACTCGTGG-3´ reverse: 5´-TGATGGAGGTGCCATCATATGA-3´
HvPap-17	forward: 5'-AGCTGCGTGTGCATTTATCATG-3' reverse: 5'-GCGGTGAAATATGCAACCCA-3'
HvPap-19	forward: 5´-CACCTTATTCATGTCTGGCGAA-3´ reverse: 5´-TGCCCGCTTAATTTGACAGG-3´
HvPap-20	forward: 5'-GGAGGTCACGCTGTCAAGTT-3' reverse: 5'-GTATCCGTCATCACCCCATC-3'