

## **Supporting Information**

### **The search for candidate genes associated with natural variation of grain Zn accumulation in barley**

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The following Supporting Information is available for this article:

**Fig. S1 Comparison of different RNA extraction methods.**

**Fig. S2 Phylogenetic tree and population genetic structure of the ICARDA panel.**

**Fig. S3 First two Principal Coordinates of the ICARDA germplasm collection.**

**Fig. S4 Variation in grain micronutrient concentration within a selection of barley accessions from the ICARDA collection.**

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**Fig. S7 Gene expression profiles of *MLOC\_40066.1* and *MLOC\_61170.4*.**

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**Table S1 Details of the ICARDA mapping panel analyzed in this study.**

**Table S2 Soil composition in pots (FT) and field plots (SE and KW).**

**Table S3 Median zinc (Zn), iron (Fe), manganese (Mn) and copper (Cu) concentrations , C [%] and total protein [%] in barley grains of landraces and cultivars.**

**Table S4 Correlation coefficients of zinc (Zn), iron (Fe), manganese (Mn) and copper (Cu) concentrations in barley grains.**

**Table S5 Correlation coefficients of zinc (Zn), iron (Fe), manganese (Mn) and copper (Cu) concentrations, C [%] and total protein [%] in barley grains in environment KW.**

**Table S6 Correlation coefficients of zinc (Zn), iron (Fe), manganese (Mn) and copper (Cu) concentrations, C [%] and total protein [%] in barley grains in environment SE.**

**Table S7 Summary of significant marker-trait associations (MTAs) found for micro- and macronutrients of barley grains grown in different environments.**

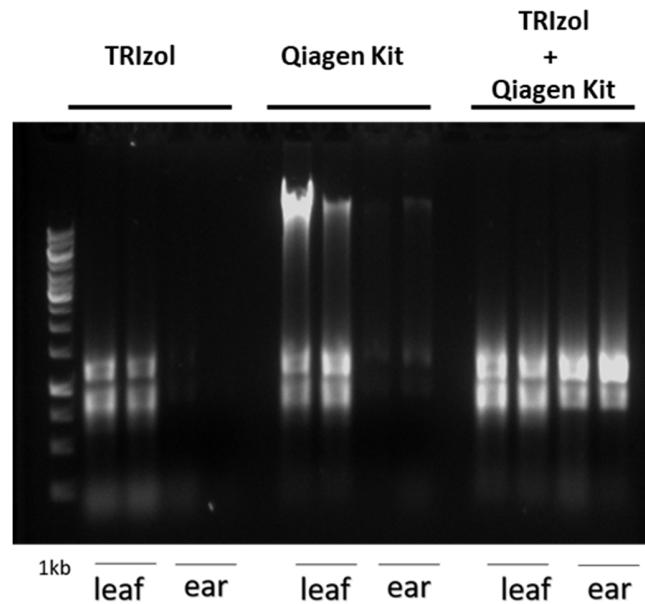
**Table S8 Summary of associated genes of identified markers for Zn concentration of grains grown in different environments.**

**Table S9 Summary of associated genes of Zn related marker 2H | bPb9754 (82.77 cM obtained by BLUEs analysis of grains grown in different environments.**

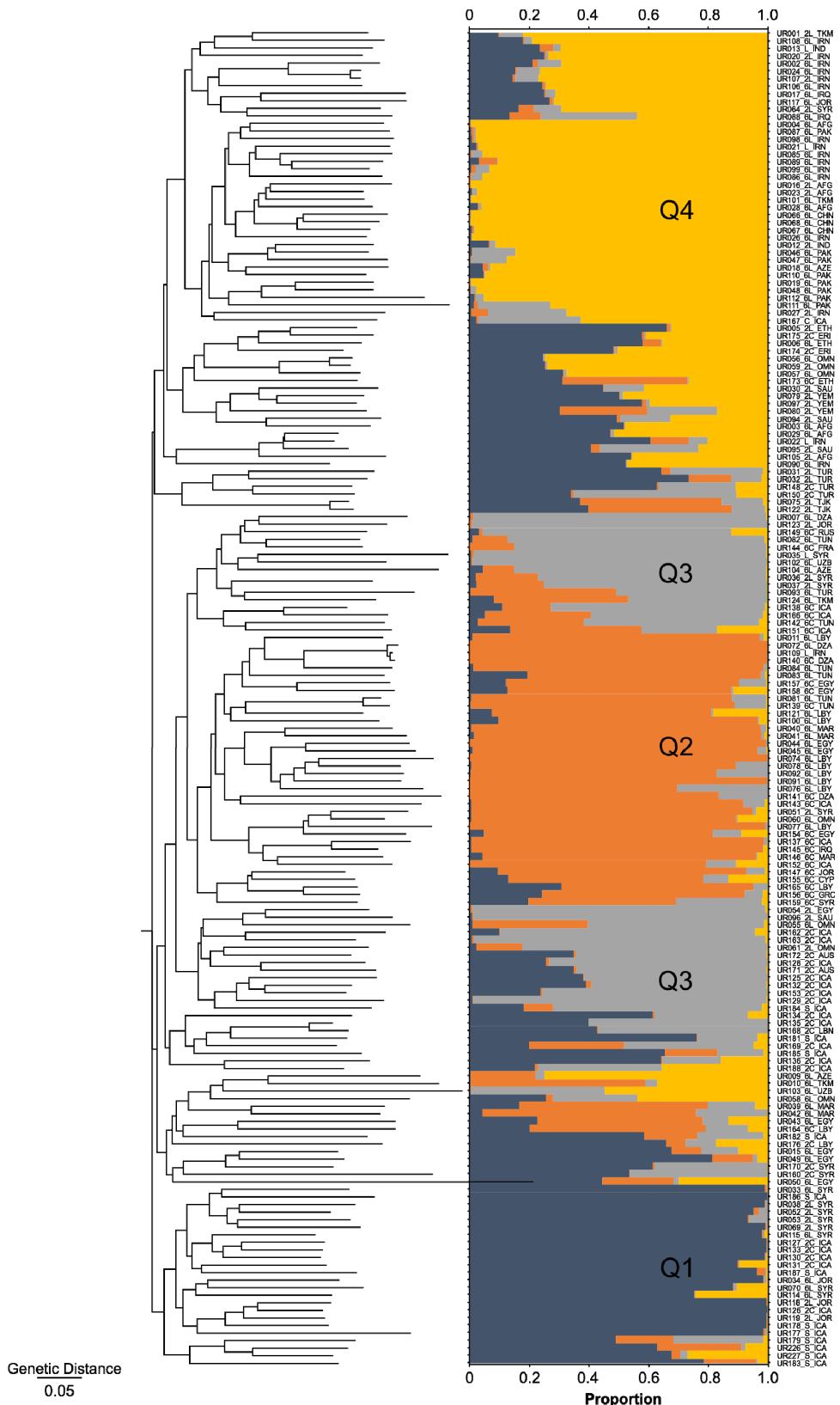
**Table S10 Differentially expressed genes in whole ears and flag leaves shared in both comparisons with lines 143 to 140 and 154 and 156 to 140 and 154.**

**Table S11 Blastx search results against exemplar sequences of significantly differentially expressed genes shared in both comparisons with lines 143 to 140 and 154 and 156 to 140 and 154.**

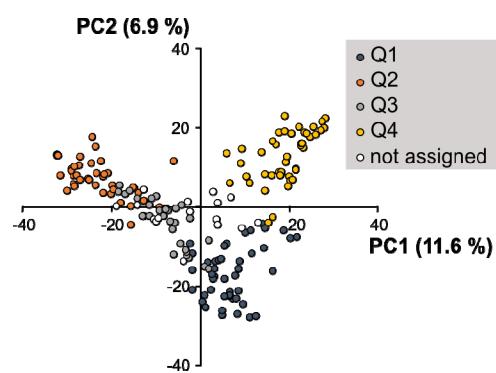
**Fig. S1 Comparison of different RNA extraction methods.** Agarose gel showing extracted RNA from leaves and ears extracted with TRIzol Reagent (Life Technologies GmbH), RNeasy Mini Kit (Qiagen) and a combination of both methods.



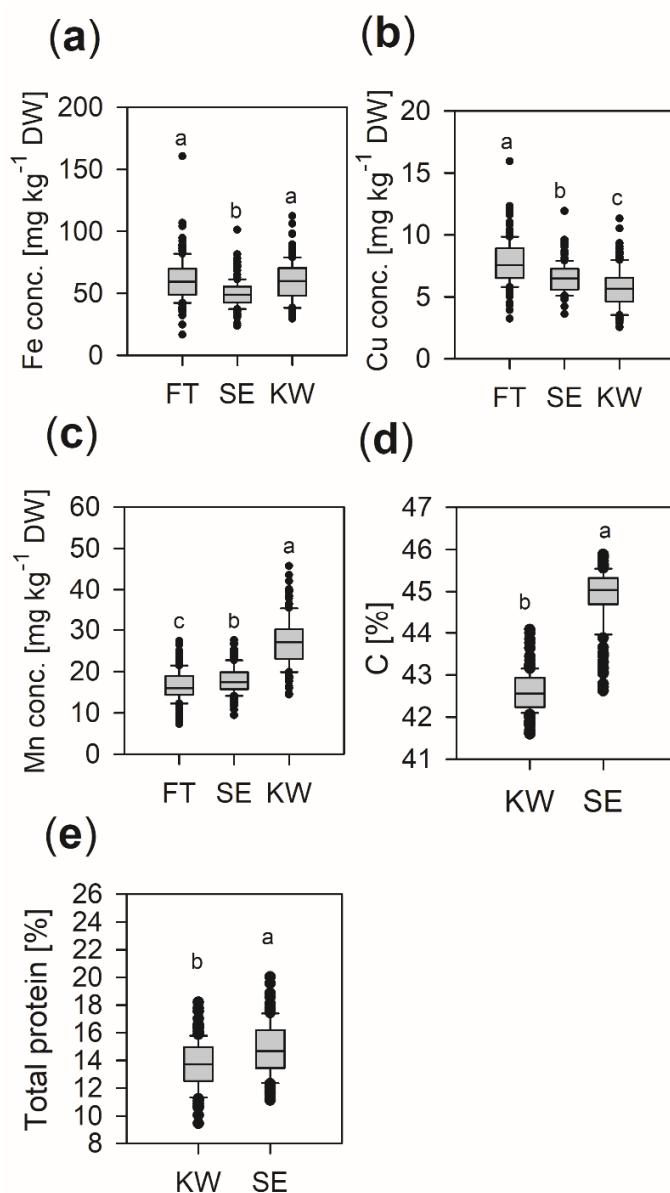
**Fig. S2 Phylogenetic tree and population genetic structure of the ICARDA panel.** Each individual is shown by a line, which is partitioned into four coloured segments, representing the individual's estimated membership coefficients in the Q-matrix. 'ICARDA' lines are sorted according to the order of the phylogenetic tree on the left. Individuals were assigned to a Q-group when the estimated coefficients were >0.5. On the right, code provides details of UR number, row-type [2 (two), 6 (six) –row], breeding status [C (cultivar), L (landrace), S (*H. spontaneum*)] and country of origin.



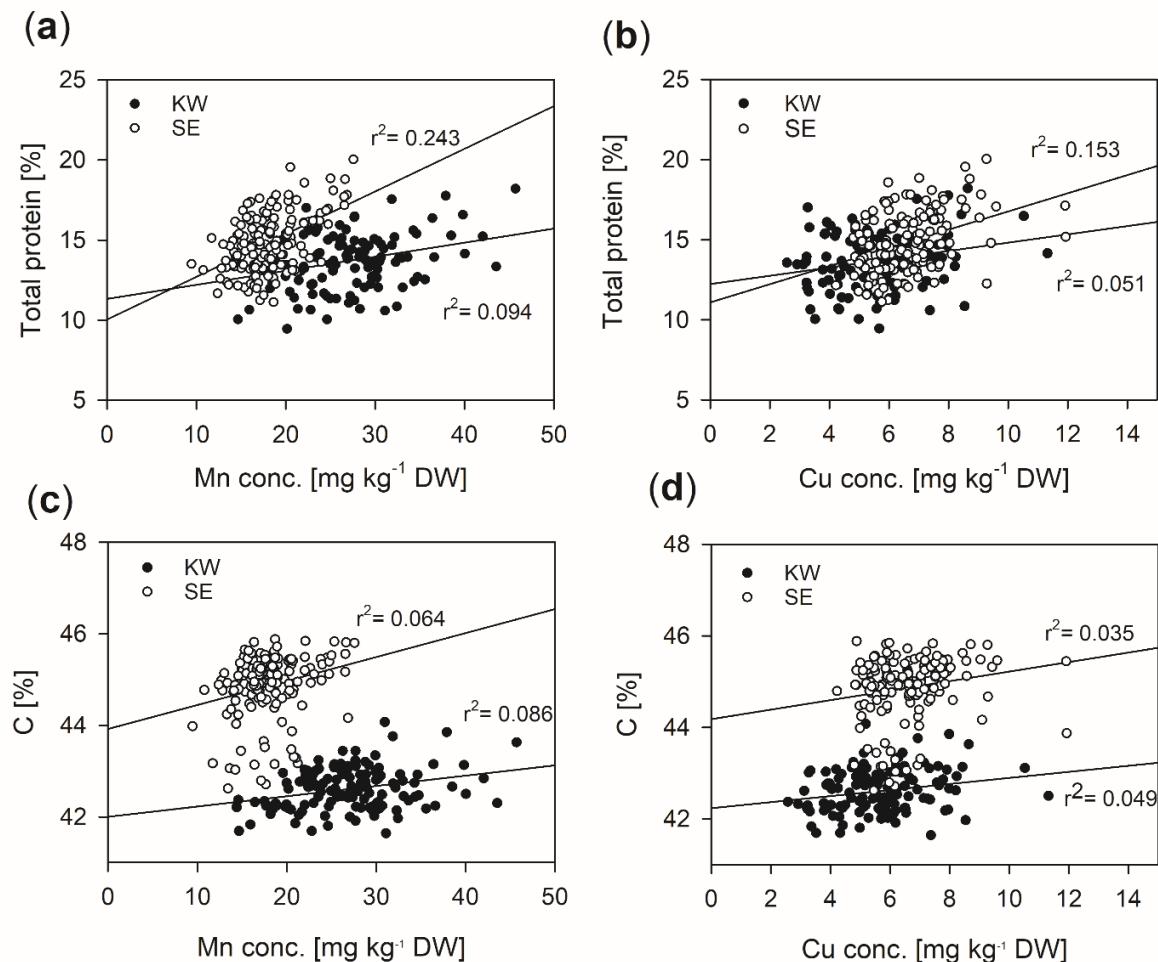
**Fig. S3 First two Principal Coordinates of the ICARDA germplasm collection.** Principal Coordinate (PCo) Analysis was applied to ICARDA lines (177 lines). Once the population structure, Q1 to Q4, was integrated, clusters were identified. Q1, two-row landraces and breeding lines from ICARDA, North Africa and East Asia; Q2, six-row landraces from North Africa; Q3, two-row cultivars from North Africa, Europe and Australia; Q4, six-row landraces from Asia.



**Fig. S4 Variation in grain micronutrient concentration within a selection of barley accessions from the ICARDA collection.** Median grain iron (Fe, **a**), copper (Cu, **b**), manganese (Mn, **c**), carbon (C [%], **d**) and total protein [%] (**e**) concentrations of barley lines grown in different environments (FT, SE, KW). Box plots display the median, the upper and lower quartile as well as extremes. The minimum number of accessions analyzed per sample group was 150. DW: Dry weight.

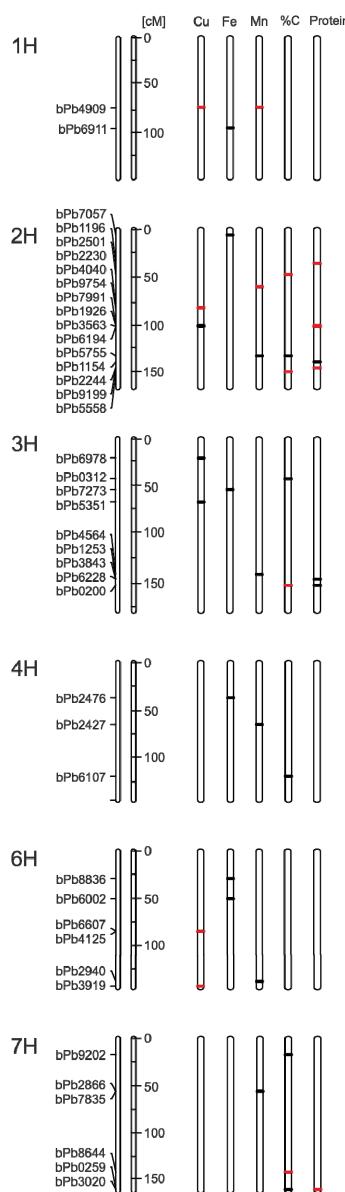


**Fig. S5 Correlations between manganese/copper (Mn/Cu) and total protein within the ICARDA collection in two environments. (a) and (b)** Regression plots of Mn (a) or Cu (b) in environment KW or SE vs total protein [%]. (c) and (d) Regression plots of Mn (a) or Cu (b) in environment KW or SE vs C [%]. Regression plots show single comparisons of the environments (KW, SE).  $r^2$ : coefficient of determination. All displayed coefficients of determination were significant,  $P \leq 0.05$  ( $n=127-148$ ). DW: Dry weight.

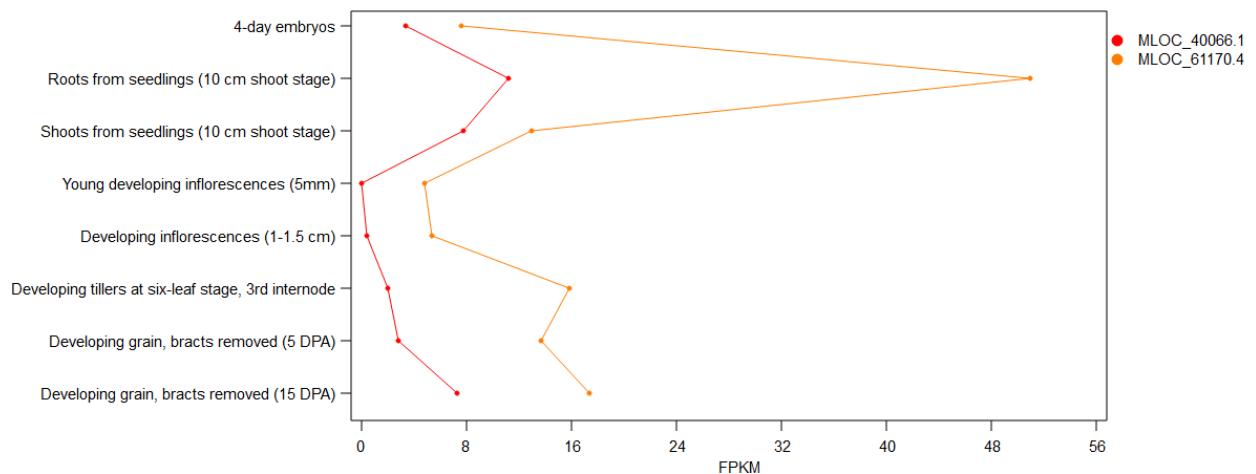


**Fig. S6 Significant marker-trait associations (MTAs) for copper (Cu), iron (Fe), carbon (%C) and total protein content in barley grains grown under different environmental conditions.**

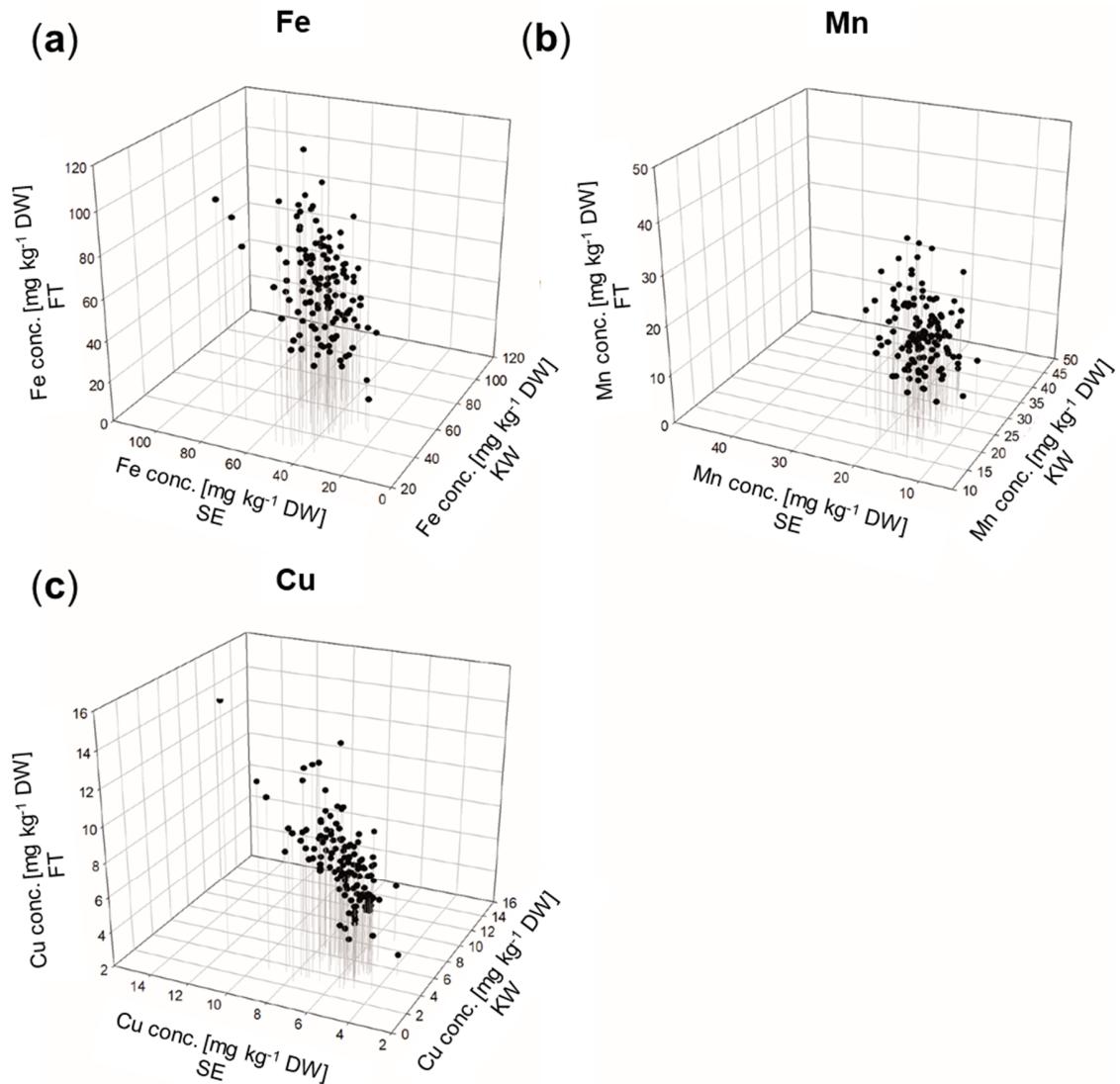
ICARDA lines were grown in three environments (FT, KW, SE). Data for each environment and best linear unbiased estimates used for genome-wide association mapping (GWAS). All significant MTAs at  $-\log(P_M) > 2.5$  (black lines) and at  $-\log(P_M) > 3$  (red lines) were mapped and associated DArT markers and marker positions (in cM) were given.



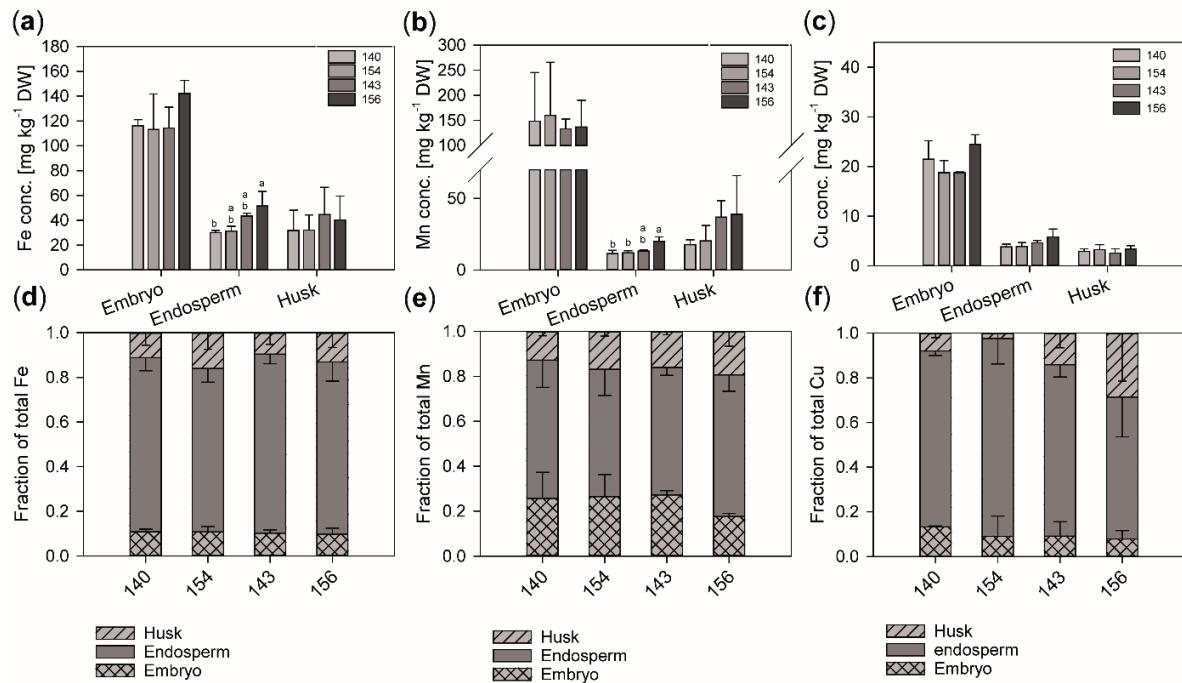
**Fig. S7 Gene expression profiles of MLOC\_40066.1 and MLOC\_61170.4.** Expression profiles based on BARLEX - the Barley Draft Genome Explorer ([https://apex.ipk-gatersleben.de/apex/f?p=284:10:::::\)](https://apex.ipk-gatersleben.de/apex/f?p=284:10:::::) of MLOC\_40066.1 (homologous to AtYSL2) and MLOC\_61170.4 (HvYSL9). FPKM: Fragments per kilo base per million mapped reads.



**Fig. S8 3D plots of grain iron (Fe), manganese (Mn) and copper (Cu) concentration within a selection of barley accessions from the ICARDA collection.** 3D scatter plots show Fe (a), Cu (b) and Mn (c) concentrations of lines grown in the three environments (FT, SE, KW).  $n=130$ . DW: Dry weight.



**Fig. S9 Micronutrient concentration in different mature seed tissues of four barley lines.** Grain iron (Fe, **a**), manganese (Mn, **b**) and copper (Cu, **c**) concentrations of embryo, endosperm and husk in four different barley lines (140, 154, 143, 156). Fractions of total Zn contributed by different tissues (embryo, endosperm and husk). Fractions were calculated by multiplying the relative weight of each tissue and the corresponding micronutrient concentration measured for each tissue and referred to the total respective micronutrient concentration of all tissues taken together. Results were obtained by dissecting and combining five grains of each independent experiment and line ( $n=3$ ). Values are means + SD. Statistical differences were calculated using ANOVA/Tukey's HSD post hoc test within one tissue and are displayed as letters if differences were detected at  $P \leq 0.05$ .



**Table S1 Details of the ICARDA mapping panel analyzed in this study.** Accession number, ICARDA IG, UR code, country of origin and subtaxa/conventional crop names are given. \*, lines selected for transcriptome analysis.

Accession number	ICARDA_IG #	UR Code	Country of origin	Subtaxa/Cultivar
37	128088	UR105	AFGHANISTAN	<i>subsp. vulgare</i> <i>convar. distichon</i>
38	27803	UR029	AFGHANISTAN	<i>subsp. vulgare</i> <i>convar. distichon</i>
39	20900	UR003	AFGHANISTAN	<i>subsp. vulgare</i> <i>convar. distichon</i>
40	25843	UR015	EGYPT	<i>subsp. vulgare</i> <i>convar. distichon</i>
41	32812	UR054	EGYPT	<i>subsp. vulgare</i> <i>convar. distichon</i>
42	32475	UR043	EGYPT	<i>subsp. vulgare</i> <i>convar. distichon</i>
43	32687	UR049	EGYPT	<i>subsp. vulgare</i> <i>convar. distichon</i>
44	32694	UR050	EGYPT	<i>subsp. vulgare</i> <i>convar. distichon</i>
45	25710	UR013	INDIA	<i>subsp. vulgare</i> <i>convar. distichon</i>
46	27655	UR022	IRAN	<i>subsp. vulgare</i> <i>convar. distichon</i>
48	112781	UR090	IRAN	<i>subsp. vulgare</i> <i>convar. distichon</i>
49	128200	UR118	JORDAN	<i>subsp. vulgare</i> <i>convar. distichon</i>
50	29097	UR034	JORDAN	<i>subsp. vulgare</i> <i>convar. distichon</i>
51	128202	UR119	JORDAN	<i>subsp. vulgare</i> <i>convar. distichon</i>
52	32978	UR061	OMAN	<i>subsp. vulgare</i> <i>convar. distichon</i>
53	32814	UR055	OMAN	<i>subsp. vulgare</i> <i>convar. distichon</i>
54	113076	UR094	SAUDI-ARABIA	<i>subsp. vulgare</i> <i>convar. distichon</i>
55	113082	UR095	SAUDI-ARABIA	<i>subsp. vulgare</i> <i>convar. distichon</i>
56	35220	UR069	SYRIA	<i>subsp. vulgare</i> <i>convar. distichon</i>
57	29057	UR033	SYRIA	<i>subsp. vulgare</i> <i>convar. distichon</i>
58	35236	UR070	SYRIA	<i>subsp. vulgare</i> <i>convar. distichon</i>
59	128172	UR114	SYRIA	<i>subsp. vulgare</i> <i>convar. distichon</i>
60	33094	UR064	SYRIA	<i>subsp. vulgare</i> <i>convar. distichon</i>
61	31396	UR035	SYRIA	<i>subsp. vulgare</i> <i>convar. distichon</i>
62	31412	UR038	SYRIA	<i>subsp. vulgare</i> <i>convar. distichon</i>
63	32774	UR053	SYRIA	<i>subsp. vulgare</i> <i>convar. distichon</i>
64	128173	UR115	SYRIA	<i>subsp. vulgare</i> <i>convar. distichon</i>
65	32756	UR052	SYRIA	<i>subsp. vulgare</i> <i>convar. distichon</i>
66	36058	UR075	TAJIKISTAN	<i>subsp. vulgare</i> <i>convar. distichon</i>
67	131668	UR122	TAJIKISTAN	<i>subsp. vulgare</i> <i>convar. distichon</i>
68	24720	no label	TURKMENISTAN	<i>subsp. vulgare</i> <i>convar. distichon</i>
69	28677	UR031	TURKEY	<i>subsp. vulgare</i> <i>convar. distichon</i>

70	28693	UR032	TURKEY	<i>subsp. vulgare convar. distichon</i>
71	37608	UR079	YEMEN	<i>subsp. vulgare convar. distichon</i>
72	113095	UR097	YEMEN	<i>subsp. vulgare convar. distichon</i>
73	37612	UR080	YEMEN	<i>subsp. vulgare convar. distichon</i>
74	22957	UR005	ETHIOPIA	<i>subsp. vulgare convar. distichon</i>
75	27892	UR030	SAUDI-ARABIA	<i>subsp. vulgare convar. distichon</i>
76	27799	UR028	AFGHANISTAN	<i>subsp. vulgare convar. vulgare</i>
77	20905	UR004	AFGHANISTAN	<i>subsp. vulgare convar. vulgare</i>
78	25883	UR016	AFGHANISTAN	<i>subsp. vulgare convar. vulgare</i>
79	27683	UR023	AFGHANISTAN	<i>subsp. vulgare convar. vulgare</i>
80	24745	UR009	AZERBAIJAN	<i>subsp. vulgare convar. vulgare</i>
81	26727	UR018	AZERBAIJAN	<i>subsp. vulgare convar. vulgare</i>
82	125827	UR104	AZERBAIJAN	<i>subsp. vulgare convar. vulgare</i>
83	33608	UR066	CHINA	<i>subsp. vulgare convar. vulgare</i>
84	33649	UR067	CHINA	<i>subsp. vulgare convar. vulgare</i>
85	33195	no label	CHINA	<i>subsp. vulgare convar. vulgare</i>
86	34263	UR068	CHINA	<i>subsp. vulgare convar. vulgare</i>
87	24634	UR007	ALGERIA	<i>subsp. vulgare convar. vulgare</i>
92	35385	UR072	ALGERIA	<i>subsp. vulgare convar. vulgare</i>
97	32482	UR044	EGYPT	<i>subsp. vulgare convar. vulgare</i>
98	32488	UR045	EGYPT	<i>subsp. vulgare convar. vulgare</i>
99	23515	UR006	ETHIOPIA	<i>subsp. vulgare convar. vulgare</i>
100	25704	UR012	INDIA	<i>subsp. vulgare convar. vulgare</i>
101	27773	UR024	IRAN	<i>subsp. vulgare convar. vulgare</i>
102	128124	UR107	IRAN	<i>subsp. vulgare convar. vulgare</i>
103	107010	UR085	IRAN	<i>subsp. vulgare convar. vulgare</i>
104	107020	UR086	IRAN	<i>subsp. vulgare convar. vulgare</i>
105	27653	UR021	IRAN	<i>subsp. vulgare convar. vulgare</i>
106	113120	UR098	IRAN	<i>subsp. vulgare convar. vulgare</i>
107	128122	UR106	IRAN	<i>subsp. vulgare convar. vulgare</i>
108	27794	UR027	IRAN	<i>subsp. vulgare convar. vulgare</i>
109	128125	UR108	IRAN	<i>subsp. vulgare convar. vulgare</i>
110	19453	UR002	IRAN	<i>subsp. vulgare convar. vulgare</i>
111	27790	UR026	IRAN	<i>subsp. vulgare convar. vulgare</i>
112	113126	UR099	IRAN	<i>subsp. vulgare convar. vulgare</i>
113	112715	UR089	IRAN	<i>subsp. vulgare convar. vulgare</i>
114	128133	UR109	IRAN	<i>subsp. vulgare convar. vulgare</i>
115	27649	UR020	IRAN	<i>subsp. vulgare convar. vulgare</i>
116	10891	UR088	IRAQ	<i>subsp. vulgare convar. vulgare</i>

117	26276	UR017	IRAQ	<i>subsp. vulgare convar. vulgare</i>
118	135258	UR123	JORDAN	<i>subsp. vulgare convar. vulgare</i>
119	128199	UR117	JORDAN	<i>subsp. vulgare convar. vulgare</i>
120	112840	UR091	LIBYA	<i>subsp. vulgare convar. vulgare</i>
121	37556	UR077	LIBYA	<i>subsp. vulgare convar. vulgare</i>
122	37576	UR078	LIBYA	<i>subsp. vulgare convar. vulgare</i>
123	115919	UR100	LIBYA	<i>subsp. vulgare convar. vulgare</i>
124	37554	UR076	LIBYA	<i>subsp. vulgare convar. vulgare</i>
125	112865	UR092	LIBYA	<i>subsp. vulgare convar. vulgare</i>
126	128218	UR121	LIBYA	<i>subsp. vulgare convar. vulgare</i>
127	36052	UR074	LIBYA	<i>subsp. vulgare convar. vulgare</i>
128	24953	UR011	LIBYA	<i>subsp. vulgare convar. vulgare</i>
129	31938	UR042	MOROCCO	<i>subsp. vulgare convar. vulgare</i>
130	31923	UR041	MOROCCO	<i>subsp. vulgare convar. vulgare</i>
131	31870	UR039	MOROCCO	<i>subsp. vulgare convar. vulgare</i>
132	31876	UR040	MOROCCO	<i>subsp. vulgare convar. vulgare</i>
133	32962	UR058	OMAN	<i>subsp. vulgare convar. vulgare</i>
134	32826	UR056	OMAN	<i>subsp. vulgare convar. vulgare</i>
135	32954	UR057	OMAN	<i>subsp. vulgare convar. vulgare</i>
136	32977	UR060	OMAN	<i>subsp. vulgare convar. vulgare</i>
137	32971	UR059	OMAN	<i>subsp. vulgare convar. vulgare</i>
138	27630	UR019	PAKISTAN	<i>subsp. vulgare convar. vulgare</i>
139	108499	UR087	PAKISTAN	<i>subsp. vulgare convar. vulgare</i>
<b>*140</b>	<b>128160</b>	<b>UR112</b>	<b>PAKISTAN</b>	<b><i>subsp. vulgare convar. vulgare</i></b>
141	32618	UR048	PAKISTAN	<i>subsp. vulgare convar. vulgare</i>
142	128158	UR110	PAKISTAN	<i>subsp. vulgare convar. vulgare</i>
<b>*143</b>	<b>128159</b>	<b>UR111</b>	<b>PAKISTAN</b>	<b><i>subsp. vulgare convar. vulgare</i></b>
144	32608	UR047	PAKISTAN	<i>subsp. vulgare convar. vulgare</i>
145	32601	UR046	PAKISTAN	<i>subsp. vulgare convar. vulgare</i>
146	113084	UR096	SAUDI-ARABIA	<i>subsp. vulgare convar. vulgare</i>
147	31410	UR037	SYRIA	<i>subsp. vulgare convar. vulgare</i>
148	31406	UR036	SYRIA	<i>subsp. vulgare convar. vulgare</i>
149	32708	UR051	SYRIA	<i>subsp. vulgare convar. vulgare</i>
150	16981	UR001	TURKMENISTAN	<i>subsp. vulgare convar. vulgare</i>
151	135528	UR124	TURKMENISTAN	<i>subsp. vulgare convar. vulgare</i>
152	120565	UR101	TURKMENISTAN	<i>subsp. vulgare convar. vulgare</i>
153	24746	UR010	TURKMENISTAN	<i>subsp. vulgare convar. vulgare</i>
<b>*154</b>	<b>37729</b>	<b>UR082</b>	<b>TUNISIA</b>	<b><i>subsp. vulgare convar. vulgare</i></b>
155	37784	UR083	TUNISIA	<i>subsp. vulgare convar. vulgare</i>

<b>*156</b>	<b>37726</b>	<b>UR081</b>	<b>TUNISIA</b>	<i>subsp. vulgare convar. vulgare</i>
157	37813	UR084	TUNISIA	<i>subsp. vulgare convar. vulgare</i>
158	112931	UR093	TURKEY	<i>subsp. vulgare convar. vulgare</i>
159	123901	UR102	UZBEKISTAN	<i>subsp. vulgare convar. vulgare</i>
160	123923	UR103	UZBEKISTAN	<i>subsp. vulgare convar. vulgare</i>
162	138212	UR226	ICARDA	<i>H. spont.</i> 41-3
163	138213	UR227	ICARDA	<i>H. spont.</i> 41-5
164	138214	UR125	ICARDA	Harmal-02/ESP/1808-4L (P2)
165	138215	UR126	ICARDA	Arta
166	138216	UR127	ICARDA	Tadmor
167	138217	UR128	ICARDA	ER/Apm
168	138218	UR129	ICARDA	WI2291
169	138219	UR130	ICARDA	Zanbaka
170	138220	UR131	ICARDA	SLB05-96
171	138221	UR132	ICARDA	Moroc9-75
172	138222	UR133	ICARDA	SLB34-40
173	138223	UR134	ICARDA	Zanbakian
174	138224	UR135	ICARDA	Harmat
175	138225	UR136	ICARDA	Sara
176	138226	UR137	ICARDA	Rihane-O3
177	138227	UR138	ICARDA	Alanda-O1
178	138228	UR139	TUNISIA	Martin
179	138229	UR140	ALGERIA	Saida
180	138230	UR141	ALGERIA	Tichedrett
181	138231	UR142	TUNISIA	Manel
182	138232	UR143	ICARDA	Badia
183	138233	UR144	FRANCE	Express
184	138234	UR145	IRAQ	IPA7
185	138235	UR146	MOROCCO	Arig8
186	138236	UR147	JORDAN	Rum
187	138237	UR148	TURKEY	Tokak
188	138238	UR149	RUSSIA	Radical
189	138239	UR150	TURKEY	Bulbul
190	138240	UR151	ICARDA	Matnan-01
191	138241	UR152	ICARDA	Assala-04
192	138242	UR153	ICARDA	Salmas
193	138243	UR154	EGYPT	CalMr
194	138244	UR155	CYPRUS	Mari/Ahs*2
195	138245	UR156	GREECE	Aths

196	138246	UR157	EGYPT	Giza 125
197	138247	UR158	EGYPT	Giza126
198	138248	UR159	SYRIA	Furat 1
199	138249	UR160	SYRIA	Furat-2
201	138251	UR162	ICARDA	Sadik-2
202	138252	UR163	ICARDA	Pamir-009
203	138253	UR164	LIBYA	Katara
204	138254	UR165	LIBYA	Barjouj
205	138255	UR166	ICARDA	Momtaz = (M 126/CM67//As/Pro/3/Alanda)
206	138256	UR167	ICARDA	Batal-01
207	138257	UR168	LEBANON	Litani
208	138258	UR169	ICARDA	Birlik
209	138259	UR170	SYRIA	Furat-3
210	138260	UR171	AUSTRALIA	Keel
211	138261	UR172	AUSTRALIA	Barque
212	138262	UR173	ETHIOPIA	Shege
213	138263	UR174	ERITREA	Demhay
214	138264	UR175	ERITREA	Atsa
215	138265	UR176	LIBYA	Tarida
216	138266	UR177	ICARDA	Arta/H.spont.41-5/Tadmor
217	138267	UR178	ICARDA	Arta/H.spont.41-5/Tadmor
218	138268	UR179	ICARDA	H.spont.41-1/Tadmor
219	138269	no label	ICARDA	H.spont.41-1/Tadmor
220	138270	UR181	ICARDA	Hml/H.spont.41-1/Tadmor
221	138271	UR182	ICARDA	Zanbaka//SLB45-40/H.spont.41-1
222	138272	UR183	ICARDA	SLB12-59//SLB45-40/H.spont.41-5
223	138273	UR184	ICARDA	SLB39-39/H.spont.41-5
224	138274	UR185	ICARDA	H.spont.41-5/Tadmor//Hml-02/Lignee131
225	138275	UR186	ICARDA	SLB05-96//H.spont.41-1/Tadmor
226	138276	UR187	ICARDA	SLB05-96/H.spont.41-5
227	138277	UR188	ICARDA	SLB34-65/Arar

**Table S2 Soil composition in pots (FT) and field plots (SE and KW).**

	Pots	SE 2007	SE 2015	KW 2007	KW 2015
pH	6,90	7,60	7,50	7,30	7,50
Organic matter (%)		-		4,00	
Phosphor (mg l <sup>-1</sup> )	502,00	6,90	7,90	17,60	18,30
Potassium (mg l <sup>-1</sup> )	1065,00	16,60	17,30	23,20	19,10
Magnesium (mg l <sup>-1</sup> )	205,00	9,80	11,10	17,10	14,60
NO <sub>3</sub> (mg l <sup>-1</sup> )	9,00	14,00		28,00	
NH <sub>4</sub> (mg l <sup>-1</sup> )	0,00	2,00		2,00	
Mineral N (mg l <sup>-1</sup> )	9,00	16,00		20,00	
B (mg kg <sup>-1</sup> )	0,99		1,04		1,48
Cu (mg kg <sup>-1</sup> )	1,40		1,20		5,90
Mn (mg kg <sup>-1</sup> )	43,00		12,00		145,00
Na (mg kg <sup>-1</sup> )	61,00		14,00		11,00
Zn (mg kg <sup>-1</sup> )	8,60		4,30		16,60
Fe (mg kg <sup>-1</sup> )			1,10		

Soil analysis of pots and field plots used for multiplication of the 'ICARDA germplasm panel was provided by AGROLAB Boden- und Pflanzenberatungsdienst GmbH ([www.agrolab.de](http://www.agrolab.de)). Both soils were clay silts, and KW was richer in nutrients.

**Table S3 Median zinc (Zn), iron (Fe), manganese (Mn) and copper (Cu) concentrations , C [%] and total protein [%] in barley grains of landraces and cultivars.** 150-162 lines of the ICARDA collection were grown in three different environments and median nutrient concentrations were calculated for landraces and cultivars (n=115-309).

	Landraces Median mg kg <sup>-1</sup> DW	Cultivars Median mg kg <sup>-1</sup> DW
Zn	50.4 (n=309)	50.8 (n=164)
Fe	52.6 (n=309)	58.9 (n=164)
Mn	18.5 (n=309)	19.6 (n=164)
Cu	6.2 (n=309)	7.1 (n=164)
C	43.1 (n=209)	44.6 (n=115)
Total protein	16 (n=209)	17.2 (n=115)

**Table S4 Correlation coefficients of zinc (Zn), iron (Fe), manganese (Mn) and copper (Cu) concentrations in barley grains.** 150-162 lines of the ICARDA collection were grown in three different environments and grain metal concentrations were correlated ( $n=473$ ). Pearson Product Moment Correlation. Signif. codes: \*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.01$ ; \*\*\*,  $P \leq 0.001$ ; ns, not significant.

	<b>Fe</b>	<b>Mn</b>	<b>Cu</b>
<b>Zn</b>	0.762	0.338	0.668
	***	***	***
<b>Fe</b>			
	0.473	0.533	
	***	***	
<b>Mn</b>		0.019	
		ns	

**Table S5 Correlation coefficients of zinc (Zn), iron (Fe), manganese (Mn) and copper (Cu) concentrations, C [%] and total protein [%] in barley grains in environment KW.** Lines of the ICARDA collection were grown in environment KW and grain metal concentrations were correlated (n=127). Pearson Product Moment Correlation. Signif. codes: \*, P ≤ 0.05; \*\*, P ≤ 0.01; \*\*\*, P ≤ 0.001; ns, not significant.

	Fe	Mn	Cu	C [%]	Total protein [%]
Zn	0.829	0.578	0.799	0.327	0.295
	***	***	***	*	***
Fe		0.702	0.798	0.278	0.332
		***	***	**	***
Mn			0.669	0.293	0.307
			***	***	***
Cu				0.222	0.233
				*	**
%C					0.658
					***

**Table S6 Correlation coefficients of zinc (Zn), iron (Fe), manganese (Mn) and copper (Cu) concentrations, C [%] and total protein [%] in barley grains in environment SE.** Lines of the ICARDA collection were in environment SE and grain metal concentrations were correlated (n=148). Pearson Product Moment Correlation. Signif. codes: \*, P ≤ 0.05; \*\*, P ≤ 0.01; \*\*\*, P ≤ 0.001; ns, not significant.

	Fe	Mn	Cu	C [%]	Total protein [%]
Zn	0.733 ***	0.598 ***	0.574 ***	0.236 **	0.511 ***
Fe		0.558 ***	0.487 ***	0.305 ***	0.639 ***
Mn			0.418 ***	0.253 **	0.493 ***
Cu				0.186 *	0.391 ***
%C					0.564 ***

**Table S7 Summary of significant marker-trait associations (MTAs) found for micro- and macronutrients of barley grains grown in different environments.** ICARDA lines were grown in three growth environments (FT, KW, SE) and results of seed composition analysis were used for GWAS. Best linear unbiased estimates (BLUEs) were calculated across the three environments. Numbers of MTAs for each chromosome is given in brackets in parallel with most significant MTA. DArT markers were considered significant at  $-\log(P_M) > 2.5$ .  $R^2$ , explained phenotypic variation.

Trait	Environment	MTAs	Most sign. marker	Chromosome	Position [cM]	$-\log(P)$	$R^2$
Zn	BLUE	2H(1)	bPb9754	2H	82.8	2.5	0.06
	FT	1H(1). 2H(3)	bPb9754	2H	82.8	3.5	0.07
	KW	1H(2). 2H(2)	bPb9199	2H	146	3.4	0.08
	SE	2H(2). 6H(2)	bPb1566	2H	149.4	3.2	0.03
Cu	BLUE	2H(6). 6H(2)	bPb3563	2H	102.4	3	0.07
	FT	2H(2)	bPb4040	2H	82.1	3.8	0.11
	KW	1H(1). 3H(1)	bPb4909	1H	72.9	3.2	0.08
	SE	3H(1). 6H(2)	bPb3919	6H	142.5	3.5	0.03
Fe	BLUE	2H(1). 4H(1)	bPb7057	2H	5.8	2.7	0.05
	KW	1H(1). 6H(1)	bPb6911	1H	94.9	2.5	0.06
	SE	3H(1). 6H(1)	bPb7273	3H	53.2	3	0.07
Mn	BLUE	3H(1)	bPb4564	3H	142.8	2.9	0.06
	FT	2H(1). 4H(1). 6H(1). 7H(1)	bPb2940	6H	137.7	2.9	0.02
	KW	1H(1). 2H(1)	bPb2230	2H	60.5	3.3	0.06
	SE	7H(2)	bPb7835	7H	55.6	2.7	0.01
%C	BLUE	2H(1). 3H(1). 7H(1)	bPb8644	7H	140.9	5.3	0.16
	KW	2H(1). 3H(1). 7H(2)	bPb2501	2H	47.4	5.6	0.05
	SE	2H(2). 3H(2). 4H(1). 7H(1)	bPb0200	3H	154.2	3.8	0.11
Protein	BLUE	2H(5). 3H(1)	bPb6194	2H	102.4	3.8	0.06
	KW	2H(5). 3H(4). 7H(2)	bPb6194	2H	102.4	4.9	0.07
	SE	2H(2). 3H(1)	bPb0200	3H	154.2	3.2	0.11

**Table S8 Summary of associated genes of identified markers for Zn concentration of grains grown in different environments.** ICARDA lines were grown in three growth environments (FT, KW, SE) and results of seed composition analysis were used for GWAS. Associated genes were searched within  $\pm$  0.5 cM of DArT markers showing LOD values  $\geq$  2.5. Barlex - The genome explorer platform (<https://apex.ipk-gatersleben.de/apex/f?p=284:10>) was used to search for associated genes. In addition, morex Genes -Barley RNA-seq Database was used to search for annotations and homologies of associated genes to other model species (<https://ics.hutton.ac.uk/morexGenes/>).

Marker	Environment	Chromosome	Position [cM]	Morex Contig	Associated genes	Position [cM]	Annotation or homology to model species (rice PP6 or TAIR PP10)
bPB-3217	FT	1H	40.5		<a href="#">AK358079</a>	40.08	UDP-Glycosyltransferase superfamily protein LENGTH=490
bPB-4909	KW	1H	72.9	<a href="#">morex contig_137689</a>	<a href="#">MLOC_6534.2</a>	72.52	BZIP transcription factor
				-	<a href="#">MLOC_6533.1</a>	72.52	Histone H3
				<a href="#">morex contig_1562468</a>	<a href="#">AK370940</a>	72.52	none
				-	<a href="#">MLOC_12065.4</a>	72.52	none
				-	<a href="#">MLOC_12066.3</a>	72.52	KRI1-like protein
bPB-6911	KW	1H	94.9	<a href="#">morex contig_2568492</a>	<a href="#">AK374068</a>	94.9	BSD domain containing protein 13105.m04603 protein IQ
				<a href="#">morex contig_243497</a>	<a href="#">MLOC_34307.3</a>	95.18	calmodulin-binding motif family protein, putative, expressed   Symbols: PDCB3
				<a href="#">morex contig_37489</a>	<a href="#">MLOC_52380.1</a>	95.18	plasmodesmata callose-binding protein 3   chr1:6419036-6420413 REVERSE LENGTH=184
				-	<a href="#">MLOC_52381.1</a>	95.18	none
				<a href="#">morex contig_47545</a>	none	95.18	
bPb-4040	FT	2H	82.1	<a href="#">morex contig_136156</a>	<a href="#">AK369206</a>	81.79	Histone-lysine N-methyltransferase E(z)
					<a href="#">AK374416</a>	81.79	none
				<a href="#">morex contig_1563715</a>	<a href="#">AK375429</a>	81.79	Protein kinase
				<a href="#">morex contig_2548955</a>	<a href="#">AK375713</a>	81.79	DNA topoisomerase VIA, putative, expressed
				<a href="#">morex contig_79282</a>	<a href="#">AK365212</a>	81.79	Glutamate dehydrogenase
				-	<a href="#">MLOC_78234.1</a>	81.79	none
				<a href="#">morex contig_136941</a>	<a href="#">MLOC_5973.1</a>	82.08	1-aminocyclopropane-1-carboxylate synthase
				<a href="#">morex contig_1576539</a>	<a href="#">MLOC_17385.1</a>	82.08	none
				<a href="#">morex contig_36893</a>	<a href="#">MLOC_51316.3</a>	82.08	none
				-	<a href="#">MLOC_51317.1</a>	82.08	none
				-	<a href="#">MLOC_51318.1</a>	82.08	none

				<a href="#">morex contig_44248</a>	<a href="#">MLOC_60276.4</a>	82.08	13104.m01655 protein histone H1, putative, expressed
				<a href="#">morex contig_455718</a>	<a href="#">HORVU0Hr1G005300.1</a>	82.08	none
				-	<a href="#">MLOC_61497.1</a>	82.08	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein LENGTH=341
				-	<a href="#">HORVU0Hr1G005310.1</a>	82.08	none
				<a href="#">morex contig_52942</a>	<a href="#">AK372027</a>	82.08	Dihydrodipicolinate synthase 2
				-	<a href="#">MLOC_67348.1</a>	82.08	none
				-	<a href="#">MLOC_67349.1</a>	82.08	C4-dicarboxylate transporter/malic acid transport family protein
				-	<a href="#">MLOC_67350.1</a>	82.08	Ankyrin repeat and zinc finger domain-containing protein
				<a href="#">morex contig_52942</a>	<a href="#">AK372027</a>	82.08	Dihydrodipicolinate synthase 2
				-	<a href="#">MLOC_67348.1</a>	82.08	NA
				-	<a href="#">MLOC_67349.1</a>	82.08	C4-dicarboxylate transporter/malic acid transport family protein
				-	<a href="#">MLOC_67350.1</a>	82.08	Ankyrin repeat and zinc finger domain-containing protein
				<a href="#">morex contig_6018</a>	<a href="#">AK356805</a>	82.08	Hydrolase, TatD family
				-	<a href="#">MLOC_71612.1</a>		NA
				<a href="#">morex contig_6109</a>	<a href="#">MLOC_72117.1</a>	82.08	GRAS family transcription factor
				<a href="#">morex contig_72043</a>	<a href="#">AK356563</a>	82.5	Subtilisin-like serine protease
				<a href="#">morex contig_2547011</a>	<a href="#">MLOC_36588.1</a>	82.08	Mechanosensitive ion channel protein LENGTH=497
				<a href="#">morex contig_39692</a>	<a href="#">MLOC_55138.1</a>	81.79	Protein kinase superfamily protein LENGTH=765
				<a href="#">morex contig_139284</a>	<a href="#">MLOC_7452.1</a>	82.08	RING finger protein
bPb-9754	FT, BLUE	2H	82.8	<a href="#">morex contig_72043</a>	<a href="#">AK356563</a>	82.5	Subtilisin-like serine protease
				<a href="#">morex contig_2547761</a>	<a href="#">AK374546</a>	82.64	Auxin response factor
bPb-2244	FT	2H	146	<a href="#">morex contig_42598</a>	<a href="#">AK362027</a>	146.1	2-succinylbenzoate-CoA ligase AMP-dependent synthetase and ligase family protein   chr3:18159031-18161294 REVERSE LENGTH=514
bPb-9199	FT	2H	146	<a href="#">morex contig_49696</a>	<a href="#">AK361917</a>	146.1	Glutathione S-transferase Regulator of chromosome condensation (RCC1) family with FYVE zinc finger domain LENGTH=1103
bPb-7211	KW	2H	146.3	<a href="#">morex contig_42598</a>	<a href="#">AK362027</a>	146.1	2-succinylbenzoate-CoA ligase AMP-dependent synthetase and ligase family protein   chr3:18159031-18161294 REVERSE LENGTH=514
				<a href="#">morex contig_49696</a>	<a href="#">AK361917</a>	146.1	Glutathione S-transferase Regulator of chromosome condensation (RCC1) family with FYVE zinc finger domain LENGTH=1103
				<a href="#">morex contig_1577592</a>	<a href="#">MLOC_17677.1</a>	146.24	Tubby-like protein
				<a href="#">morex contig_42008</a>	<a href="#">MLOC_57705.1</a>	146.38	Protein of unknown function (DUF793) LENGTH=382

				<a href="#">morex contig 39396</a>	<a href="#">AK356062</a>	146.52	Nodulin MtN21 /EamA-like transporter protein
					<a href="#">MLOC 54777.1</a>	146.52	NA
				<a href="#">morex contig 39447</a>	<a href="#">AK365404</a>	146.52	Protein kinase, putative
					<a href="#">MLOC 54851.1</a>	146.52	NA
				<a href="#">morex contig 39476</a>	<a href="#">MLOC 54892.1</a>	146.52	Peroxidase 10
					<a href="#">MLOC 54893.1</a>	146.52	Peroxidase 12
				<a href="#">morex contig 50241</a>	<a href="#">MLOC 65476.1</a>	146.52	NA
					<a href="#">MLOC 65477.1</a>	146.52	Peroxidase 10
				<a href="#">morex contig 62700</a>	<a href="#">MLOC 72957.1</a>	146.52	NA
				<a href="#">morex contig 8198</a>	<a href="#">AK366558</a>	146.52	UPF0235 protein C15orf40-like protein
				<a href="#">morex contig 49247</a>	<a href="#">MLOC 64730.1</a>	146.52	F-box domain containing protein
					<a href="#">MLOC 64731.1</a>	146.52	30S ribosomal protein S11, putative
bPb-1184	SE	2H	149.4	<a href="#">morex contig 158818</a>	<a href="#">MLOC 20130.1</a>	149.15	Tubulin folding cofactor B
bPb-1566	SE	2H	149.4		<a href="#">MLOC 20131.1</a>	149.15	NA
					<a href="#">MLOC 20132.1</a>	149.15	Receptor kinase 2
					<a href="#">MLOC 20133.1</a>	149.15	NA
bPb-8836	SE	6H	28.8	<a href="#">morex contig 44344</a>	<a href="#">AK365904</a>	28.47	Symbols: SYP51, ATSYP51   syntaxin of plants 51   chr1:5555183-5556340 REVERSE LENGTH=232
bPb-9651	SE	6H	28.8		<a href="#">MLOC 60395.3</a>	28.47	Carboxypeptidase B2, putative
				<a href="#">morex contig 49611</a>	<a href="#">HORVU0Hr1G015030.29</a>	28.47	NA
					<a href="#">HORVU0Hr1G015040.1</a>	28.47	NA
					<a href="#">MLOC 65000.4</a>	28.47	Ubiquitin thioesterase OTUB1
					<a href="#">MLOC 65001.1</a>	28.47	NA
					<a href="#">MLOC 65002.1</a>	28.47	NA
				<a href="#">morex contig 1563669</a>	<a href="#">AK359031</a>	28.54	Copine, putative
					<a href="#">MLOC 12590.1</a>	28.54	NA
					<a href="#">MLOC 12591.1</a>	28.54	NA
					<a href="#">MLOC 12593.1</a>	28.54	NA
				<a href="#">morex contig 268203</a>	<a href="#">AK248811.1</a>	28.54	NA
					<a href="#">MLOC 43169.1</a>	28.54	NA
				<a href="#">morex contig 272003</a>	<a href="#">MLOC 43672.2</a>	28.86	Protein kinase

**Table S9 Summary of associated genes of Zn related marker 2H | bPb9754 (82.77 cM**

**obtained by BLUEs analysis of grains grown in different environments.** ICARDA lines were grown in three growth environments (FT, KW, SE) and results of seed composition analysis were used for GWAS. Associated genes were searched within  $\pm$  2.5 cM of DArT marker bPb9754 showing a LOD value of 2.5. Barlex - The genome explorer platform (<https://apex.ipk-gatersleben.de/apex/f?p=284:10>) was used to search for associated genes. In addition, morex Genes -Barley RNA-seq Database was used to search annotations and homologies of associated genes to other model species (<https://ics.hutton.ac.uk/morexGenes/>).

Position [cM]	Morex Contig	Associated genes	Annotation or homology to model species (rice PP6 or TAIR PP10
80.38	<a href="#">morex contig_38722</a>	<a href="#">AK368045</a>	Elongation factor 4
80.38		<a href="#">MLOC_53896.1</a>	Protein kinase superfamily protein LENGTH=349
80.59	<a href="#">morex contig_161508</a>	<a href="#">AK358911</a>	Trihelix transcription factor
80.88	<a href="#">morex contig_2556062</a>	<a href="#">MLOC_40066.1</a>	Symbols: YSL2, ATYSL2   YELLOW STRIPE like 2   chr5:8324098-8326525 FORWARD LENGTH=664
80.88	<a href="#">morex contig_47039</a>	<a href="#">MLOC_62860.1</a>	NA
80.88	<a href="#">morex contig_135983</a>	<a href="#">MLOC_5187.5</a>	Ubiquitin-specific protease family C19-related protein   chr4:11783199-11785730 REVERSE LENGTH=445
80.95	<a href="#">morex contig_45176</a>	<a href="#">MLOC_61170.4</a>	<b>Yellow stripe-like 9 transporter</b>
81.23	<a href="#">morex contig_1570881</a>	<a href="#">MLOC_15510.1</a>	GATA transcription factor 29
81.23	<a href="#">morex contig_37206</a>	<a href="#">AK360700</a>	Domain of unknown function (DUF3598) LENGTH=500
81.23	<a href="#">morex contig_55447</a>	<a href="#">MLOC_68842.2</a>	Poly(U)-specific endoribonuclease
81.23		<a href="#">MLOC_68843.4</a>	Protein kinase superfamily protein   chr5:19118683-19120528 REVERSE LENGTH=410
81.52	<a href="#">morex contig_224145</a>	<a href="#">MLOC_31990.1</a>	NA
81.52		<a href="#">MLOC_31991.1</a>	BTB/POZ domain-containing protein LENGTH=548
81.52	<a href="#">morex contig_274579</a>	<a href="#">MLOC_44349.1</a>	13102.m02510 protein expressed protein
81.52	<a href="#">morex contig_36846</a>	<a href="#">MLOC_51071.2</a>	RNA binding protein, putative
81.52	<a href="#">morex contig_38448</a>	<a href="#">MLOC_53540.1</a>	Casein kinase II subunit beta-4
81.52		<a href="#">MLOC_53541.1</a>	Eukaryotic translation initiation factor 4 gamma, 1
81.52	<a href="#">morex contig_39457</a>	<a href="#">MLOC_54863.1</a>	13104.m04612 protein transcription factor like protein, putative, expressed
81.52		<a href="#">MLOC_54864.10</a>	Cationic amino acid transporter, putative
81.80	<a href="#">morex contig_136156</a>	<a href="#">AK369206</a>	Histone-lysine N-methyltransferase E(z)
81.80		<a href="#">AK374416</a>	NA
81.80	<a href="#">morex contig_1563715</a>	<a href="#">AK375429</a>	Protein kinase

81.80	<a href="#">morex contig 2548955</a>	<a href="#">AK375713</a>	DNA topoisomerase VIA, putative, expressed
81.80	<a href="#">morex contig 79282</a>	<a href="#">AK365212</a>	Glutamate dehydrogenase
81.80		<a href="#">MLOC_78234.1</a>	NA
81.80	<a href="#">morex contig 39692</a>	<a href="#">MLOC_55138.1</a>	Protein kinase superfamily protein LENGTH=765
82.08	<a href="#">morex contig 136941</a>	<a href="#">MLOC_5973.1</a>	1-aminocyclopropane-1-carboxylate synthase
82.08	<a href="#">morex contig 1576539</a>	<a href="#">MLOC_17385.1</a>	NA
82.08	<a href="#">morex contig 36893</a>	<a href="#">MLOC_51316.3</a>	NA
82.08		<a href="#">MLOC_51317.1</a>	NA
82.08		<a href="#">MLOC_51318.1</a>	NA
82.08	<a href="#">morex contig 44248</a>	<a href="#">MLOC_60276.4</a>	Symbols: HIS1-3   histone H1-3   chr2:7846095-7846670 FORWARD LENGTH=167
82.08	<a href="#">morex contig 455718</a>	<a href="#">HORVU0Hr1G005300.1</a>	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein
82.08		<a href="#">HORVU0Hr1G005310.1</a>	HAT family dimerisation domain containing protein, expressed
82.08		<a href="#">MLOC_61497.1</a>	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein LENGTH=341
82.08	<a href="#">morex contig 52942</a>	<a href="#">AK372027</a>	Dihydrodipicolinate synthase 2
82.08		<a href="#">MLOC_67348.1</a>	NA
82.08		<a href="#">MLOC_67349.1</a>	C4-dicarboxylate transporter/malic acid transport family protein
82.08		<a href="#">MLOC_67350.1</a>	Ankyrin repeat and zinc finger domain-containing protein
82.08	<a href="#">morex contig 6018</a>	<a href="#">AK356805</a>	Hydrolase, TatD family
82.08		<a href="#">MLOC_71612.1</a>	NA
82.08	<a href="#">morex contig 6109</a>	<a href="#">MLOC_72117.1</a>	GRAS family transcription factor
82.08	<a href="#">morex contig 2547011</a>	<a href="#">MLOC_36588.1</a>	Mechanosensitive ion channel protein LENGTH=497
82.08	<a href="#">morex contig 139284</a>	<a href="#">MLOC_7452.1</a>	RING finger protein
82.51	<a href="#">morex contig 72043</a>	<a href="#">AK356563</a>	Subtilisin-like serine protease
82.65	<a href="#">morex contig 2547761</a>	<a href="#">AK374546</a>	Auxin response factor
		<a href="#">MLOC_62861.3</a>	Sphingosine kinase

**Table S10 Differentially expressed genes in whole ears and flag leaves in comparisons of high vs low zinc (Zn) accumulating barley lines.** Log fold changes and gene description of genes showing higher or lower transcript abundance in each comparison of high (143 or 156) vs low (140 or 154) Zn barley lines 15 days after pollination (dap) in whole ears and flag leaves. Microarray data were analyzed with RobiNA using following settings: Normalization method: GcRMA, p-value correction: Bonferroni Hochberg, multiple testing: nested.F. Statistical parameters of transcript analysis: *P*-value cutoff: 0.05, log fold change min=1. Color key shows less (yellow) or more (blue) abundant transcripts in high Zn lines compared to low Zn lines.

Whole ears										Flag Leaves				Identifier	Bins	Biological Process	Cellular Component	Molecular Function	<i>e</i> value	Target Description (Blastx)	Annotation Grade
143	143	156	156	143	143	156	156	-	-	-	-	-	-								
140	154	140	154	140	154	140	154														
4.03	4.38	4.50	4.85	4.16	4.60	4.35	4.79							Contig3642_at	26.17 misc.dynamin			6.00E-98	putative cytochrome P450 [Oryza sativa (japonica cultivar-group)]	A	
4.18	3.61	4.29	3.71	3.18	3.56	3.61	3.99							Contig253_s_at	29.2.2 protein.synthesis.misc ribosomal protein	translation	intracellular, ribosome		3.00E-28	RIBOSOMAL PROTEIN S30 homolog; protein id: At4g29390.1 [Arabidopsis thaliana]	A
1.92	1.92	2.03	2.03	2.74	2.76	3.03	3.05							Contig13836_at	35.1 not assigned.no ontology				≤none>	R	
8.50	7.98	7.59	7.07											HB09A04w_s_at	33.50 development.inhibitor proteins			2.00E-28	Alpha-amylase/trypsin inhibitor CMB precursor (Chloroform/methanol-soluble protein CMB)	A	
3.43	4.19	4.50	5.27											EBro03_SQ008_F20_at	35.2 not assigned.unknown				≤none>	A	
2.89	3.17	3.21	3.49											Contig14203_s_at	35.2 not assigned.unknown				≤none>	A	
1.29	1.76	1.10	1.57											Contig17501_at	35.2 not assigned.unknown			1.00E-09	(AY055743) wound-responsive protein 10.1 [Castanea sativa]	R	
				1.56	1.33	1.72	1.50							Contig15482_at	28.1 DNA.synthesis/chromatin structure			1.00E-31	(NM_102889) hypothetical protein; protein id: At1g31500.1 [Arabidopsis thaliana]	R	
				1.45	1.27	1.64	1.46							HW03O03u_at	9.7 mitochondrial electron transport / ATP synthesis.cytochrome c oxidase	membrane, integral component of membrane	copper ion binding	4.00E-49	hypothetical protein T6A9.10 - Arabidopsis thaliana gb AAG00893.1 AC064879_11 Similar to cytochrome-c oxidase assembly protein [Arabidopsis thaliana]	A	
-1.50	-1.93	-1.75	-2.17	-1.61	-1.99	-1.80	-2.19							HU11B09u_s_at	35.2 not assigned.unknown			8.00E-08	OSJNBb0024F06.4 [Oryza sativa (japonica cultivar-group)]	A	
-1.90	-2.72	-2.90	-3.72	-2.55	-3.28	-3.43	-4.16							Contig10826_at	35.2 not assigned.unknown			≤none>		A	

-	1.82	-	2.00	-	1.40	-	1.58	-	2.62	-	2.61	-	2.61	-	2.61	Contig16760_at	35.2 not assigned.unknown	nucleobase-containing compound metabolic process, nucleic acid phosphodiester bond hydrolysis	nucleic acid binding, 3'-5' exonuclease activity	1.00E-11	(AJ404476) exonuclease [Arabidopsis thaliana]	A
-	2.68	-	3.85	-	2.88	-	4.05	-	2.78	-	3.09	-	2.50	-	2.81	Contig5646_at	35.2 not assigned.unknown		3.00E-76	hypothetical protein; protein id: At1g34220.1 [Arabidopsis thaliana]	A	
-	3.38	-	1.85	-	3.50	-	1.97	-	3.43	-	2.61	-	3.25	-	2.43	HU08A18u_at	35.2 not assigned.unknown		3.00E-09	stem rust resistance protein Rpg1 [Hordeum vulgare subsp. vulgare] gb AAM81972.1 AF509748_1 barley stem rust resistance protein [Hordeum vulgare]	R	
-	4.04	-	4.09	-	4.04	-	4.09	-	4.97	-	5.43	-	4.93	-	5.39	Contig679_s_at	29.2.3 protein.synthesis.initiation		3.00E-62	ELONGATION FACTOR 1-ALPHA (EF-1-ALPHA) pir JC1454 translation elongation factor eEF-1 alpha chain - wheat	R	
-	1.55	-	1.99	-	2.56	-	3.00									Contig9140_at	35.2 not assigned.unknown		1.00E-34	OJ000223_09.1 [Oryza sativa (japonica cultivar-group)]	A	
-	2.14	-	1.36	-	3.49	-	2.70									Contig250_at	28.1.3 DNA.synthesis/chromatin structure.histone	nucleosome assembly, nucleosome, nucleus, chromosome	DNA binding, protein heterodimerization activity	2.00E-48	histone H4 (TH091) - wheat gb AAA34292.1  histone H4	A
-	2.34	-	2.31	-	1.62	-	1.58									HU11B09u_at	35.2 not assigned.unknown		8.00E-08	OSJNBB0024F06.4 [Oryza sativa (japonica cultivar-group)]	R	
-	2.45	-	2.78	-	2.38	-	2.71									rbags22p06_s_at	35.2 not assigned.unknown		<none>		R	
-	2.56	-	1.39	-	2.66	-	1.49									HO14A23S_at	35.2 not assigned.unknown		0.004	(AJ245900) CAA30371.1 protein [Oryza sativa]	A	
-	2.76	-	2.60	-	2.75	-	2.58									Contig12289_at	35.2 not assigned.unknown		<none>		A	
-	3.00	-	3.28	-	3.31	-	3.59									Contig6310_at	35.2 not assigned.unknown		<none>		A	
						-	2.42	-	3.48	-	2.42	-	3.48			Contig15571_at	35.2 not assigned.unknown	ADP binding	7.00E-05	(AC096691) putative disease resistance gene [Oryza sativa (japonica cultivar-group)]	A	
						-	3.36	-	2.48	-	3.32	-	2.44			Contig14184_at	35.2 not assigned.unknown		5.00E-61	csAtPR5 [Aegilops tauschii]	A	
						-	3.53	-	2.73	-	7.18	-	6.38			Contig7943_at	35.2 not assigned.unknown		8.00E-27	(AF325034) AT5g02160 [Arabidopsis thaliana]	R	
						-	3.85	-	2.95	-	2.52	-	1.62			Contig10162_s_at	35.2 not assigned.unknown	membrane, integral component of membrane	1.00E-25	zhb0003.1 [Oryza sativa (indica cultivar-group)]	A	

**Table S11 Blastx search results against exemplar sequences of significantly differentially expressed genes shared in comparisons of high vs low zinc (Zn) barley lines.** Exemplar sequences of significantly differentially expressed transcripts of comparisons of high (143 or 156) vs low (140 or 154) Zn accumulating barley lines 15 days after pollination (dap) in whole ears and flag leaves. Blastx searches partially revealed potential functions based on conserved domains/superfamilies.

Identifier	Conserved domains/ Superfamilies	Highest similarity to (blastx)	Max score	Total score	Query cover	E value	Ident	Accession
Contig3642_at	p450 superfamily, PRK0, Motor_, P-loop_NTPase	PREDICTED: cytochrome P450 72A15-like [ <i>Brachypodium distachyon</i> ]	612	612	47%	0	87%	<a href="#">XP_003566917.1</a>
Contig253_s_at	Ribosomal_S30 superfamily	Ribosomal protein S30 [ <i>Cynara cardunculus var. scolymus</i> ]	126	126	37%	9.00E-35	100%	<a href="#">KVI01226.1</a>
Contig13836_at	no result	<a href="#">predicted protein [<i>Hordeum vulgare subsp. vulgare</i>]</a>	73.6	73.6	12%	1.00E-12	77%	<a href="#">BAK03229.1</a>
HB09A04w_s_at	no result	RecName: Full=Alpha-amylase/trypsin inhibitor CMb; AltName: Full=Chloroform/methanol-soluble protein CMb; Flags: Precursor	123	123	44%	2.00E-33	97%	<a href="#">P32936.2</a>
EBro03_SQ008_F20_at	no result	<a href="#">hypothetical protein TRIUR3_01380 [<i>Triticum urartu</i>]</a>	86.3	86.3	42%	1.00E-18	76%	<a href="#">EMS55232.1</a>
Contig14203_s_at	no result	<a href="#">hypothetical protein TRIUR3_01380 [<i>Triticum urartu</i>]</a>	92.8	92.8	45%	3.00E-21	75%	<a href="#">EMS55232.1</a>
Contig17501_at	no result	<a href="#">predicted protein [<i>Hordeum vulgare subsp. vulgare</i>]</a>	261	261	51%	3.00E-83	99%	<a href="#">BAJ99367.1</a>
Contig15482_at	EEP superfamily	<a href="#">predicted protein [<i>Hordeum vulgare subsp. vulgare</i>]</a>	209	209	50%	5.00E-63	100%	<a href="#">BAK04055.1</a>
HW03O03u_at	CtaG_Cox11 Superfamily	<a href="#">cytochrome c oxidase assembly protein COX11, mitochondrial [<i>Aegilops tauschii subsp. tauschii</i>]</a>	211	211	56%	5.00E-66	99%	<a href="#">XP_020168420.1</a>
HU11B09u_s_at	no result	<a href="#">predicted protein [<i>Hordeum vulgare subsp. vulgare</i>]</a>	264	264	64%	3.00E-83	98%	<a href="#">BAK01532.1</a>
Contig10826_at	no result	<a href="#">predicted protein [<i>Hordeum vulgare subsp. vulgare</i>]</a>	321	321	47%	1.00E-106	99%	<a href="#">BAK08002.1</a>
Contig16760_at	DnaQ_like_exo Superfamily	<a href="#">Werner syndrome ATP-dependent helicase [<i>Aegilops tauschii</i>]</a>	127	127	34%	2.00E-33	97%	<a href="#">EMT21210.1</a>

Contig5646_at	Ist1 superfamily	<a href="#">predicted protein [Hordeum vulgare subsp. vulgare]</a>	875	875	81%	0	100%	<a href="#">BAJ94091.1</a>
HU08A18u_at	no result	<a href="#">predicted protein [Hordeum vulgare subsp. vulgare]</a>	131	131	46%	8.00E-33	67%	<a href="#">BAK01052.1</a>
Contig679_s_at	SelB Superfamily (EF1_alpha_III)	<a href="#">elongation factor 1-alpha [Aegilops tauschii subsp. tauschii]</a>	266	266	54%	6.00E-84	96%	<a href="#">XP_020173542.1</a>
Contig9140_at	no result	<a href="#">predicted protein [Hordeum vulgare subsp. vulgare]</a>	1000	1000	71%	0	97%	<a href="#">BAK01532.1</a>
Contig250_at	H4 Superfamily	<a href="#">histone H4-like [Aegilops tauschii subsp. tauschii]</a>	187	187	66%	6.00E-58	83%	<a href="#">XP_020164542.1</a>
HU11B09u_at	no result	<a href="#">predicted protein [Hordeum vulgare subsp. vulgare]</a>	264	264	64%	3.00E-83	98%	<a href="#">BAK01532.1</a>
rbags22p06_s_at		No significant similarity found.						
HO14A23S_at	no result	<a href="#">predicted protein [Hordeum vulgare subsp. vulgare]</a>	66.6	66.6	42%	3.00E-10	100%	<a href="#">BAJ86088.1</a>
Contig12289_at	no result	<a href="#">uncharacterized protein LOC109763383 [Aegilops tauschii subsp. tauschii]</a>	205	205	68%	1.00E-58	76%	<a href="#">XP_020177815.1</a>
Contig6310_at	no result	<a href="#">unnamed protein product [Triticum aestivum]</a>	83.6	83.6	61%	5.00E-15	65%	<a href="#">CDM86087.1</a>
Contig15571_at	no result	<a href="#">predicted protein [Hordeum vulgare subsp. vulgare]</a>	143	143	45%	5.00E-37	100%	<a href="#">BAK07911.1</a>
Contig14184_at	no result	<a href="#">predicted protein [Hordeum vulgare subsp. vulgare]</a>	317	317	70%	7.00E-102	99%	<a href="#">BAK07732.1</a>
Contig7943_at	no result	<a href="#">predicted protein [Hordeum vulgare subsp. vulgare]</a>	188	188	35%	1.00E-56	100%	<a href="#">BAJ99506.1</a>
Contig10162_s_at	DUF247 Superfamily	<a href="#">predicted protein [Hordeum vulgare subsp. vulgare]</a>	407	407	79%	2.00E-138	100%	<a href="#">BAJ92639.1</a>