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# Iron and zinc grain density in common wheat grown in Central Asia

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10 Abstract Sixty-six spring and winter common wheat genotypes from Central Asian breeding 11 12 programs were evaluated for grain concentrations 13 of iron (Fe) and zinc (Zn). Iron showed large variation among genotypes, ranging from 14  $25 \text{ mg kg}^{-1}$  to  $56 \text{ mg kg}^{-1}$  (mean  $38 \text{ mg kg}^{-1}$ ). 15 Similarly, Zn concentration varied among geno-16 types, ranging between 20 mg kg<sup>-1</sup> and 39 mg kg<sup>-1</sup> 17 (mean 28 mg kg<sup>-1</sup>). Spring wheat cultivars pos-18 19 sessed higher Fe-grain concentrations than winter 20 wheats. By contrast, winter wheats showed higher

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Zn-grain concentrations than spring genotypes. 21 Within spring wheat, a strongly significant positive 22 correlation was found between Fe and Zn. Grain 23 protein content was also significantly (P < 0.001)24 correlated with grain Zn and Fe content. There 25 were strong significantly negative correlations 26 between Fe and plant height, and Fe and glutenin 27 content. Similar correlation coefficients were 28 29 found for Zn. In winter wheat, significant positive correlations were found between Fe and Zn, and 30 31 between Zn and sulfur (S). Manganese (Mn) and phosphorus (P) were negatively correlated with 32 both Fe and Zn. The additive main effects and 33 multiplicative interactions (AMMI) analysis of 34 genotype  $\times$  environment interactions for grain Fe 35 and Zn concentrations showed that genotype 36 effects largely controlled Fe concentration, 37 whereas Zn concentration was almost totally 38 dependent on location effects. Spring wheat 39 genotypes Lutescens 574, and Eritrospermum 78; 40 and winter wheat genotypes Navruz, NA160/ 41 HEINEVII/BUC/3/F59.71//GHK, Tacika, DU-42 CULA//VEE/MYNA, JUP/4/CLLF/3/ 43 and II14.53/ODIN//CI13431/WA00477, are promising 44 materials for increasing Fe and Zn concentrations 45 in the grain, as well as enhancing the concentra-46 tion of promoters of Zn bioavailability, such as S-47 containing amino acids. 48

KeywordsBreeding  $\cdot$  Central Asia  $\cdot$  G  $\times$  E  $\cdot$ 49Iron  $\cdot$  Wheat  $\cdot$  Zinc50



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### 51 Introduction

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52 The Central Asia region comprises five countries (Kazakhstan, Kyrgyzstan, Turkmenistan, Tajiki-54 stan and Uzbekistan) which grow a total of more than 15 million ha of wheat (Triticum aestivum). 56 In northern Kazakhstan (48-55° N), spring wheat is grown on steppe lands under dry-land conditions. Throughout the southern region (36-44° N), occupying 5-6 million ha, winter or facultative wheat is grown primarily under irrigation (60-70%) (Fig. 1). Rainfed wheat is planted on 62 the remaining 30-40% of the area, mostly on hillsides or mountainous areas where irrigation is 64 not possible (Morgounov et al. 2001).

65 For both spring and winter wheat improve-66 ment, regional and international cooperation 67 have been established with the objectives of 68 strengthening national breeding programs by 69 exchanging information and breeding materials. 70 Since 1994 wheat research in the region was 71 substantially influenced by the development of 72 international linkages, especially in breeding. 73 CIMMYT and ICARDA established germplasm 74 exchange networks through the TURKEY-CI-75 MMYT-ICARDA Winter and Facultative Wheat 76 Improvement Program located in Turkey, and 77 more recently through the Kazakhstan-Siberia 78 Wheat Improvement Network on Spring

(KASIB), a CIMMYT Central Asia and Caucasus 79 initiative. By 2003 several wheat lines from both 80 programs were being tested in the region for 81 possible release. The advantages of the new lines 82 are higher grain yield, and better resistance to leaf 83 diseases, especially yellow rust. These efforts 84 contribute significantly to improving food security 85 and self-sufficiency of grain production in these 86 countries. Uzbekistan achieved self-sufficiency in 87 2002 and 2003, whereas Tajikistan and Turkmen-88 istan have improved national wheat production 89 substantially during the past decade (Morgounov 90 et al. 2005). 91

However, nutritional problems related to 92 cereal-based diets throughout the region, espe-93 cially those linked to vitamin and mineral defi-94 ciencies in vulnerable groups, such as children 95 96 under five years and women in reproductive age, are national concerns. UNICEF and the Micro-97 nutrient Initiative (2004) estimated iron defi-98 ciency anemia ranging from 33% to 49% in 99 children under 5 years of age and 31-63% in 100 women aged 15-49 for countries in the Central 101 Asian region. In Central Asia, as in large parts of 102 the developing world, micronutrient deficiencies 103 are widespread. It is estimated that two billion 104 people worldwide suffer from micronutrient defi-105 ciencies, especially children and women (Cakmak 106 et al. 2002; Welch and Graham 2004). High and 107



Fig. 1 Wheat-producing areas in the former Soviet Union. Winter wheat is grown during October-June and spring wheat during May-August

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108 monotonous consumption of cereal-based foods 109 has been shown to be a major reason for such 110 widespread occurrence of micronutrient deficien-111 cies in the developing world. Cereal grains are 112 inherently poor in concentration of micronutrients, and rich in compounds depressing the 113 bioavailability of micronutrients such as phytic 114 115 acid. In Central Asian countries, wheat is the most important staple food contributing greatly to 116 daily calorie intake. Between 50% (Kazakhstan) 117 and 65% (Tajikistan) of daily calorie intake 118 119 comes solely from wheat and this rate can be 120 greater than 75% in rural regions (Cakmak et al. 121 2004).

122 This situation has led to the formation of the 123 "Anemia Prevention and Control" (APC) pro-124 gram in Kazakhstan, Kyrgyzstan, Uzbekistan, Tajikistan and Turkmenistan under the support 125 126 of UNICEF, which among other activities fosters 127 the universal fortification of wheat flour with 128 minerals and vitamins (Gleason and Sharmanov 129 2002). However, fortification efforts are highly 130 dependent on funding, and the scope is restricted 131 to a single geographical area. Standard fortifica-132 tion programs must be sustained at the same 133 level of funding year after year; and if the investments are not sustained, the benefits 134 disappear (Bouis et al. 2000). By contrast, 135 investment in research in plant breeding has 136 137 multiplicative effects; the benefits may accrue to 138 a number of countries and moreover, the benefits 139 from breeding advances typically do not disap-140 pear after initial successful investment and 141 research, as long as an effective domestic agri-142 cultural research infrastructure is maintained 143 (Bouis et al. 2000).

144 Recently, the CGIAR launched the Harvest-145 Plus initiative, a challenge program on biofortification of staple crops (breeding crops with high 146 micronutrient contents). Under this initiative, 147 148 CIMMYT is developing high yielding disease 149 resistant wheat germplasm with enhanced levels 150 of iron (Fe) and zinc (Zn), and this germplasm is 151 now being tested by national program partners.

The objectives of the present study were to (i)
determine the levels of Fe and Zn in the grain of
current wheat lines and cultivars used in breeding
programs in Central Asia, (ii) analyze the

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# Materials and methods

Sixty-six spring and winter wheat cultivars and 161 advanced lines from Central Asian national 162 breeding programs were selected for this study 163 (Table 1). Grain samples from each germplasm 164 included in a Kazakhstan-Siberia Network for 165 Spring Wheat Improvement Regional Nursery 166 (5th KASIB) grown at five locations in Kazakh-167 stan in 2004 were analyzed for Fe and Zn 168 concentration at Waite Analytical Services, Uni-169 versity of Adelaide, Australia, based on the nitric/ 170 perchloric acid digestion method using an induc-171 tively coupled plasma optical emission spectrom-172 eter (ICP-OES) (Zarcinas et al. 1987). Grain 173 from field trials, grown at nine locations in 174 Kazakhstan, Kyrgyzstan and Tajikistan in 2005, 175 were also analyzed for micro (Fe, Zn and Mn) 176 and macro-elements (Mg, P, and S) at Sabanci 177 University, Istanbul, Turkey. Measurements of 178 the mineral nutrients were conducted using an 179 ICP-OES after digesting samples in a closed 180 microwave system (Zarcinas et al. 1987; USEPA 181 1998; Ryan 2005). Agronomic and grain quality 182 data for spring wheat were available from 183 "Results of the 4th and 5th Kazakhstan-Siberia 184 Network Trials for Spring Wheat Improvement" 185 (CIMMYT 2005). For winter wheat, data on grain 186 yield additional to the mineral analyses were 187 available only for Tajikistan. 188

Data were evaluated statistically using one-way 189 analyses of variance; means were compared using 190 a least significant difference (LSD) procedure. 191 Associations among variables were evaluated 192 using Pearson correlation and linear regression 193 techniques. Genotype × environment was ana-194 lyzed independently by trials and country using 195 the additive main effects and multiplicative 196 interactions analysis (AMMI). The AMMI model 197 postulates additive components for the main 198 effects of genotypes ( $\alpha i$ ) and environments ( $\beta i$ ) 199 and multiplicative components for the effect of 200



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 Table 1
 Concentrations of Fe and Zn in seeds of spring and winter wheat genotypes

Genotype	$Fe (mg kg^{-1})^b$	$Zn (mg kg^{-1})$	Test environments <sup>a</sup>
Spring wheat			
Chelyaba	56	32 (4)	A, B, C, D, E
Lutescense 148-97-16	48	32 (2)	A, B, C, D, E
Iren	48	32 (3)	A, B, C, D, E
Eritrospermum 78	48	29 (11)	A, B, C, D, E
Omskaya 35	47	29 (6)	A, B, C, D, E
Lutescense 574	47	29 (8)	A, B, C, D, E
For a	47	33 (1)	A, B, C, D, E
Eritrospermum 727	47	29 (12)	A, B, C, D, E
Novosibirsk 15	46	29 (7)	A, B, C, D, E
Altaiskaya 50	46	28 (30)	A, B, C, D, E
Tertsia	46	29 (10)	A, B, C, D, E
Lutescense 424	45	29 (9)	A, B, C, D, E
Shortandikskaya uluchshennaya	45	31 (5)	A, B, C, D, E
GVK 1857-9	45	26 (19)	A, B, C, D, E
Lutescense 13	43	27 (15)	A, B, C, D, E
Lutescense 29-94	43	24 (22)	A, B, C, D, E
Lutescense 53-95	43	25 (21)	A, B, C, D, E
GVK 1369-2	43	26 (18)	A, B, C, D, E
Atubenka	42	24 (24)	A, B, C, D, E
Glubokovskaya	41	27 (14)	A, B, C, D, E
Bayterek	41	25 (20)	A, B, C, D, E
Lutescense 54	40	26 (17)	A, B, C, D, E
Stepnaya	40	24 (23)	A, B, C, D, E
Aktobe 32	40	23 (25)	A, B, C, D, E
Astana	39	26 (16)	A, B, C, D, E
Spring wheat mean	45	28	
LSD (0.05)	5.6	12.72	
Winter wheat			
VORONA/HD2402	43	30 (16)	F, G, H
Navruz	42	39 (1)	F, G, H
Tacika	42	34 (6)	F, G, H
Alex	41	34 (7)	F, G, H
Naz	40	29 (19)	L, M
Ormon	39	32 (11)	F, G, H
DUCULA//VEE/MYNA	39	33 (9)	F, G, H
JUP/4/CLLF/3/II14.53/ODIN// CI13431/WA00477	39	32 (12)	F, G, H
Kauz	38	35 (4)	F, G, H
Norman	38	31 (13)	F, G, H
KINACI	38	28 (21)	F, G, H
NA160/HEINE VII/BUC/3/ F59.71//GHK	38	38 (2)	F, G, H
TX71A 1039.1VI*3	38	28 (22)	F, G, H
Chakbol	37	30 (15)	F, G, H
Krasnodar 99	37	36 (3)	F, G, H
Atilla <sup>c</sup>	36	34 (5)	F, G, H
1D13.1/MLT//TUI	36	33 (10)	F, G, H
SHARK/F4105W2.1	36	30 (18)	F, G, H
7C/CNO//CAE/3/YMH/4/VP	36	27 (25)	F, G, H
MV 218-98	36	34 (8)	F, G, H
Eritrosp.750	35	25 (31)	L, M
BOCRO 4	34	30 (17)	F, G, H
CEBECO 148//CNO/TNIA//	34	31 (14)	F, G, H
NORKAN/TJB406.892/MON	33	27 (24)	F, G, H
Almaly	33	25 (30)	L, M
Adyr	32	28 (23)	I, J, K
Kazakhstan 10	32	27 (26)	L, M
Zhetisu	32	25 (32)	L, M, N

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Table 1         Continued	
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Genotype	Fe (mg kg <sup>-1</sup> ) <sup>b</sup>	Zn (mg kg <sup>-1</sup> )	Test environments <sup>a</sup>
Akdan	31	29 (20)	L, N
Tilek	30	24 (36)	I, J, K
Asyl	29	23 (38)	I, J, K
Djamin	29	24 (34)	I, J, K
Nikonia	29	27 (28)	L, N
Intensivanaya	28	26 (29)	I, J, K
Kayrak	27	24 (35)	I, J, K
Kyial	27	23 (39)	I, J, K
Mironovskaya 35	27	25 (33)	L, N
Azibrosh	26	23 (37)	I, J, K
Zubkov	26	22 (40)	I, J, K
Mambo	26	27 (27)	L, N
Swindi	25	20 (41)	I, J, K
Winter wheat mean	34	29	
LSD (0.05)	7.54	7.89	
Grand mean	38	28	

Genotypes are listed in descending order for Fe. Numbers in parentheses indicate Zn ranking

<sup>a</sup> Spring wheat environments in Kazakhstan: A = Almaty 2004, B = Kartabalyk 2004, C = Pavlodar 2004, D = Astana 2004, and E = Aktobe 2004. Winter wheat environments in Tajikistan: F = Gissar 2005, G = Isfara 2005 and H = Vakhsh 2005. Winter wheat environments in Kyrgyzstan: I = Karasu-Osh 2005, J = Sokuluk-Chu 2005, and K = Bakay-Atip-Talas 2005. Winter wheat environments in Kazakhstan: L = Uzun-Agash 2005, M = Almalibak 2005, and N = Shymkent 2005 <sup>b</sup> Environment B (Karabalyk 2004) was not included in the Fa analysis for spring wheat

<sup>b</sup> Environment B (Karabalyk 2004) was not included in the Fe analysis for spring wheat

<sup>c</sup> Spring wheat genotype Atilla performed well under autumn-sowing conditions in Tajikistan, therefore it was included with winter wheat in our study

206	the interaction $(\phi i j)$ . Thus, the mean response of
207	genotype <i>i</i> in environment <i>j</i> is modeled by

$$\hat{Y} = \mu + \alpha i + \beta j + \sum_{k=1}^{m} \lambda k \gamma i k \delta j k + \rho i j + \varepsilon i j$$

209 where  $\mu$  is the grand mean,  $\alpha i$  is the main effect of 210 the *i*th genotype,  $\beta j$  is the main effect of the *j*th 211 environment, and  $\phi i j$  is the interaction between 212 genotype *i* and environment *j*; in which  $\phi i j$  is 213 represented by

$$\sum_{k=1}^m \lambda k \gamma i k \delta j k$$

where  $\lambda k$  is the size,  $\gamma i k$  is the normalized 215 216 genotype vector of the genotype scores or sensi-217 tivities,  $\delta i k$  is the normalized environmental 218 vector of the scores describing environments,  $\rho i j$ 219 are the AMMI residuals, and *ij* is the error term. 220 All calculations were performed by IRRISTAT 221 4.3 software (International Rice Research Insti-222 tute 2003).

### **Results and discussion**

Wheat grain composition

225 Table 1 shows mean concentrations of Fe and Zn in mature grain from 66 genotypes. The amount 226 of Fe in the grain showed a large variation among 227 genotypes and ranged from 25 mg  $kg^{-1}$  to 56 mg 228  $kg^{-1}$  (mean 38 mg kg<sup>-1</sup>). As with Fe, the concen-229 tration of Zn varied among genotypes and 230 ranged from 20 mg kg<sup>-1</sup> to 39 mg kg<sup>-1</sup> (mean 231  $28 \text{ mg kg}^{-1}$ ). 232

Comparing the 12 spring and 12 winter wheat 233 genotypes with the highest iron and zinc concen-234 trations, it was clear that spring wheat genotypes 235 possessed higher grain Fe concentrations. By 236 contrast, grain Zn concentrations were higher in 237 winter wheat than spring wheat. Comparing the 238 top 12 Fe genotypes with the best 12 Zn genotypes 239 for winter wheat, there were six genotypes in 240 common (Navruz, Tacika, Alex, Ormon, NA160/ 241 HEINE VII/BUC/3/ F59.71//GHK and JUP/4/ 242 CLLF/3/II14.53/ODIN//CI13431/WA00477). For 243



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244 spring wheat, 11 genotypes were among the top 12 for high Fe and high Zn concentrations. 245 246 Table 2 and Fig. 2A show that the concentration 247 of Fe and Zn in the grain of spring wheat cultivars were strongly and positively corre-248 lated [Fe = (17.2011) + (0.9917) Zn,  $R^2 = 0.6335$ ; 249 250  $P = \langle 0.001 ]$ . A strong correlation between grain 251 Zn and Fe concentrations occurred in germplasm 252 containing both wild wheat (Cakmak et al. 2004) 253 and cultivated wheats (Peterson et al. 1986). In 254 winter wheat this association was equally strong 255 [Table 3 and Fig. 2B; Fe = (8.5300) + (0.8855)256 Zn,  $R^2 = 0.6270$ ; P < 0.001]. The relationship between Fe and Zn was not so strong for the 257 258 combined spring and winter wheat data [Fig. 2C; Fe = (16.8126) + (0.7452) $R^2 = 0.1856;$ 259 Zn, 260 P < 0.001]. Considering independently spring and winter wheat, our findings support other 261 262 findings, that it is possible to combine high-iron 263 and high-zinc traits during breeding (Monasterio and Graham 2000; Cakmak et al. 2004). 264

265 Table 2 shows the Pearson correlations 266 between Fe, Zn and other nine agronomic and 267 grain quality traits for spring wheat. A strong 268 positive significant correlation was found between 269 Fe and grain protein content (r = 0.65). Strong negative significant correlations occurred be-270 271 tween Fe and plant height (r = -0.6), and Fe and glutenin content (r = -0.48), indicating that 272 273 shorter plants with lower glutenin content favor higher grain-Fe concentration. Weak but 274

Table 2 Pearson correlation coefficients among grain iron
content (Fe), grain zinc content (Zn), grain yield, grain
protein content, glutenin content, gliadin content, days to
heading, plant height, thousand-grain weight (1000-K),

significant negative correlations between Fe and 275 grain yield (r = -0.41), and Fe and grain number 276 per  $m^2$  (r = -0.38), confirmed that modern culti-277 vars with high grain yield and grain yield compo-278 nent traits tend to have lower concentrations of 279 micronutrients in the grain. Similar correlation 280 coefficients were found between Zn and other 281 traits (Table 2), but the negative correlations 282 between Zn and yield (r = -0.64), and Zn and 283 grain number per  $m^2$  (r = -0.55) were stronger 284 than those observed for Fe. Another slight 285 difference was the positive significant correlation 286 between Zn and gliadin content (r = 0.44). For 287 Zn, the strongest correlation was with protein 288  $(r = 0.68^{***})$ . A very strong correlation between 289 grain Zn and grain protein was also shown 290 previously (Peterson et al. 1986; Feil and Fossati 291 1995), indicating that grain protein may be a sink 292 for Zn. In agreement with these results, Distelfeld 293 294 et al. (2006) recently showed that a locus (e.g., Gpc-B1 affecting grain protein concentration) on 295 the short arm of chromosome 6B in wheat was 296 also effective in increasing accumulation of Zn 297 and Fe in grain. In wheat seed, Zn is predom-298 inantly localized in the embryo and aleurone 299 layer (up to 150 mg per kg seed) whereas endo-300 sperm contains much less (around 15 mg Zn per 301 kg seed) (Ozturk et al. 2006). The embryo and 302 aleurone are rich in protein, supporting the 303 suggestion that high protein in seed represents 304 an important sink for Zn. This association 305

grain number per  $m^2$  (KNO), and test weight of 25 spring wheat genotypes grown across locations in Kazakhstan in 2004

	Zn	Yield	Grain protein	Glutenin	Gliadin	Days to heading	Height	1000- K	KNO	Test weight
Fe Zn Yield Grain Protein Glutenin Gliadin Days to Heading Height 1000-K KNO	0.79***	-0.41* -0.64***	0.65*** 0.68*** -0.46*	-0.48** -0.51** 0.32 -0.85***	0.34 0.44* -0.18 0.29 -0.20	0.05 -0.13 0.53** -0.11 -0.03 0.02	-0.60*** -0.62*** 0.73*** -0.61*** 0.51** -0.06 0.57**	$\begin{array}{c} 0.05\\ 0.03\\ -0.04\\ -0.35\\ 0.45^{*}\\ 0.03\\ 0.18\\ 0.10\\ \end{array}$	-0.38* -0.55** 0.87*** -0.22 0.04 -0.17 0.37 0.57** -0.52**	$\begin{array}{c} -0.26 \\ -0.37 \\ 0.65^{***} \\ -0.50^{**} \\ 0.51^{**} \\ -0.10 \\ 0.57^{**} \\ 0.72^{***} \\ 0.15 \\ 0.46^{*} \end{array}$

\*Significant at P = 0.05; \*\*significant at P = 0.01; \*\*\*significant at P = 0.001

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Fig. 2 Linear regressions: (a) Fe vs. Zn for 25 spring genotypes, (b) Fe vs. Zn for 42 winter wheat genotypes, and (c) Fe vs. Zn for the combined spring-winter wheat genotypes

between Zn and protein should be considered in
breeding programs aimed at improving cereal
grains for Zn and Fe contents.

For winter wheat, only data on micro and 309 macro-nutrient concentrations in grain were 310 available for all trials. Significant positive corre-311 312 lation coefficients were found between Fe and Zn, 313 S, and Mg; between Zn and S; and between 314 Mn, Mg and P. An important point was the 315 negative correlation between P and both Fe 316 and Zn (r = -0.18 and r = -0.11, respectively

**Table 3** Pearson correlation coefficients among grain iron content (Fe), grain zinc content (Zn), grain manganese content (Mn), grain magnesium content (Mg), grain phosphorous content (P), and grain sulfur content of 42 winter wheat genotypes across locations in central Asia in 2005

	Zn	Mn	Mg	Р	S
Fe Zn Mn Mg P	0.79***	-0.46*** -0.46***	0.29* 0.16 0.31*	-0.18 -0.11 0.59*** 0.50***	0.67*** 0.71*** 0.05 0.47*** 0.04

\*Significant at P = 0.05; \*\*significant at P = 0.01; \*\*\*significant at P = 0.001

(Table 3, Fig. 3C). The contents of P in winter 317 wheat analyzed ranged from 2627 mg kg<sup>-1</sup> to 318  $3694 \text{ mg kg}^{-1}$  (mean  $3177 \text{ mg kg}^{-1}$ ). Approxi-319 mately 75% of total P in wheat grain is stored 320 as phytic acid, particularly in the germ and 321 aleurone layers (Lott and Spitzer 1980; Raboy 322 2000). At physiological pH, phytic acid is a poly-323 anion, with each molecule containing six to eight 324 negative charges distributed among six phosphate 325 esters. This relatively small molecule with a high 326 charge density is a strong chelator of positively 327 charged mineral cations such as calcium, iron and 328 zinc (Raboy 2000). In terms of human health and 329 nutrition, dietary phytate can have both negative 330 and positive outcomes. It can contribute to 331 mineral depletion and deficiency in populations 332 that rely on whole grains and legume-based 333 products as staple foods; however, phytic acid 334 can also function as an antioxidant and anticancer 335 agent and may have other beneficial effects on 336 health (Cakmak et al. 2002; Welch and Graham 337 2004). 338

Welch and Graham (2004) highlighted the 339 importance of promoters, mostly organic acids 340 and S-containing amino acids, for the bioavail-341 ability of Zn. Biologically, increasing the content 342 of promoters which serve as catalysts, is an 343 attractive option to increase Zn bioavailability, 344 because marginal increases are likely to have 345 large effects. Minor changes in the promoter 346 content are unlikely to have negative effects on 347 the food quality. This is in contrast to selecting for 348 a lower anti-nutrient content, which may have 349 negative effects on food quality due to potential 350 anti-carcinogenic and anti-mutant functions. 351

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Fig. 3 Linear regressions of 42 winter wheat genotypes: (a) Fe vs. Mn, (b) Fe vs. Mg, (c) Fe vs. P, and (d) Fe vs. S

In this study, Fe-grain and S-grain, and Zn-352 353 grain and S-grain, were positively and signifi-354 cantly correlated, suggesting a possible positive correlation between high grain micronutrients 355 356 and high S-containing amino acid concentrations 357 in grain. From the best 12 genotypes with S-grain content ranging from  $1140 \text{ mg kg}^{-1}$  to 358 1558 mg kg<sup>-1</sup>, seven were among the best 12 359 Fe-grain genotypes (Navruz, Naz, DUCULA// 360 VEE/MYNA, NA160/HEINEVII/BUC/3/F59.71 361 362 //GHK, Norman, JUP/4/CLLF/3/II14.53/ODIN// 363 CI13431/WA00477, and Tacika), and nine were within the top 12 Zn-grain genotypes (Navruz, 364 365 NA160/HEINEVII/BUC/3/F59.71//GHK, Kauz, 366 DUCULA//VEE/MYNA, JUP/4/CLLF/3/II14.53 /ODIN// CI13431/WA00477, Atilla, Krasnodar 367 99, MV 218-98 and Tacika). Five genotypes with 368 369 high S-grain concentration were among both high

Fe-grain and high Zn-grain groups. Thus, the370development of new winter wheat genotypes with371higher grain Fe and grain Zn concentrations and372promoters that affect both Fe and Zn bioavail-373ability appears feasible.374

# Genotype $\times$ environment interactions 375

The AMMI analysis of variance of Fe and Zn 376 grain concentrations (mg kg<sup>-1</sup>) carried out inde-377 pendently for each trial and presented in Tables 4 378 379 and 5, show the relative magnitudes of the genotype (G), location (L), and genotype  $\times$  loca-380 tion (GL) variance terms. Generally, the expres-381 sions of Fe and Zn levels were controlled to a 382 383 large extent by location (especially true for Zn). However, for Fe in spring wheat, and in winter 384 wheat in Kazakhstan (trial 1), genotype (G) was 385

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Source	df	SS	MS	Explained (%)
Spring wheat, Kazakhstan				
Genotypes	24	1213.09	50.55	50.53
Locations	3	758.53	252.84	31.59
Genotypes $\times$ location	72	428.91	5.96	17.86
Total	99	2400.53		
Winter wheat, Tajikistan				
Genotypes	21	405.98	19.33	22.72
Locations	2	752.38	376.19	42.11
Genotypes $\times$ location	42	628.21	14.95	35.16
Total	65	1786.58		
Winter wheat, Kyrgyzstan				
Genotypes	9	124.43	13.82	22.23
Locations	2	283.08	141.54	50.58
Genotypes $\times$ location	18	152.11	8.45	27.18
Total	29	559.62		
Winter wheat, Kazakhstan				
Genotypes <sup>a</sup>	4	83.26	20.81	51.31
Locations	1	16.03	16.03	9.88
Genotypes $\times$ location	4	62.96	15.74	38.80
Total	9	162.27		
Genotypes <sup>b</sup>	4	34.73	8.68	22.83
Locations	1	43.37	43.37	28.51
Genotypes $\times$ location	4	73.98	18.49	48.64
Total	9	152.10		

**Table 4** Additive main effects analysis of variance from the AMMI model for grain iron density (mg kg<sup>-1</sup>) of the genotypes for independent trials

<sup>a</sup> Trial 1: locations Uzun-Agash and Almalibak; and genotypes Almaly, Naz, Kazakhstan 10, Eritrosp.750, and Zhetisu

<sup>b</sup> Trial 2: locations Uzun-Agash and Shymkent; and genotypes Zhetisu, Akdan, Mambo, Nikonia, and Mironovskaya 35

386the most important source of grain Fe concentra-387tion accounting for over 50% of the G + L + GL.388For all trials, the grain Fe- genotype effect was389never less than 22% indicating that genotype was390an important contributor to overall variability.

In contrary to Fe, in the AMMI analysis of 391 variance of grain Zn concentrations, genotype 392 393 (G) was the most important source of variation only in Tajikistan, accounting for 35.13% of the 394 395 G + L + GL. For the other trials (with the 396 exception of Kyrgyzstan where G accounted for 397 about 32%) the genotypic effect was minor, 398 explaining 4-9% of the G + L + GL variation. 399 The genotype × location effect (GL) was impor-400 tant for both Fe and Zn. For Fe, GL ranged from 17.6% to 48.64% across the trials, and for Zn, 401 402 from about 7.3% to 48.77%. This implies that for both Fe and Zn, the rankings of winter wheat 403 genotypes in Tajikistan and Kazakhstan were 404 influenced by location. 405

# Conclusions

There were strong positive correlations between 407 the Fe and Zn grain concentrations for both 408 spring and winter materials. For spring wheat, 409 positive correlations between grain Fe and grain 410 Zn concentrations and grain protein content 411 indicated that breeding and selection for one of 412 these traits could simultaneously improve the 413 others. Negative correlations between the micro-414 nutrient concentrations, plant height and grain 415 yield does not necessarily imply that a strategy for 416 reducing plant height could produce gains in grain 417 yield and grain element concentrations. The 418 observed negative correlations between grain 419 element concentrations, plant height and grain 420 yield might be at least partially explained in that 421 shorter and lower yielding genotypes have a lower 422 dilution effect of minerals in the grain, and thus 423 express higher grain Fe and Zn concentrations. 424



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Source	df	SS	MS	Explained (%)
Spring wheat, Kazakhstan				
Genotypes	24	976.58	40.69	8.67
Locations	4	9443.27	2360.82	83.88
Genotypes $\times$ location	96	836.96	8.72	7.43
Total	124	11256.80		
Winter wheat, Tajikistan				
Genotypes	21	703.49	33.49	35.13
Locations	2	636.51	318.25	31.79
Genotypes $\times$ location	42	662.51	15.77	33.08
Total	65	2002.52		
Winter wheat, Kyrgyzstan				
Genotypes	9	125.40	13.93	32.59
Locations	2	162.50	81.25	42.24
Genotypes $\times$ location	18	96.80	5.38	25.16
Total	29	384.69		
Winter wheat, Kazakhstan				
Genotypes <sup>a</sup>	4	25.80	6.45	8.58
Locations	1	128.28	128.28	42.65
Genotypes xlocation	4	146.69	36.67	48.77
Total	9	300.77		
Genotypes <sup>b</sup>	4	15.88	3.97	4.68
Locations	1	209.56	209.56	61.73
Genotypes × location	4	114.01	28.50	33.59
Total	9	339.46		

**Table 5** Additive main effects analysis of variance from the AMMI model for grain zinc density (mg kg<sup>-1</sup>) of the genotypes for independent trials

<sup>a</sup> Trial 1: locations Uzun-Agash and Almalibak; and genotypes Almaly, Naz, Kazakhstan 10, Eritrosp.750, and Zhetisu

<sup>b</sup> Trial 2: locations Uzun-Agash and Shymkent; and genotypes Zhetisu, Akdan, Mambo, Nikonia, and Mironovskaya 35

However, some genotypes with optimum plant
height and above average Fe and Zn (Lutescens
574 and Eritrospermum 78) were found.

In the winter wheats, the strong positive corre-429 430 lations among grain Fe, grain Zn and grain S 431 together with high concentrations of each (Navruz, 432 NA160/HEINEVII/BUC/3/F59.71//GHK, Tacika, DUCULA//VEE/MYNA, and JUP/4/CLLF/3/ 433 II14.53/ODIN//CI13431/WA00477) should be 434 435 important for the development of new breeding populations targeting the enhancement of Fe and 436 437 Zn bioavailability by increasing the concentration 438 of promoters such as S-containing amino acids 439 (i.e., methionine, histidine, and lysine).

Breeding for increased grain yield may simultaneously increase grain element concentration
and could take three approaches: (i) to identify
lines with improved ability to allocate mineral
nutrients into the grain without changes in root
uptake of nutrients, (ii) to select lines with greater
ability for root uptake of mineral nutrients, whilst

maintaining current high efficiencies of partition-447 ing to the grain, and (iii) to identify lines that 448 have both features (Calderini and Ortiz-Monas-449 terio 2003). Regarding genotype  $\times$  environment 450 interactions grain Fe concentration was to an 451 important extent, controlled by genotype effects, 452 whereas grain Zn concentration was almost 453 454 totally dependent on location. Thus, genotypes having a greater genetic ability for root uptake of 455 Fe and Zn (CIMMYT 2005) could be important 456 sources of germplasm for increasing micronutri-457 ent concentration in Central Asian wheats. 458

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