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Investigation in qualitative characteristics of breed wheat cultivars using biochemical-molecular markers and micronutrients

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Key words: Bakery property, Gluten, HMW-GS, Micronutrient, LMW-GS

Abstract

This experiment was aimed to investigate the genetic diversity of high and low molecular weight glutenin subunits along with Fe and Zn concentrations in grains of 17 wheat cultivars/lines. For this, Total grain protein was extracted and electrophoresis of HMW-GS was carried out using SDS-PAGE on poly acrylamide gels. Specific primers were selected for LMW-GS and glidine subunits and PCR products were separated on agarose gels. Grain samples were analyzed for zinc and iron using atomic absorption spectroscopy. The most common composition of HMW-GS subunits was (null, 7+8, 2+12). Also, compositions (2*, 7+8, 5+10), (1, 7+8/6+8, 5+10) and (null, 17+18, 5+10) that give the maximum quality grade 10, 10/8 and 8 to the genotypes possessing them, were observed in Sasyon, Gaspard, and LineA, respectively. Maximum LMW-GS and gliadin alleles number, 11, 10, 10, 10, 9, 9, 9, that give highly potential quality to the genotypes possessing them, were observed in Niknejad, Sasyon, Shahpasand, Bayat, Shahriyar, Sorkhtokhm and Darya, respectively. Among studied genotypes, the concentrations of grain Fe varied by 1.80 fold, ranging from 25.28 (Sorkhtokhm) to 45.62 (LineA) $\mu g.g^{-1}$, and grain Zn varied by 2.49 fold, from 28.85 (Sepahan) to 72.04 (Alamut) $\mu g.g^{-1}$. Also, highly positive correlation was observed between grains Fe with Zn. In conclusion Niknejad, Sasyon and LineA with high potential of the bakery property and iron and zinc contents altogether were introduced as appropriate candidate parents in future wheat breeding programs.

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Introduction

Wheat is one of the most important cereals and a major source of energy, protein, and dietary fiber in human nutrition. Wheat is consumed in many different forms such as bread, macaroni, spaghetti and cakes. The flour ability of wheat to be processed into different foods is largely determined by the gluten proteins (Weegels *et al*., 1996). Mature wheat grains contain 8-20% proteins.

The gluten constitutes up to 80-85% of total flour protein, and confers properties of extensibility that are essential for functionality of wheat flours (Shewry *et al*., 1995). Glutenins and gliadins, constitute each around 50% of the gluten proteins and determine the quality of various enduse products of wheat. Glutenin subunits base on their differential mobility on SDS-PAGE are divided into high molecular weight glutenin subunits (HMW-GS) and Low molecular weight glutenin subunits (LMW-GS) (Singh and Shepherd, 1988; Gupta and Shepherd, 1990). Gliadin subunits loci control the synthesis of a group of proteins, which they further were separated into three types: α/β-gliadin, γ-gliadin and ω-gliadin (Payne, 1987; Kawaura *et al*., 2005).

Many studies have been conducted in assessing the role of HMW-GS in imparting dough strength and bread making quality (Luo *et al*., 2001; Liu *et al*., 2005; Figueroa *et al*., 2009) and the additively or combined role of LMW-GS in improving bread making quality (Gupta *et al*., 1989). Based on the composition of HMW-GS and LMW-GS alleles, models were developed for predicting dough strength and extensibility (Cornish *et al*., 2006). However, inconsistent results obtained for the nomenclature of Glu-3 alleles across the various experiments made it more complicated the assessment of the role of individual LMW-GS alleles and their interactive effects with HMW glutenins (Ikeda *et al*., 2008). This has been primarily due to the complexity in LMW-GS allele's large number and overlapping mobility with the gliadins on SDS-PAGE (Igrejas *et al*., 1999). Genetic studies of HMW-GS (Payne *et al*., 1987) reported subunits with both positive (5+10) and negative (2+12) effects on bread making quality. In general, a null at Glu-A1 locus, subunit 6+8 at Glu-B1and 2+12 at Glu-D1are negatively related with the quality parameters (Weegels *et al*., 1996). Many breeders (Igregas *et al*., 1999; Nakamura, 2001) have used the HMW-GS composition and released lines/cultivars in relation with end-use quality and a screening test to ensure that good bread making alleles (1, 2*, 5+10, 7+8, 7+9) are incorporated in new lines/cultivars (Lukow, 1991). many studies have been performed on the gliadin and glutenin variation of different *Triticum* species, e.g., *T. urartu* (Caballero *et al*., 2008; Ahmadi and Pour-Aboughadareh, 2015; Cuesta *et al*., 2017), *T. monococcum* (An *et al*., 2006) and *T. boeoticum* (Ahmadi and Pour-Aboughadareh, 2015).

The availability of appropriate mineral nutrients in the human diet depends on mineral nutrient concentration in staple food crops such as cereal grains (Cakmak *et al*., 2010). The important mineral elements lacking in human diets are iron (Fe) and zinc (Zn) (Stein, 2010).

The widespread deficiencies of Fe and Zn in developing countries are mostly due to monotonous consumption of cereal foods with low content and reduced bioavailability of Fe and Zn (Graham *et al*., 2001). Therefore, biofortification of cereals is considered the most promising and cost-effective approach to combat malnutrition and related health problems (Peleg *et al*., 2009; Saltzman *et al*., 2013).

Genetic diversity of grain micronutrients in adapted genetic materials is the basic requirement for biofortification breeding programs, and thus needs to be assessed beforehand. Several studies have reported the existence of a large variation in grain micronutrients in wheat (Morgounov *et al*., 2007; Peleg *et al*., 2008; Ficco *et al*., 2009; Chatzav *et al*., 2010; Velu *et al*., 2011; Zhao *et al*., 2011; Harmankaya *et al*., 2012; Badakhshan *et al*., 2013). Improvement of wheat grains in terms of dough properties and

bread making quality along with highly iron and zinc micronutrients is an important wheat breeding program around the world, especially in Asian countries. To this end, this experiment was aimed to investigate the genetic diversity of high and low molecular weight glutenin subunits along with Fe and Zn concentrations in grains of wheat cultivars/lines.

Materials and methods

Protein isolation and SDS-PAGE for HMW-GS

High and Low molecular weight glutenin subunits were assessed in 17 Iranian wheat cultivars/lines prepared from Iranian Seed and Plant Improvement Institute. Total grain protein was extracted and electrophoresis of HMW glutenin subunits was carried out using SDS-PAGE on 10% poly acrylamide gels following Payne *et al*., (1981) method. Gels were stained by Commassie Brilliant Blue R-250 in the presence of Tri-chloroacetic acid (TCAA) and ethanol, over night. Gels were destained by a 3% NaCl solution. To separate subunits 2* and 2 which normally overlap in 10% SDS-PAGE gels, in genotypes carrying 2+12 subunits, 7.5% poly acrylamide gels were employed.

Primers designing for LMW-GS

Specific primers for Low molecular weight glutenin and glidine subunits were selected according to Benmoussa *et al.* (2000), Long *et al.* (2005), Tanaka *et al.* (2005), Ikeda *et al.* (2002), Masoudi-Nejad *et al.* (2002), Anderson *et al.* (2009), Von Buren (2001) reaches results (Table 1).

DNA isolation and PCR amplification for LMW-GS

Genomic DNA was isolated from fresh leaf tissues using a CTAB modified method as reported by Doyle and Doyle (1987). Polymerase chain reactions (PCR) were run in a volume of 20 µl containing 1X PCR buffer, 50 ng sample DNA, 0.2 µM of each primer, 200 µM of each dNTPs, 2 mM MgCl2 and 1 unit of Taq DNA polymerase. PCR program was started with first denaturing at 95°C for 5 min followed by 35 cycles including: denaturing at 94°C for 1 min, annealing at 52-68°C (depending on primer annealing temperatures) for 1 min, extension at 72°C for 1 min and the final extension at 72°C for 5 minutes. The PCR products were separated on 1.6% agarose gels.

Determination of grains Fe and Zn concentration

Grain samples were digested in a mixture of Nitric acid 0.1 N and Citric acid 10%. Digested samples were analyzed for zinc and iron using atomic absorption spectroscopy. Grain micronutrients (zinc, Zn; iron, Fe) were determined by Gupta (2000) method. Mineral concentrations were expressed as µg/g dry weight. Appropriate quality controls were performed for each set of measurements.

Statistical analysis

Identification of HMW-GS was according to Payne and Lawernce (1983). The LMW-GS alleles scored by presence or absence of each single glutenin subunits as one and zero respectively. Correlation between Fe and Zn contents was established by the Pearson correlation coefficient. Statistical calculations were performed using SPSS V15.0 software.

Results and discussion

Surveying allelic diversity of HMW glutenin subunits

Among 17 studied breed wheat, 11 different HMW subunits were identified, three of which were related to *Glu-A1*, five to *Glu-B1* and three were related to *Glu-D1*. Null allele of *Glu-A1* locus was observed in 82.4% of cultivars.

Also the frequency of allele 2* was 11.8% in this genetic locus (Table 2). In *Glu-B1* locus, subunits 7+8, 7+9, 17+18, 13+16 and 7+8/6+8 were observed. Each of subunits 7+8 and 7+9 were present in 35.3% of cultivars. We found the most abundant alleles for subunits 7+8 and 7+9 with 35.3 and 35.3% frequency, respectively (Table 2).

Concurrently, diversities of subunits 2+12 and 5+10, encoded by Glu-D1 locus, were 58.8 and 35.3%, respectively. According to Morgunov *et al.* (1993) studies, subunit 5+10 is the most valuable subunit for

flour quality and bakery. Its desirability in comparison with subunit 2+12 is due to excessive cysteine amino acid in subunit 5 in comparison with subunit 2. As a result it would create opposite peptide chains reactions and increase dough strength (Shewry *et al.*, 1997). Interestingly, the subunit 2.1+10* encoded by *Glu-D1* locus was reported as a new allele with a very low diversity in Pakistan native varieties (Tahir *et al.*, 1994), this allele was also observed in one cultivar (5.9%) in this study. Bahraei *et al.* (2004) has also reported a high diversity of this subunit in Iranian landraces grown in Sistan and Balouchestan province.

Table 1. Specific primers for low molecular weight glutenin subunits designed according to wheat genome.

Gene locus	Primer sequence (5'-3')		Annealing Tm (°C)
LMWG-1D1	ATGAAGACCTTCCTCGTCTTTGC TCAGTAGGCACCAACTCCGGTGC	Benmoussa *et al.*, 2000	68
Glu3D.3	ATGGAGACTAGATGCATCCCT AGATTGGATGGAACCCTGAAC	Long *et al.*, 2005	60
Glu3D.4	ATGGAGACTAGCTGCATCT CTGCAAAAAGGTACCCTGTA	Long *et al.*, 2005	52
Glu-D3	CCACATCCCTAGCTTGGAGAA ATGGTATTTGTTGTTGCGGA	Tanaka *et al.*, 2005	61
Glu-D3	CGTCTTGCTAGGTCGCAAATG CAGATTGACATCCACACAATGCC	Ikeda *et al.*, 2002	52
ωgli1	TTCCATTATCCAAACTTCAA GAACCCTCCATTGACTAAAC	Masoudi-Nejad *et al.*, 2002	64
ωgli2	AGATCGCCACTGCCGCTAGTG CATTGGCCACCGATGCTTGTAAG	Anderson *et al.*, 2009	67
γgli1	ACAAT GGCCA CAACA ACAAC TGCCC TGRCC CTGGR C	Von Buren, 2001	68
γgli2	GCAAC CACAA CAACA ATTTT CT GATAT AGTGG CAGCA GGATA TG	Von Buren, 2001	67

Table 2. Allelic combination and genotypic scores based on glutenin high molecular weight subunits for 17 breed wheat cultivars/lines.

Genotype name	Glu-A1	Glu-B1	Glu-D1	Allelic score	Genotypic Score
Moghan1	null	7+8	2+12	1+3+2	6
Bayat	null	17+18	2+12	1+3+2	6
Bezostaya	null	7+9	5+10	1+2+4	7
Sepahan	null	17+18	2+12	1+3+2	6
Marvdasht	null	7+8	2+12	1+3+2	6
DN-11	null	7+9	5+10	1+2+4	7
Darya	2*	7+9	2+12	3+2+2	7
LineA	null	17+18	5+10	1+3+4	8
Sorkhtokhm	null	7+8	2.1+10*	1+3+nd	-
Gaspard	1	7+8/6+8	5+10	3+3/1+4	10 / 8
Rushan	null	7+8	2+12	1+3+2	6
Sasyon	2*	7+8	5+10	3+3+4	10
Sahriyar	null	7+9	2+12	1+2+2	5
Shahpasand	null	7+8	2+12	1+3+2	6
Alamut	null	7+9	2+12	1+2+2	5
Niknejad	null	7+9	5+10	1+2+4	7
Zagros	null	13+16	2+12	1+3+2	6

Among 17cultivars, the most common composition of HMW glutenin subunits was null, 7+8, 2+12 and observed in four cultivars (Table 3). Also, compositions (2*, 7+8, 5+10), (1, 7+8/6+8, 5+10) and (null, 17+18, 5+10) that give the maximum grade 10, 10/8, 8 quality to the genotypes possessing them, were observed in Sasyon, Gaspard, and LineA cultivars, respectively. More importantly, subunit 7+8 which has the great effect on dough expansion was observed in Moghan1, Marvdasht, Sorkhtokhm, Rushan, Sasyon and Shahpasand cultivars, while subunit 6+8 that is the glutenin low quality indicator (Payne *et al.*, 1984) was only observed in Gaspard.

Table 3. High molecular weight glutenin subunits (HMW-GS) and their composition patterns in 17 breed wheat cultivars/lines.

Locus	subunit	Number of genotypes	Frequency %	Subunit composition	Number of genotypes	Frequency %
Glu- A1	null	14	82.4	null, 7+9, 2+12	2	11.8
	2*	2	11.8	null, 7+8, 2+12	4	23.5
	1	1	5.9	null, 17+18,2+12	2	11.8
				2+122.1+10*		
				null, 17+18,5+10	1	5.9
Glu- B1	7+8	6	35.3	2*, 7+8, 5+10	1	5.9
	7+9	6	35.3	null, 7+8, 2.1+10*	1	5.9
	6+8	1	5.9	null, 13+16, 2+12	1	5.9
	13+16	1	5.9	2*, 7+9, 2+12	1	5.9
	17+18	3	17.6	1, 7+8/6+8, 5+10	1	5.9
Glu- D1	2+12	10	58.8	null, 7+9, 5+10	3	17.6
	5+10	6	35.3	null, 20, 5+10	1	5.9
	2.1+10*	1	5.9			

Table 4. Allelic combination and genotypic scores based on glutenin low molecular weight allels for 17 breed wheat cultivars/lines.

Genotype name	Glu3D.4	Glu3D.3	GluD3		GluD3	LMWG-1D1	ωgli1	ωgli2	γgli1			γgli2	Genotypic Score
	Gene allele												
	a	b	c_1	c_2	d	e	f	g	h_1	h_2	h_3	i	
Moghan1	+	+	+	+	+	+	o	o	o	o	+	+	8
Bayat	+	o	+	o	+	+	+	+	+	+	+	+	10
Bezostaya	o	o	o	o	o	o	+	o	+	+	+	+	5
Sepahan	+	o	+	o	+	+	oo	+	o	o	+	+	7
Marvdasht	o	+	o	o	o	+	o	o	o	o	+	+	4
DN-11	+	o	+	o	+	+	o	+	+	o	+	+	8
Darya	+	+	+	o	+	+	+	+	o	o	+	+	9
LineA	o	+	+	o	+	+	o	+	o	o	o	+	6
Sorkhtokhm	+	+	+	o	+	+	o	o	+	+	+	+	9
Gaspard	o	+	o	o	+	o	+	o	o	o	o	+	4
Rushan	+	o	+	o	o	+	o	o	o	o	o	+	4
Sasyon	+	+	+	o	+	+	+	o	+	+	+	+	10
Sahriyar	+	+	+	o	+	+	o	o	+	+	+	+	9
Shahpasand	+	o	+	+	+	+	+	o	+	+	+	+	10
Alamut	+	+	o	o	+	+	+	o	o	o	o	+	6
Niknejad	+	+	+	o	+	+	+	+	+	+	+	+	11
Zagros	+	o	+	o	+	+	o	o	o	o	+	+	6

Glutenin and gliadin alleles detected using locus-specific markers

The different alleles detected at the loci analyzed and their frequency was indicated in Tables 4 and 5. A total of 12 alleles were detected for the Glu3D, GluD3, ωgli and γgli. For Glu3D.4, one allele was detected by the Glu3D.4 specific primer pairs. The size of amplified segment was 700bp and observed in 13 genotypes (Table 4).

Based on Glu3D.3, one allele was detected across 10 cultivars sized 600bp. Furthermore, locus-specific marker GluD3 amplified two fragments (alleles a and b) sized 479 and 300bp with one fragments recognized as new allele, which had the abundances of 76.4 and 11.8%, respectively (Table 5).

Table 5. The frequencies of low molecular weight glutenin subunit (LMW-GS) gene alleles in 17 breed wheat cultivars/lines.

Primer	Allele	Allele size	Number of genotypes	Frequency %
Glu3D.4	a	700	13	76.4
Glu3D.3	a	600	10	58.42
GluD3	a	479	13	76.4
	b	300	2	11.8
GluD3	a	600	15	88.2
LMWG-1D1	a	915	14	82.3
ωgli1	a	1419	8	47
ωgli2	a	1135	6	35.3
γgli1	a	>800	13	76.4
	b	780	7	41.2
	c	650	8	47
γgli2	a	800	17	100

Based on Glu3D.3, one allele was detected across 14 cultivars sized 600 bp. For LMWG-1D1, one allele sized 915 bp was detected by its specific primer pairs and observed in 15 cultivars. At ωgli1 and ωgli2 loci, two allelic variants with the sizes 1419 and 1135 bp were observed, respectively. At γgli1 locus, three allelic variants were discovered, which its alleles ranged between 650 to >800bp (Tables 4 and 5). For γgli2, one allele was detected by its specific primer pairs sized 800bp.

The maximum LMW-GS and gliadin allele compositions that give the maximum quality grade (11, 10, 10, 10, 9, 9, 9) to the genotypes possessing them, were observed in Niknejad, Sasyon, Shahpasand, Bayat, Shahriyar, Sorkhtokhm and Darya, respectively. Based on our results, all glutenin and gliadin loci analyzed were polymorphic, indicating presence of genetic diversity between cultivars.

Similar results were reported by Moghaddam *et al.* (2000) and Ahmadi and Pour-Aboughadareh (2015) in a group of Iranian landraces and wild wheat accessions and by Aguiriano *et al.* (2006) in Spanish durum wheat landraces.

Genetic diversity for grain micronutrients

The concentrations of two micronutrients (Fe and Zn) were determined in wheat genotypes on grain dry weight bases and the concentrations are presented on the Fig. 1.

Among the 17 studied cultivars/lines of bread wheat, the concentration of grain iron (Fe) varied by 1.80 fold, ranging from 25.28 (Sorkhtokhm) to 45.62 (LineA) $\mu g.g^{-1}$, and the concentration of grain zinc (Zn) by 2.49 fold, from 28.85 (Sepahan) to 72.04 (Alamut) $\mu g.g^{-1}$ (Fig. 1).

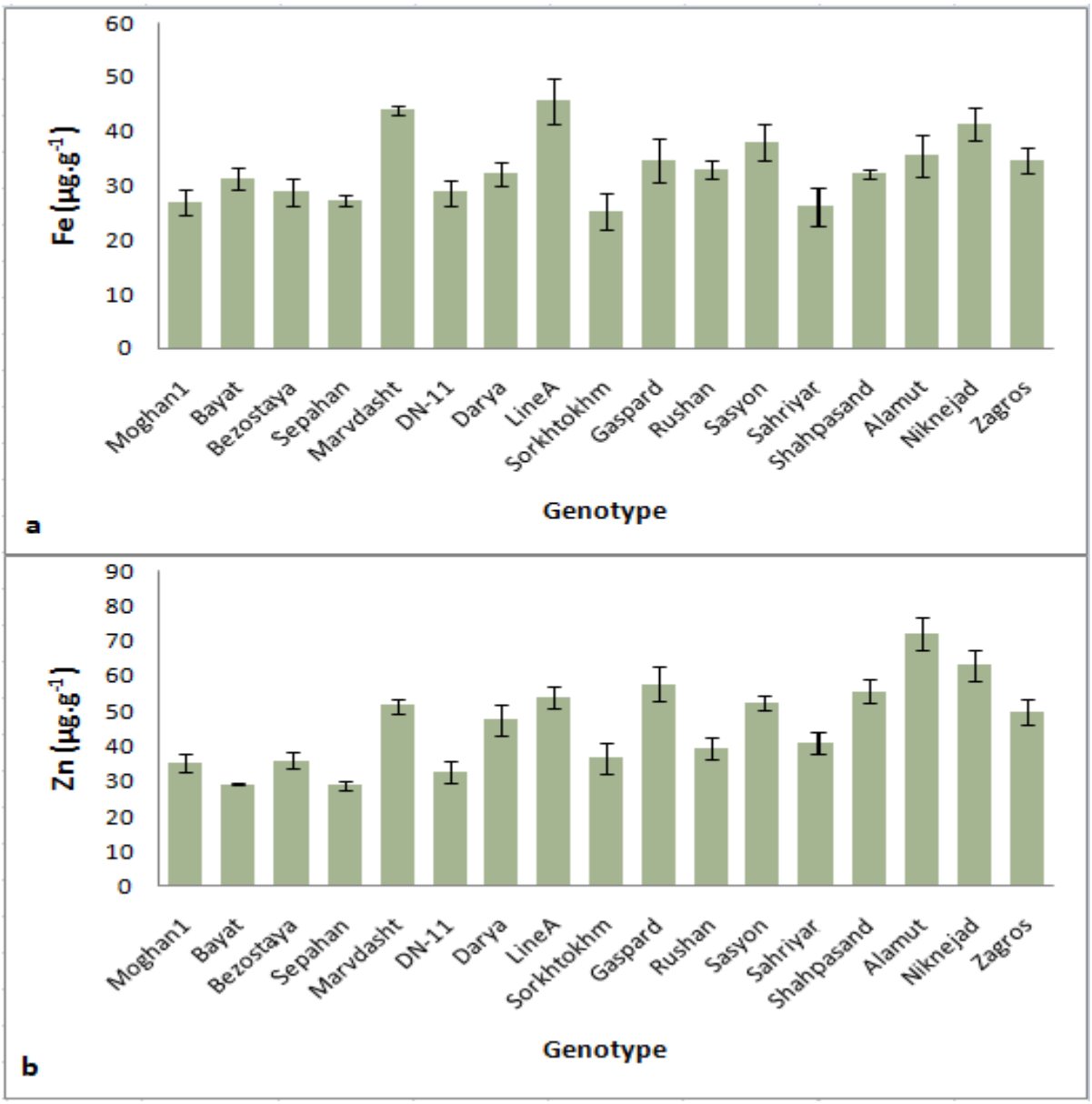

Fig. 1. Micronutrient concentrations (mean±S.E) of a) iron (Fe), b) zinc (Zn) in 17 wheat cultivars/lines grains evaluated in this research.

The range of Fe and Zn content for bread wheat determined in our study was in accordance to that reported by previous studies with 27.3–41.9 and 16.1–27.2 µg.g^{-1} (Oury *et al.*, 2006), 28.8–50.8 and 13.5–34.5 µg.g^{-1} (Zhao *et al.*, 2009), 21.3–30.6 and 14.9–19.3 µg.g^{-1} (Rawat *et al.*, 2009), 24.2–43.1 and 10.4–38.2 µg.g^{-1} (Harmankaya *et al.*, 2012), respectively. Mean comparison using t-student tests (mean±S.E) showed highly significant differences between wheat cultivars for grains Fe and Zn concentrations (Fig. 1).

Previous studies on wheat landraces have demonstrated considerable variation in grain Zn and Fe contents (Genc *et al.*, 2005; Gomez-Becerra *et al.*, 2010a, b).

It has been suggested that in order to have a measureable biological impact on human health, grain concentrations of Zn and Fe should be increased (Cakmak, 2008; Chatzav *et al.*, 2010). The targets for Fe and Zn biofortification in wheat grains are around 40 and 60 µg.g^{-1}, respectively (Ortiz-Monasterio *et al.*, 2007).

Therefore, LineA (45.6 µg.g^{-1}), Marvdasht (43.9 µg.g^{-1}) and Niknejad (41.3 µg.g^{-1}) in term of Fe potential and Alamut (72 µg.g^{-1}), Niknejad (62.9 µg.g^{-1}), Gaspard (57.6 µg.g^{-1}), Shahpasand (55.5 µg.g^{-1}) and LineA (54.01 µg.g^{-1}) in term of Zn potential had high levels of mentioned microelements, respectively.

Correlation between wheat micronutrients (Fe and Zn)

Highly significant and positive correlation between grains Fe with Zn (r=0.67**) was observed. Significant correlation between wheat grains Fe and Zn were reported in most of researches (Cakmak *et al.,* 2004; Morgounov *et al.,* 2007; Zhao *et al.,* 2009; Demirkiran, 2009; Peleg *et al.,* 2009; Chatzav *et al.,* 2010; Wang *et al.,* 2011). QTL mapping in various wheat populations confirmed co-localization of high Zn and high Fe (Peleg *et al.,* 2009). Also co-localization of QTLs for Zn and Fe contents has been reported in rice (Garcia-Oliveira *et al.,* 2009). Positive correlation was reported between grain protein content with the Fe and Zn accumulation in wheat grains (Oury *et al.,* 2006; Cakmak, 2008; Zhao *et al.,* 2009; Wang *et al.,* 2011). Similar relationship among grain protein, Fe and Zn has been reported in bean (Gelin *et al.,* 2007) and rice (Garcia- Oliveira *et al.,* 2009).

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References

Ahmadi J, Pour-Aboughadareh A. 2015. Allelic variation of glutenin and gliadin genes in Iranian einkorn wheat. Journal of Biodiversity and Environmental Sciences **7,** 168–179.

Aguiriano E, Ruiz M, Fite R, Carrillo JM. 2006. Analysis of genetic variability in a sample of the durum wheat (*Triticum durum*) Spanish collection based on gliadin markers. Genetic Resources and Crop Evolution **53**, 1543–1552.

An X, Zhang Q, Yan Y, Li Q, Zhang Y, Wang A, Pei Y, Tian J, Wang H, Hsam SLK, Zeller FJ. 2006. Cloning and molecular characterization of three novel LMW-i glutenin subunit genes from cultivated einkorn (*Triticum monococcum* L.). Theoretical and Applied Genetics **113,** 383–395.

Anderson OD, Yong QG, Xiuying K, Gerard RL and Jiajie W. 2009. The wheat ω-gliadin genes: structure and EST analysis. Functional & Integrative Genomics **9(3),** 397–410. http://dx.doi.org/10.1007/s10142-009-0122-2.

Badakhshan H, Moradi N, Mohammadzadeh H, Zakeri MR. 2013. Genetic variability analysis of grains Fe, Zn and beta-carotene concentration of prevalent wheat varieties in Iran. International journal of agriculture and crop sciences **6(2),** 57.

Bahraei S, Saidi A, Alizadeh D. 2004. High molecular weight glutenin subunits of current bread wheats grown in Iran. Euphytica **137**, 173–179.

Benmoussa M, Vezina LP, Page M, Yelle S, Laberge S. 2000. Genetic polymorphism in low-molecular weight glutenin genes from *Triticum aestivum*, variety Chinese Spring. Theoretical and Applied Genetics **100**, 789-793.

Caballero L, Martin MA, Alvarez JB. 2008. Allelic variation for the high- and low-molecular-weight glutenin subunits in wild diploid wheat (*Triticum urartu*) and its comparison with durum wheats. Australian Journal of Agricultural Research **59,** 906–910.

Cakmak I. 2008. Enrichment of cereal grains with zinc: agronomic or genetic biofortification. Plant Soil **302,** 1-17. http://dx.doi.org/10.1007/s11104-007-9466-3

Cakmak I, Tourn A, Millet E, Feldman M, Fahima T, Korol A, Nevo E, Braun HJ, Ozkan H. 2004. Triticumdicoccoides: an important genetic resource for increasing zinc and iron concentration in modern cultivated wheat. Soil Science and Plant Nutrition **50(7),** 1047-1054.

Cakmak I, Pfeiffer WH, McClafferty B. 2010.Biofortification of durum wheat with zinc and iron. Cereal Chemistry **87**, 10–20. https://doi.org/10.1094/CCHEM-87-1-0010.

Chatzav M, Peleg Z, Ozturk L, Yazici A, Fahima T, Cakmak I, Saranga Y. 2010. Genetic diversity of grain nutrients in wild emmer wheat: potential for wheat improvement. Annals of Botany **105** (7), 1211-1220. https://doi.org/10.1093/aob/mcq024.

Cornish GB, Békés F, Eagles HA, Payne PI. 2006. Prediction of dough properties for bread wheats. In: Wrigley CW, Bekes F, Bushuk W (eds), Gliadin and Glutenin. The Unique Balance of Wheat Quality. AACCI Press. St Paul, Min., USA. 243–280 P.

Cuesta S, Alvarez JB, Guzman C. 2017. Identification and molecular characterization of novel LMW-m and –s glutenin genes, and a chimeric –m/-I glutenin gene in 1A chromosome of three diploid Triticum species. Journal of Cereal Science **74**, 46–55.

Demirkiran AR. 2009. Determination of Fe, Cu and Zn contents of wheat and corn grains from different growing site. Journal of Animal and Veterinary Advances **8(8),** 1563-1567.

Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin **19,** 11–15.

Ficco DBM, Riefolo C, Nicastro G, De Simone V, Di Gesu AM, Beleggia R, Platani C, Cattivelli L, De Vita P. 2009. Phytate and mineral elements concentration in a collection of Italian durum wheat cultivars. Field Crops Resarch **111(3),** 235–242. http://dx.doi.org/10.1016/j.fcr.2008.12.010.

Figueroa JDC, Maucher T, Reule W, Peña RJ. 2009. Influence of high molecular weight glutenins on viscoelastic properties of intact wheat kernel and relation to functional properties of wheat dough. Cereal Chemestry **86**,139–144. http://dx.doi.org/10.1094/CCHEM-86-2-0139.

Garcia-Oliveira AL, Tan L, Fu Y, Sun C. 2009. Genetic identification of quantitative trait loci for contents of mineral nutrients in rice grain. Journal of Integrative Plant Biology **51(1),** 84-92.

Graham RD, Welch RM, Bouis HE. 2001. Addressing micronutrient malnutrition through enhancing the nutritional quality of staple foods: principles, perspectives and knowledge gaps. Advances in Agronomy **70**, 77-142.

Gelin JR, Forster S, Grafton KF, McClean PE, Rojas-Cifuentes GA. 2007. Analysis of seed zinc and other minerals in a recombinant inbred population of navy bean (*Phaseolus vulgaris* L.). Crop Science **47,** 1361-1366.

Genc Y, Humphries JM, Lyons GH, Graham RD. 2005. Exploiting genotypic variation in plant nutrient accumulation to alleviate micronutrient deficiency in populations. Journal of Trace Elements in Medicine and Biology **18**, 319-324.

Gomez-Becerra HF, Erdem H, Yazici A, Tutus Y, Torun B, Ozturk L, Cakmak I. 2010a. Grain concentrations of protein and mineral nutrients in a large collection of spelt wheat grown under different environments. Journal of Cereal Science **52,** 342-349. http://dx.doi.org/10.1016/j.jcs.2010.05.003.

Gomez-Becerra HF, Yazici A, Ozturk L, Budak H, Peleg Z, Morgounov A, Fahima T, Saranga Y, Cakmak I. 2010b. Genetic variation and environmental stability of grain mineral nutrient concentrations in *Triticum dicoccoides* under five environments. Euphytica **171,** 39-52. http://dx.doi.org/10.1007/s10681-009-9987-3.

Gupta RB, Shepherd KW. 1990. Two-step one dimensional SDS-PAGE analysis of LMW subunits of glutenin. I. Variation and genetic control of the subunits in hexaploid wheats. Theoretical and Applied Genetics **80**, 65–74.

Gupta RB, Singh NK and Shepherd KW. 1989. The cumulative effect of allelic variation in LMW and HMW glutenin subunits on physical dough properties in progeny of two bread wheats. Theoretical and Applied Genetics **77**, 57-64.

Gupta PK. 2000. Soil, plant, water and fertilizer analysis. Agrobios, New Dehli, India. P. 438.

Harmankaya M, Ozcan MM, Gezgin S. 2012. Variation of heavy metal and micro and macro element concentrations of bread and durum wheats and their relationship in grain of Turkish wheat cultivars. Environmental Monitoring and Assessment **184**(9), 5511–5521. http://dx.doi.org/10.1007/s10661-011-2357-3.

Ikeda TM, Branlard G, Peña RJ, Takata K, Liu L, He Z, Lerner SE, Kolman MA, Yoshida H, Rogers WJ. 2008. International collaboration for unifying Glu-3nomenclature system in common wheats. In: International Wheat Genetics Symposium, Brisbane, Australia. http://ses.library.usyd.edu.au/bitstream/2123/3281/1/O42.pdf

Ikeda TM, Nagamine T, Fukuoka H, Yano H. 2002. Identification of new low-molecular-weight glutenin subunit genes in wheat. Theoretical and Applied Genetics **104**, 680-687.

Igrejas G, Guedes-Pinto H, Cardine V, Branlard G. 1999.The high and low molecular weight glutenin subunits and omega gliadin composition of bread and durum wheats commonly grown in Portugal. Plant Breeding **118,** 297–302.

Kawaura K, Mochida K, Ogihara Y. 2005. Expression profile of two storage-protein gene families in hexaploid wheat revealed by large-scale analysis of expressed sequence tags. Plant Physiology **139,** 1870–1880.

Masoudi-Nejad A, Nasuda S, Kawabe A, Endo TR. 2002. Molecular cloning, sequencing, and chromosome mapping of a 1A-encoded ω-type prolamin sequence from wheat. Genome **45**, 661–669.

Moghaddam M, Ehdaie B, Waines JG. 2000. Genetic diversity in population of wild diploid wheat *Triticum urartu* revealed by isozyme markers. Genetic Resources and Crop Evolution **47,** 323–334.

Morgunov AI, Pena RJ, Crossa J, Rajam S. 1993. Worldwide distribution of Glu-1 alleles in bread wheat. Journal of Genetics and Breeding **47,** 53-60.

Morgounov A, Gomez-Becerra HF, Abugalieva A, Dzhunusova M, Yessimbekova M, Muminjanov H, Zelenskiy Y, Ozturk L, Cakmak I. 2007. Iron and zinc grain diversity in common wheat grown in central Asia. Euphytica **155**, 193-203.

Nakamura H. 2001. Genetic diversity of high molecular-weight glutenin subunits compositions of in landraces of hexaploid wheat from Japan. Euphytica **120,** 227-234.

Liu L, He ZH, Yan J, Zhang Y, Xia X, Pena RJ. 2005. Allelic variation at the Glu-1and Glu-3loci, presence of the 1B.1R translocation, and their mixographic properties in Chinese bread wheats. Euphytica **142**,197–204.

Long H, Wei YM, Yan ZH, Baun B. 2005. Classification of wheat low molecular weight glutenin subunit genes and its chromosome assignment by development LMW-GS group specific primers. Theoretical and Applied Genetics **111**, 1251-1259.

Lukow OM. 1991. Screening of bread wheats formilling and baking quality- A Canadian perspective. Cereal Foods World **36(6),** 497-501.

Luo C, Griffin WB, Branlard G, McNeil DL. 2001. Comparison of low and high molecular weight wheat glutenin allele effects on flour quality. Theoretical and Applied Genetics **102,** 1088–1098.

Ortiz-Monasterio JI, Palacios-Rojas E, Meng E, Pixley K, Trethowan R, Pena RJ. 2007. Enhancing the mineral and vitamin content of wheat and maize through plant breeding. Journal of Cereal Science **46,** 293- 307. http://dx.doi.org/10.1016/j.

Oury FX, Leenhardt F, Remesy C, Chanliaud E, Duperrier B, Balfourier F, Charmet G. 2006. Genetic variability and stability of grain magnesium, zinc and iron concentrations in bread wheat. European Journal of Agronomy **25,** 177-185. http://dx.doi.org/10.1016/j.eja.2006.04.011.

Payne PI, Corfield KG, Blackman JA. 1981. Correlation between the inheritance of certain high-molecular-weight subunits of glutenin and bread-making quality in progenies of six crosses of bread wheat. Journal of the Science of Food and Agriculture **32,** 51–60.

Payne PI, Lawrence GJ. 1983. Catalogue of alleles or the complex loci, Glu-A1, Glu-B1and Glu-D1which coded for high-molecular-weight subunits of glutenin in hexaploid wheat. Cereal Research Communications **11**, 29–35.

Payne IP, Jackson EA, Holt LM, Law CN.1984. Genetic linkage between endosperm storage protein genes on each of the short arms of chromosomes 1A and 1B in wheat. Theoretical and Applied Genetics **67**, 235–243.

Payne PI, Mark AN, Krattiger AF, Holt LM. 1987. The relationship between HMW glutenin subunits composition and the bread making quality of British-grown wheats. Journal of the Science of Food and Agriculture **40**, 51-65.

Payne PI. 1987. Genetic of wheat storage proteins and the effect of allelic variation on bread making quality. Annual Reviews in Plant Physiology **8,** 141-153.

Peleg Z, Saranga Y, Yazici A, Fahima T, Ozturk L, Cakmak I. 2008. Grain zinc, iron and protein concentrations and zinc-efficiency in wild emmer wheat under contrasting irrigation regimes. Plant Soil **306(1–2),** 57–67. http://dx.doi.org/10.1007/s11104-007-9417-z.

Peleg Z, Cakmack I, Ozturk L, Yazici A, Budak H, Korol AB, Fahima T, Saranga Y. 2009. Quantitative trait loci conferring grain mineral nutrient concentrations in durum wheat × wild emmer wheat RIL population. Theoretical and Applied Genetics **119,** 353-369. http://dx.doi.org/10.1007/s00122-009-1044-z.

Rawat N, Tiwari VK, Singh N, Randhawa GS, Singh K, Chhuneja P, Dhaliwal HS. 2009. Evaluation and utilization of Aegilops and wild Triticum species for enhancing iron and zinc content in wheat. Genetic Resources and Crop Evolution **56(1),** 53–64.

Saltzman A, Ekin B, Howarth EB, Erick B, Fabiana FDM, Yassir I, Wolfgang HP. 2013. Biofortification: Progress toward a more nourishing future. Global Food Security **2,** 9-17. http://dx.doi.org/10.1016/j.gfs.2012.12.003.

Shewry PR, Tatham AS. 1997. Disulphide bonds in wheat gluten proteins. Journal of Cereal Science **25**, 207-227.

Shewry PR, TathamAS, Barro F, Barcelo P, Lazzeri P. 1995. Biotechnology of unravelling and manipulating the multi-protein gluten complex. Biotechnology **13**, 1185–1190.

Singh NK, Shepherd KW. 1988. Linkage mapping of genes controlling endosperm storage proteins in wheat I. Genes on the short arms of group 1 chromosomes. Theoretical and Applied Genetics **75,** 628–641.

Stein AJ. 1994. Global impacts of human mineral malnutrition. Plant and Soil **335,** 133-154.

Tahir M, Lafiandra D. 1994. Assessment of genetic variability in hexaploid wheat landraces of Pakistan based on polymorphism for HMW-glutenin subunits. In: Biochemical Evaluation of Plant Fenetic Resources, Final Technical Report, Dept. of Agrobiology and Agrobiochemistry, University of Tuscia, Viterbo, Italy, 33-44 P.

Tanaka H, Toyoda S, Tsujimoto H. 2005. Diversity of low-molecular-weight glutenin subunit genes in asian common wheat. Breeding Science **55**, 349-354.

Velu G, Ortiz-Monasterio I, Singh R, Payne T. 2011. Variation for grain micronutrients concentration in wheat core-collection accessions of diverse origin. Asian Journal of Crop Science **3**, 43–48.

Von Buren M. 2001. Polymorphisms in two homeologous γ-gliadin genes and the evolution of cultivated wheat. Genetic Resources and Crop Evolution **48**, 205–220.

Wang S, Yin L, Tanaka H, Tanaka K, Tsujimoto H. 2011. Wheat- Aegilops chromosome addition lines showing high iron and zinc contents in grains. Breeding Science **61,** 189-195.

Weegels PL, Van de Pijpekamp AM, Graveland A, Hamer RJ, Schofield JD. 1996. Depolymerisation and re-polymerisation of wheat glutenin during dough processing. I. Relationships between glutenin macropolymer content and quality parameters. Journal of Cereal Science **23**,103–11.

Zhao FJ, Su YH, Dunham SJ, Rakszegi M, Bedo Z, McGrath SP, Shewry PR. 2009. Variation in mineral micronutrient concentrations in grain of wheat lines of diverse origin. J Cereal Sci 49 (2):290-295. http://dx.doi.org/10.1016/j.jcs.2008.11.007.

Zhao H, Guo B, Wei Y, Zhang B, Sun S, Zhang L, Yan J. 2011. Determining the geographic origin of wheat using multielement analysis and multivariate statistics. Journal of Agricultural and Food Chemistry **59(9),** 4397–4402. http://dx.doi.org/10.1021/jf200108d.