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Variations in micronutrients, bread quality and agronomic traits of wheat landrace varieties and commercial cultivars

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Abstract

Micronutrients malnutrition causes global health problems specifically in less developed and developing countries. The objectives of this study were to investigate variations in micronutrients, bread making quality and their relationships with grain yield (GY) in wheat. Fifty landrace varieties and 10 commercial cultivars were grown in a RCBD with no micronutrient fertilizer. Zinc (Zn^{+2}) and iron (Fe^{+2}) contents as mg kg^{-1} dry weight, dry gluten (Glu), sodium do-decyl sulfate (SDS) volume, grain hardness (GH), zeleny sedimentation volume (ZSV), grain protein content (Gpc), hectoliter weight (HW) and agronomic traits were assayed. Iron and Zinc concentrations were measured using atomic absorption spectroscopy (Shimadzu AA-670). Landraces had higher Fe^{+2} (24.93 to 66.51), Zn^{+2} (18.68 to 38.66) and GY (6.2 to 11.8 g) compared with commercial cultivars. This indicates the fact that breeding for micronutrients and bio-fortification of wheat cultivars have been forgotten. Higher Gpc of commercial cultivars (11.8% to 15.1%) than landrace varieties (9.8% to 14.03%) and SDS data showed insufficient bread-making quality in landraces. The highest correlations among baking-quality traits and micronutrients were observed between Gpc and SDS volume (r=0.82**) and grain Fe^{+2} (0.55**) and Zn^{+2} (0.52**) concentrations with Gpc. These were followed by correlations between grain Fe^{+2} and Zn^{+2} concentrations (0.51**) and between dry gluten content and SDS volume (0.30**). In conclusion, results showed that the hybridization of genotypes for incorporation of higher micronutrient, grain yield and better bread making quality would be more efficient than surveying for single superior plants via direct selection.

Keywords: Fe^{+2}; grain yield; landrace; protein; wheat; Zn^{+2}.
Abbreviations: ANOVA_Analysis of variance; GCV_ Genetic coefficient of variation; Gpc_grain protein content; GH_ Grain hardness; GI_Genetic improvement; Glu_gluten; LSD_Least significant differences; MA_maturity; PCA_Principal component analysis; PCV_ Phenotypic coefficient of variation; SDS_Sodium dodecyl sedimentation; HW_ Hectoliter weight; RCBD_Randomized complete block design; TGW_thousand grain weight; GY_Grain yield.

Introduction

Micronutrient malnutrition causes global health problems in less developed and developing countries. More than 2 billion people around the world suffer from the deficiencies of iron (Fe^{+2}), zinc (Zn^{+2}) and other micronutrients (Cakmak, 2008; Wang et al., 2011). Micronutrients play key roles in plant and human metabolisms. Deficiencies of micronutrients lead to dysfunctions and diseases such as impairments in physical development, immune system and brain function in human (Cakmak, 2008). Zn^{+2} and Fe^{+2} deficiencies are responsible for 3.2% and 3.1% of illnesses in low income countries, respectively (Cakmak, 2008; Taheri et al., 2011). Micronutrient deficiency also affect growth and structural development of crop plants (Taheri et al., 2011). Cereals lay an important role in providing micronutrients and protein and daily calorie intake in developing world. Wheat which is consumed as the major staple food in many parts of the world contributes to around 60% of daily energy intake in most of developing countries (Cakmak, 2008; Wang et al., 2011). In Iran, daily calorie intake from wheat grain is higher than 1300 Kcal/capita/day (Cakmak, 2008). Therefore, the composition and nutritional quality of the wheat grain affects human health while cultivated wheat is insufficient in Fe^{+2} and Zn^{+2} contents (Wang et al., 2011). Variation in micronutrients and protein of wheat grain is highly attributed to genetic effects (Gomez-Becerra et al., 2010a, b). Several strategies have been suggested for the reduction of micronutrients and protein malnutrition. The nutritional value of cereals needs to be improved through the general use of less refined flour and the selection of wheat varieties with high mineral diversity. Comparatively, plant breeding has been identified as more sustainable and less expensive, since seeds could reach a larger number of people without the necessity of changing consumer's behavior (Cakmak, 2008; Ng'uni et al., 2012). A comprehensive exploration of genetic resources is a preliminary step in breeding for higher micronutrients in crop plants. Compared to cultivated crops, wild and primitive wheat genotypes are better genetic resources rich in Zn^{+2} or other micronutrients (Cakmak, 2008). The results of a genetic diversity assay in 80 wheat genotypes showed that breeders attention to enhancing grain yield caused the production of low quality wheat for iron, zinc and protein concentrations during 70 years (Amiri et al., 2015). Screening spring wheat germplasm showed that wild relatives, primitive wheats and landraces were the most promising sources for enhanced zinc and iron concentrations (Velu et al., 2011; Xu et al., 2011). In analyzing a number of *T. dicoccoides* accessions, concentration of Zn^{+2} varied from 14 to 190 mg kg^{-1} dry weight and Fe^{+2} content ranged between 15 and 109 mg kg^{-1}(Cakmak et al., 2004). By evaluating 82 wheat varieties, Badakhshan et al. (2013)

showed that highly significant and positive correlations were found between Fe^{+2}, Zn^{+2} and protein contents indicating concurrently improvement of these nutrients is possible. Non-significant correlation among yield components and grain micronutrient concentration showed that wheat varieties with high micronutrient not necessarily tend to produce lower yield (Badakhshan et al., 2013). In a study by Chatzav et al. (2010), the concentrations of grain Fe^{+2} and Zn^{+2} and protein in wild accessions were about two-fold greater than in the domesticated genotypes. The same study showed that concentrations of grain zinc, iron and protein were positively correlated, with no clear association with plant productivity, suggesting that all three nutrients can be improved concurrently with no yield penalty. In the Hruskova et al. (2012) report, wheat grain hardness was significantly correlated with gluten content and flour yield. Assessing CIMMYT wheat germplasms indicated that grain hardness had negative association with iron content while its correlations with protein, zinc and grain weight were positive (Velu et al., 2011). Evidently, the genetic diversity is sufficient for the development of wheat cultivars for increased micronutrients and bread making quality (Xu et al., 2011). Landrace varieties are promising sources that have not been fully explored for grain yield potential and micronutrient concentrations. Therefore, the main objectives of this study were (1) to investigate variations in Zn^{+2}, Fe^{+2} contents, bread making quality and agronomic traits in 50 wheat landrace varieties and 10 commercial cultivars and (2) to evaluate the relationships of agronomic and quality related traits.

Results and Discussion

Analysis of variance and traits variation

Variations of agronomic, bread- making quality traits and micronutrients were significant in wheat genotypes (Table 1). Mean squares of landraces versus commercial cultivars were also significant indicating difference of these two types for both agronomic and grain quality properties. Means for agronomic and bread- making quality traits and micronutrients are presented in Supplementary Table 1. Landraces and cultivars reached physiological maturity during a 17 day period. Among landrace varieties, KC4570, KC4607, KC4638, KC4576, KC4679, KC 4558 and KC4602 matured earlier. Chamran was an early- matured cultivar among commercial cultivars. Single plant GY ranged from 6.2 to 11.8 g in landrace varieties. The figure for commercial cultivars was 6.06 to 8.06 g. These results show the high potential of landraces in grain yield breeding programs.

Figure 1 and 2 show variations in Fe^{+2} and Zn^{+2} contents, grain yield and protein content in wheat genotypes. Fe^{+2} content increased from 24.93 (mg kg^{-1}dry weight) in KC4830 to 66.51 in KC120 (Supplementary Table 1). In commercial cultivars, Fe^{+2} content varied between 38.92 (mg kg^{-1}dry weight) in Navid and 54.94 in Alvand. A variation from 41.36 to 67.67 mg kg^{-1} previously was reported for Fe^{+2} content in wheat (Badakhshan et al., 2013). In a study in wheat, $Fe^{+2}$and Zn^{+2} contents varied from 47.5 to 60 mg $kg^{-1}$and from 32.3 to 44.2 mg $kg^{-1}$of grain dry weight, respectively (Distelfeld et al., 2007). Grain Fe^{+2} and Zn^{+2} contents varied between 24.68 and 26.20 mg kg^{-1} and between 15.99 and 19.22 mg kg^{-1} in different cropping systems and fertilizer application in wheat (Wozniak and Makarski, 2013). Some reports have shown an average concentration of Zn^{+2} from 20 to 35 mg kg^{-1} in wheat grain in different countries (Rengel et al., 1999; Cakmak et al., 2004). Survey studies also showed that Zn^{+2} content in wheat grown on Zn^{+2} -sufficient soils varies between 20 and 30 mg kg^{-1} compared with 5 to 12 mg kg^{-1} in wheat growing on Zn^{+2}-insufficient soils (Erdal et al., 2002; Cakmak et al., 2004). In present study, measurements for Zn^{+2} content indicated that landraces had higher Zn^{+2} than commercial cultivars. Of the landraces, KC4684 had highest Zn^{+2} (38.66 mg kg^{-1}dry weight) but the highest Zn^{+2} (29.05) in commercial cultivars was measured in Falat. This indicates that in breeding programs the bio-fortification of crop plants to alleviate micronutrients malnutrition has been forgotten. This may be primarily due to the higher cost of micronutrient measurements and difficulties of screening high number of genotypes. The range of Fe^{+2} and Zn^{+2} concentrations of bread wheat in the present study was similar to those reported in earlier studies (Oury et al., 2006; Morgounov et al., 2007; Zhao et al., 2009). Studies with rice and wheat, and preliminary studies with wild relatives and wheat landraces have demonstrated that considerable variations exist in grain Zn^{+2} and Fe^{+2} concentrations (Genc et al., 2005; Gomez-Becerra et al., 2010a, b).

GH varied between 40.3 (g mm^{-2}) and 53.33 in landraces and between 46.3 and 53.66 in commercial cultivars. Landrace varieties had more variation for HW (74.61 to 83.65 kg) than commercials (71.04 to 77.96). The landrace KC4558 had significantly higher Glu (18.8%) than Chamran (16.96%). Commercial cultivars showed higher ZSV (from 17 ml in Tajan to 20.33 in Falat) than landraces (from 9.6 ml to 18.0). SDS volume was from 43.3 ml to 57.6 in landrace varieties and from 47.2 ml to 64.6 in commercial cultivars. Similar results indicated higher Gpc of commercial cultivars (11.8% to 15.1%) than landrace varieties (9.8% to 14.3%). These results show that landrace varieties had insufficient bread-making quality compared with commercial cultivars despite the fact that some landraces had higher grain yield.

Heritability and genetic variation parameters

The highest heritability estimated for GY, MA, SDS, Fe^{+2} and Zn^{+2} contents (Table 2). The lowest heritability belonged to TGW and HW. Lower heritability reflects the contribution of non-additive effects or environment in variations of traits. The estimation of heritability showed that the variation of grain yield was primarily connected with the genetic attributes of the genotypes and the environment had only a minor influence on variation of this trait. PCV was highest for GY, Fe^{+2} and Zn^{+2} contents and ZSV. The lowest PCV belonged to MA, HW and GH. The highest GCV belonged to GY, Fe^{+2} and Zn^{+2} contents and ZSV. Regarding bread quality characters, SDS volume had the highest GCV among wheat genotypes. The GI index of SDS, Fe^{+2} and Zn^{+2} contents, ZSV, and GY was higher than GI of other traits. High GI index of grain yield accompanied by high heritability, PCV and GCV show the efficacy of selection in further breeding programs of grain yield.

Correlations of traits

The highest correlations were those between Gpc and SDS volume, Glu and SDS volume, Fe^{+2} and Zn^{+2} contents with Gpc, and between $Fe^{+2}$and Zn^{+2} contents (Table 3).

Negative correlations were found between grain yield and both Fe^{+2} and Zn^{+2} contents. Fe^{+2} and Zn^{+2} contents and bread making quality traits showed non- significant or negative correlations with MA. This shows high- Fe^{+2} and Zn^{+2}-genotypes matured earlier than low- Fe^{+2} and Zn^{+2} varieties. An early senescence association has been found with higher grain Fe^{+2}, Zn^{+2} and protein lines in wheat (Distelfeld et al., 2007). In the Distelfeld et al. (2007) study, results showed

Table 1. Analysis of variances for the effect of wheat genotype on agronomic, bread making quality traits and micronutrients.

		Mean Squares										
Source	df	MA	GY	TGW	GH	HW	Glu	ZSV	Fe^{+2}	Zn^{+2}	SDS	Gpc
Block	2	0.7	0.15	12.7	0.41	10.8	0.57	0.97	4.7	1.3	6.5	0.14
Genotype	59	26.4**	8.5**	34.2**	33.0**	32.1**	10.5**	24.0**	354.4**	81.8**	55.7**	4.0**
Landrace Vs. Commercials	1	2.0**	251.5**	69.7**	533.6**	1042.4**	46.0**	541.3**	489.0**	464.5**	172.4**	63.0**
Error	118	0.5	0.15	8.2	2.0	6.4	0.6	0.55	2.7	1.1	7.2	0.26

**: significant at 0.01, df: degree of freedom, Fe^{+2}: iron content, MA: day to maturity, Gpc: grain protein content, GY: grain yield, TGW: thousand grain weight, GH: grain hardness, HW: hectoliter weight, Glu: dry gluten, Vs: versus, SDS: sodium dodecyl sedimentation, Zn^{+2}: zinc content, ZSV: zeleny sedimentation volume

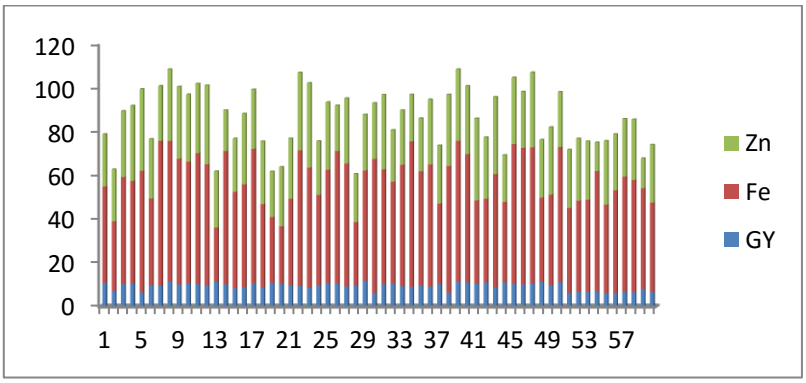

Fig 1. Variations of grain (GY) yield (g), grain Zn and Fe (mg kg^{-1} dry weight) contents (vertical axis) in wheat landrace varieties and commercial cultivars (horizontal axis). Numbers in horizontal axis refer to the name of genotypes in Table 6.

Table 2. Mean, variance and heritability of agronomic, bread-making quality traits and micronutrients.

Trait	Mean	Min	Max	CV (%)	GCV (%)	PCV (%)	Environmental variance	Heritability (%)	GI (%)
MA	207.0±3.0	200	217	0.34	1.42	1.46	0.5	94.47	2.84
TGW	38.32±4.1	26.8	55	7.4	7.6	10.7	1.23	51.27	11.3
GY	9.333±1.7	5.8	12.3	4.2	17.9	18.4	0.15	94.74	36.0
GH	46.08±3.5	40	55	3.0	6.9	7.6	1.99	83.83	13.17
HW	78.54±3.9	65.4	89.52	3.2	3.7	4.9	6.36	57.43	5.82
Glu	15.78±2.0	9.88	19.68	4.8	11.5	12.5	0.59	84.81	21.86
ZSV	14.82±2.9	9	21	5.0	18.6	19.5	0.55	93.38	37.55
Fe^{+2}	49.32±10.9	23.74	67.6	3.3	21.9	22.2	2.69	97.75	44.71
Zn^{+2}	27.98±5.3	12.4	39.45	3.7	18.5	18.9	1.1	96.04	37.43
SDS	52.2±4.8	42.0	66.0	5.1	7.7	9.2	7.22	97.7	44.7
Gpc	11.8±1.2	8.7	15.76	4.4	9.5	10.4	0.26	82.3	17.7

CV: coefficient of variation, Fe^{+2}: grain iron (mg kg^{-1}dry weight), GCV: genotypic coefficient of variation, MA: day to maturity, GI: genetic improvement index, Gpc: grain protein content (%), GY: grain yield (g per plant), PCV: phenotypic coefficient of variation, TGW: thousand grain weight (g), GH: grain hardness (g mm^{-2}), HW: hectoliter weight (kg), Glu: dry gluten (%), SDS: sodium dodecyl sedimentation volume (ml), Zn^{+2}: grain zinc content (mg kg^{-1} dry weight), (ZSV: zeleny sedimentation volume (ml)

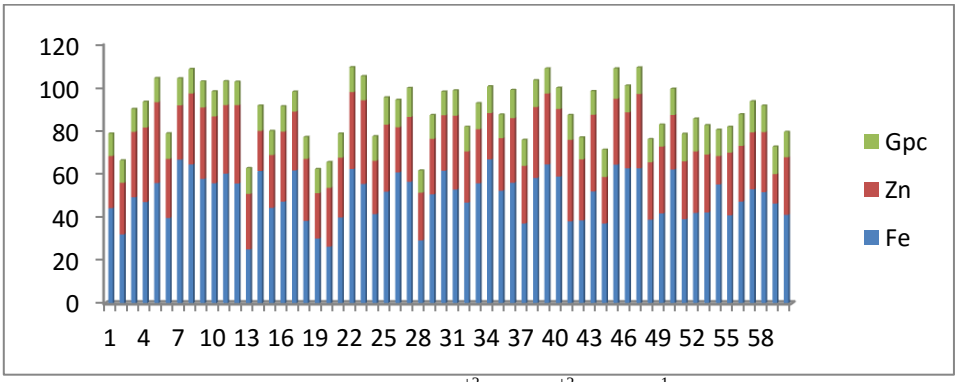

Fig 2. Variations of grain protein (%) content (Gpc), grain Zn^{+2} and Fe^{+2} (mg kg^{-1} dry weight) contents (vertical axis) in wheat landrace varieties and commercial cultivars (horizontal axis). Numbers in horizontal axis refer to the name of genotypes in Table 6.

Table 3. Phenotypic (above diagonal) and genetic (under diagonal) correlations of micronutrient, bread-making quality and agronomic traits in wheat genotypes.

Trait	MA	TGW	GY	GH	HW	Glu	ZSV	Fe^{+2}	Zn^{+2}	SDS	Gpc
MA	1	-0.28	-0.22	-0.01	-0.08	-0.27	-0.07	-0.06	0.09	-0.20	-0.22
TGW	-0.25	1	0.46	0.06	0.19	0.19	-0.0002	-0.44	-0.49	0.06	-0.08
GY	-0.22	0.39	1	-0.37	0.61	0.37	-0.45	-0.44	-0.49	-017	-0.37
GH	-0.01	0.04	-0.39	1	-0.57	-0.30	0.41	-0.07	-0.28	-0.12	0.07
HW	-0.07	0.16	0.47	-0.49	1	0.37	-0.6	0.08	0.23	-0.17	-0.46
Glu	-0.26	0.16	0.26	-0.28	0.33	1	-0.16	0.18	0.048	0.30	0.13
ZSV	-0.06	-0.002	-0.32	0.4	-0.53	0.15	1	-0.19	-0.45	0.02	0.41
Fe^{+2}	-0.06	-0.02	-0.4	-0.07	0.06	0.18	-0.19	1	0.51	0.20	0.55
Zn^{+2}	0.09	-0.15	-0.41	-0.26	0.2	0.04	0.44	0.51	1	0.04	0.52
SDS	-0.19	0.16	-0.08	-0.10	-0.10	0.30	0.20	0.18	0.04	1	0.82
Gpc	-0.21	-0.07	-0.36	0.07-	-0.40	0.24	0.24	0.54	-0.48	0.75	1

Fe^{+2}: grain iron (mg kg^{-1}dry weight), MA: day to maturity, Gpc: grain protein content (%), GY: grain yield (g per plant), TGW: thousand grain weight (g), GH: grain hardness (g mm^{-2}), HW: hectoliter weight (kg), Glu: dry gluten (%), SDS: sodium dodecyl sedimentation volume (ml); Zn^{+2}: grain zinc content (mg kg^{-1} dry weight), (ZSV: zeleny sedimentation volume (ml), absolute values higher than 0.24 and 0.33 are significant for phenotypic and genetic correlations respectively.

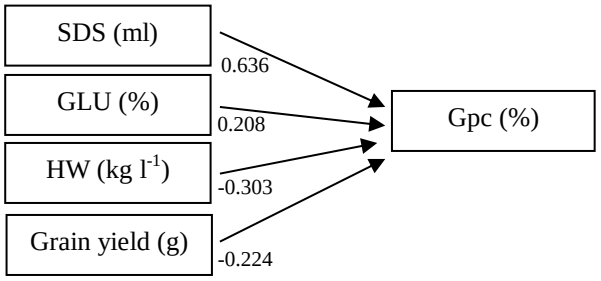

Fig 3. Diagram for direct effects (numbers on arrows) of sodium dodecyl sedimentation (SDS), dry gluten (Glu), hectoliter weight (HW) and grain yield on grain protein content (Gpc) of wheat genotypes.

Table 4. The indirect effects of the most important traits on Gpc (%) in wheat genotypes.

	coefficients for indirect effects of traits on protein content			
Trait	X1	X2	X3	X4
SDS (X1)	-	0.032	0.02	0.064
HW (X2)	-0.068	-	-0.106	0.069
GY (X3)	-0.057	-0.143	-	0.055
Glu (X4)	0.197	-0.1	0.059	-

Gpc: grain protein content (%), GY: grain yield (g per plant), GH: grain hardness (g mm^{-2}), HW: hectoliter weight (kg), Glu: dry gluten, SDS: sodium dodecyl sedimentation volume (ml)

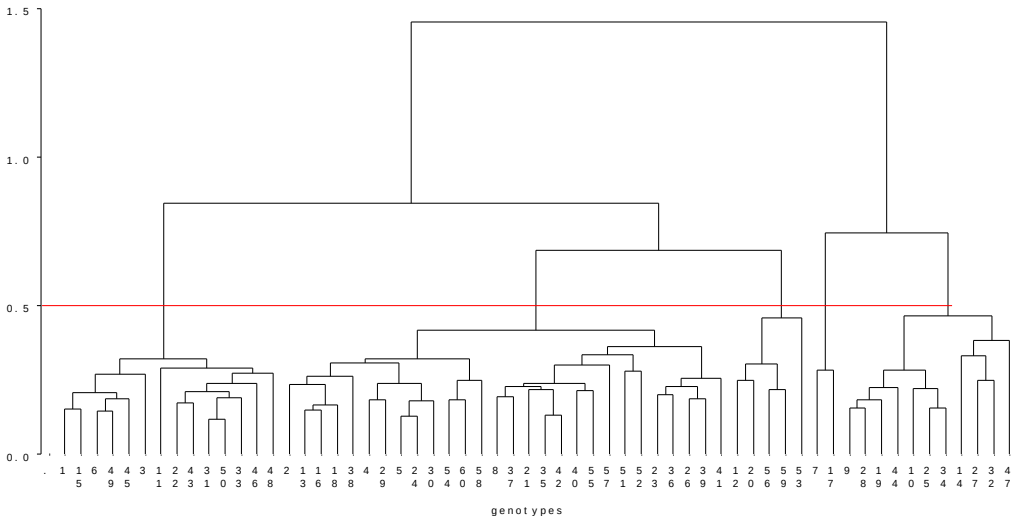

Fig 4. Dendrogram of cluster analysis based on all of the traits. The vertical axis shows Euclidean distances and the red line at 0.5 shows 5 main groups based on lowest distances between genotypes. The numbers refer to the name of genotypes in Table 6. Number of genotype must be read vertical.

Table 5. Traits mean and analysis of variance between 5 groups of wheat genotypes in cluster analysis.

Trait	MS (between groups)	MS (within groups)	Mean				
			Group 1	Group 2	Group 3	Group 4	Group 5
MA	26.468**	0.507	206.26c	206.54bc	208.13b	209.00a	208.27b
TGW	34.242**	8.238	38.63a	38.33a	38.69a	38.30a	37.76a
GY	8.581**	0.156	9.47b	9.35b	8.01c	8.65bc	9.84a
GH	32.143**	6.367	79.58a	78.00ab	76.47ac	78.53a	79.56a
HW	33.069**	1.998	44.90bc	46.44b	48.87a	45.83bc	45.45bc
Glu	10.528**	0.593	15.98a	15.82ab	14.17c	15.35ab	16.26a
ZSV	24.016**	0.554	14.40bc	14.96b	17.67a	14.67b	13.73c
SDS	55.783**	7.227	51.86ab	52.95a	52.00a	46.83c	52.03a
Fe^{+2}	354.435**	2.694	47.54bc	50.34b	46.62bc	40.46d	51.87a
Zn^{+2}	81.862**	1.109	28.99b	26.95c	27.03bc	27.00bc	30.71a
Gpc	4.019**	0.269	11.58b	11.96a	12.01a	10.51c	11.59b

Fe^{+2}: grain iron (mg kg^{-1} dry weight), Gpc: grain protein content (%), GY: grain yield (g per plant), MA: day to maturity, MS: mean square, TGW: thousand grain weight (g), GH: grain hardness (g mm^{-2}), HW: hectoliter weight (kg), Glu: dry gluten (%), SDS:sodium dodecyl sedimentation volume (ml); Zn^{+2} : grain zinc content (mg kg^{-1} dry weight), ZSV: zeleny sedimentation volume (ml).

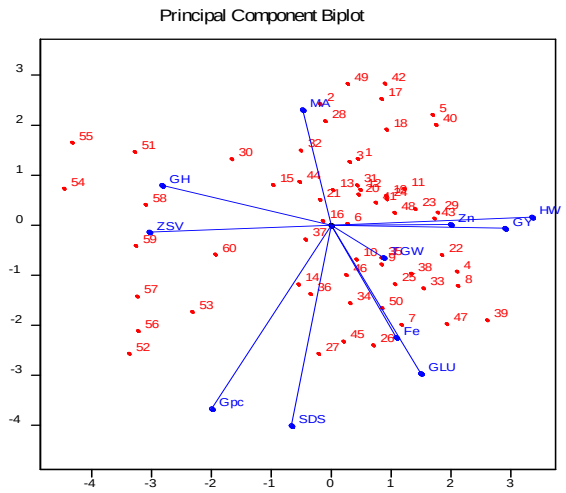

Fig 5. Principal component analysis for distribution of wheat genotypes based on the associations of micronutrients, bread making quality and agronomic traits. Narrow angles between vectors show stronger relationship of traits and genotypes close to each vector have higher values for corresponded vector. Gpc: grain protein content, GH: grain hardness, Glu: dry gluten weight, HW: hectoliter weight, MA: maturity, SDS: sodium dodecyl sedimentation, TGW: thousands grain weight, ZSV: zeleny sedimentation volume. Numbers refer to the name of genotypes in Table 6.

Table 6. Wheat genotypes consisting of 50 landrace varieties and 10 commercial cultivars.

Number	Genotype	Type	Number	Genotype	Type	Number	Genotype	Type
1	KC4818	Landrace	21	KC4542	Landrace	41	KC4692	Landrace
2	KC4580	Landrace	22	KC4606	Landrace	42	KC2623	Landrace
3	KC4608	Landrace	23	KC4806	Landrace	43	KC4845	Landrace
4	KC4800	Landrace	24	KC4803	Landrace	44	KC4585	Landrace
5	KC4697	Landrace	25	KC4689	Landrace	45	KC4674	Landrace
6	KC4631	Landrace	26	KC4607	Landrace	46	KC4820	Landrace
7	KC126	Landrace	27	KC4575	Landrace	47	KC4780	Landrace
8	KC4696	Landrace	28	KC4856	Landrace	48	KC4793	Landrace
9	KC4559	Landrace	29	KC4632	Landrace	49	KC4684	Landrace
10	KC4682	Landrace	30	KC4834	Landrace	50	KC224	Landrace
11	KC4569	Landrace	31	KC4570	Landrace	51	Alvand	Comercial
12	KC4779	Landrace	32	KC4830	Landrace	52	Azar2	Comercial
13	KC4687	Landrace	33	KC4644	Landrace	53	Chamran	Comercial
14	KC4840	Landrace	34	KC4815	Landrace	54	Niknejad	Comercial
15	KC4601	Landrace	35	KC4548	Landrace	55	Moghan3	Comercial
16	KC4703	Landrace	36	KC4858	Landrace	56	Shirodi	Comercial
17	KC4810	Landrace	37	KC4558	Landrace	57	Falat	Comercial
18	KC4602	Landrace	38	KC4638	Landrace	58	Navid	Comercial
19	KC4848	Landrace	39	KC120	Landrace	59	Zarin	Comercial
20	KC4881	Landrace	40	KC4634	Landrace	60	Tajan	Comercial

Landraces provided by the Seed and Plant Improvement Institute (SPII), Karaj, Iran.

that high- GpcB1a lines senesced 3 days earlier than low-GpcB1b lines. Correlation coefficients of quantitative traits and baking quality and micronutrients indicated significant and negative relationship between grain yield and protein content (Chee et al., 2001; Cantrell and Joppa, 1991). In Distelfeld et al. (2007) study, no association was found between GY or TGW with Gpc and micronutrients. Peterson et al. (1986) evaluated variation of mineral elements in grains of 27 wheat varieties and results indicated that grain yield had highly significant and negative correlations with Zn^{+2} and Fe^{+2} contents. They also indentified that mineral and micronutrient concentrations were more influenced by variations in grain weight than by variation in flour. Analysis of genes for Gpc and micronutrients confirmed that *Gpc-B1a* allele, already known to increase Gpc, is highly important in mineral accumulation in wheat and incorporation of this gene into commercial cultivars has the potential to increase both protein and micronutrients (Distelfeld et al., 2007).

Relationships of traits and similarities between genotypes

Stepwise regression model for elucidation of Gpc variations based on agronomic and baking quality traits and micronutrients is shown in the following equation (R^2= 0.74). This equation proved that SDS, HW, GY and Glu were the major traits contributing to the total Gpc variations.

Gpc= 21.14 + 0.163 (SDS) – 0.126 (HW) – 0.166 (GY) + 0.12 (Glu)

The Fe^{+2} and Zn^{+2} contents were not entered into the model. Coefficients of Glu and SDS show the positive relation of these traits with Gpc. HW and GY had negative relations with Gpc due to negative regression coefficients.

Path analysis with Gpc as dependent variable indicating that SDS volume had the highest direct effect on Gpc variation, followed by Glu (Fig 3). HW and GY showed direct negative effects on protein content. This shows that by accumulation of starch and increasing grain yield, less energy and supply were available for increasing Gpc. Glu had the largest indirect effect through SDS volume which shows its strong relation with higher Gpc (Table 4). Based on cluster analysis, genotypes were classified into 5 main groups (Fig 4). Between groups mean squares were significant confirming the best possible classification of genotypes based on traits scores (Table 5). Group 2 was the largest and comprised of 28 genotypes while only two genotypes assigned to group 3. Group 5 had the highest Fe^{+2} (51.87 mg kg^{-1}dry weight), Zn^{+2} (30.71 mg kg^{-1}dry weight), Glu (16.26%) and grain yield (9.84 g per plant). Regarding SDS volume (52.03 ml) and Gpc (11.59%), group 5 ranked second after group 2. As compared with Gpc, SDS volume is more associated with better bread-making properties and has been recognized as the best indicator of gluten strength and bread baking quality (Dick and Quick, 1983; Kovacs et al., 1993, 1995). Therefore, selection for breeding micronutrients, bread-making quality and grain yield can be performed using group 5 genotypes. Genotypes in group 1 were early- matured showing the possibility of transferring earliness from these genotypes to the genotypes of group 5 through hybridization. This could result in combining higher grain yield, better bread making quality and higher Fe^{+2}, and Zn^{+2} contents in early- matured genotypes.

Principal component analysis for distribution of genotypes between traits vectors

The scatter plot of the first two principal components shows that genotypes were distributed between the vectors of micronutrients, bread making quality and agronomic traits (Fig 5). Very tight and acute angles among the vectors of Zn^{+2}, GY and HW represent strong associations of these traits. Selection of genotypes located between the vectors increases Zn^{+2}, HW and grain yield concurrently. Narrow angles show that selection of genotypes scattered between Gpc and Glu vectors leads to higher Gpc, Fe^{+2}, SDS and Glu. Orientations of MA vector with the vectors of bread making quality traits indicated that late- mature genotypes had lower bread quality than early- matured ones. Wide angles between the vectors corresponding to MA, SDS, Gpc, Glu and Fe^{+2} indicated that focusing on genotypes distributed between MA and Fe^{+2} vectors reduce protein and Fe^{+2} content in late-matured plants.

Materials and Methods

Plant materials and experimental design

Plant materials included 60 wheat genotypes consisting of 50 landrace varieties and 10 commercial cultivars (Table 6). The landrace varieties were provided by the Seed and Plant Improvement Institute (SPIF), Iran. All genotypes were sown in a Randomized Complete Block Design (RCBD) with three replications. Field site was located at the Research Farm, College of Agriculture (29^o 50 N', 52^o 46' E, 1810 m alt), Shiraz University, Iran. In November 2012, the seeds were sown on 2- m long rows spaced 5 cm with row spacing of 20 cm. The soil texture was sandy clay with pH 7. Prior to sowing, the field was fertilized with 50 kg N ha^{-1} and 110 kg triple superphosphate ha^{-1}. The total amount of 100 kg N ha^{-1} was added at the stem elongation and heading stages. Weeding was performed using 40 g ha^{-1} the herbicide Total® (sulfosulfuron+metsulfuron methyl) at tillering stage and by hand pulling at all stages of wheat growth. Irrigation practices were performed normally throughout the growing season and no micronutrient fertilizer was added.

Determination of Zn^{+2} ***and*** Fe^{+2} ***contents***

The seeds were oven dried at 70 ˚C and were fine powdered for quantifying micronutrients. Powders were exposed to 550 ˚C in electric furnace and were digested in 5 ml Chloridric acid (HCl).The solution was subsequently filtered (Zarcinas et al., 1987). After filtration, the solution was boiled and it's Zn^{+2} and Fe^{+2} contents were measured using an atomic absorption spectroscopy instrument (Shimadzu AA-670). Zn^{+2} and Fe^{+2} contents of grains were calculated as mg kg^{-1} dry weight.

Assay for bread making quality

Ten ml distilled water was added to 25 g flour in a beaker and gluten content (%) was determined using the standard procedure no. 38-10/01 of AACC (1983). Zeleny sedimentation volume (ZSV) was also quantified based on AACC standard method 56-61.02 (1983). In this procedure, 3.2 g flour was added to 50 ml bromophenol blue (BPB) in a tube and mixed in a Zeleny-specific shaker for 5 min. Sodium Dodecyl Sedimentation (SDS) test was performed by using AACC approved methods no.56-70.01. SDS test was started by adding 50 ml distilled water to 6.3 g flour that mixed for 2 min. Subsequently, 50 ml lactic acid (85%) –sodium dodecyl sulphate (3%) solution was added to the samples in each tube and sediment (ml) was measured after 20 min.

Grain protein content (Gpc) was quantified based on Kjeldahl method (Bremner and Mulvaney, 1982). In this procedure, 15

ml sulphuric acid and 1 Kjeldahl tablet were added to 1 g flour and the solution was transferred to a Kjeldahl instrument (Gerhardt, Germany). Samples were transferred to a distillation instrument and the released nitrogen via titration with chloridric acid (0.1 N) was used to measure Gpc based on the following equation as below:

Gpc (%) = amount of consumed acid × 0.1 × 1.4 × 6.25

Hectoliter weight (HW) in kilogram per hectoliter (kg hl^{-1}) was determined using the Schopperchondrometer equipped with a 1 litter container. Grain hardness (g mm^{-2}) was also measured by an infrared informatics instrument.

Agronomic traits

Variations of agronomic traits in both types of genotypes were also recorded in order to analyze their relations with micronutrient contents and bread-making quality traits. Data were recorded for day to maturity (MA), grain yield (GY) as g per plant and thousand grain weight (TGW) as g.

Data analysis

Data were subjected to the analysis of variance (ANOVA) in SAS software. Means were statistically compared using the least significant differences ($LSD_{0.01}$) test. Genetic, phenotypic and environmental variances were calculated based on the expected mean squares of the source of variations in RCBD design. Genetic improvement (GI) index was also calculated using the equation proposed by Falconer and MacKay (1996) as follow:

$GI= ih^2\sigma_p$

Where, i, h^2 and σ_p are selection intensity (2.06), heritability estimate and root of phenotypic variance, respectively. The phenotypic (PCV) and genetic (GCV) coefficients of variation were also estimated based on the expected mean squares in ANOVA. For better understanding of the interrelationship between traits, genetic correlations and path analysis of traits were performed in SAS software. Heritability of traits was estimated according to the expected mean squares in ANOVA. Clustering of genotypes was performed using complete linkage method to assign similar genotypes into the same group based on agronomic, bread making quality and micronutrients data. Principal component analysis (PCA) was conducted to capture most of the variability in the original data in agronomic and bread making quality traits and micronutrients.

Conclusion

Micronutrients, gluten and agronomic traits variations were investigated in commercial cultivars and landrace varieties. Results indicated the higher potential of landraces compared with commercial cultivars. Landrace varieties accumulated higher micronutrients in grain indicating the potential of such germplasm and the fact that bio-fortification of commercial cultivars to alleviate micronutrients malnutrition has been forgotten. This may primarily due to difficulties and the higher cost of micronutrient assay in large crop populations. The correlation between grain Fe^{+2} and Zn^{+2} contents with grain yield was also negative. Regression model indicated that grain protein was highly associated with SDS volume, grain hardness, gluten dry weight and grain yield. Investigations for bread-making quality using grain hardness, SDS volume and zeleny sedimentation volume revealed that landrace varieties had insufficient bread-making quality compared with commercial cultivars. In general, results showed that crossing genotypes for incorporation of higher micronutrient, grain yield and better bread making quality would be more efficient than surveying for single superior plants via direct selection.

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