

# Association between Grain Size and Shape and Quality Traits, and Path Analysis of Thousand Grain Weight in Iranian Bread Wheat Landraces from Different Geographic Regions

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## Abstract

Grain characteristics, particularly grain weight, grain morphology, and grain protein content (GPC), are important components of grain yield and quality in wheat. A set of 98 bread wheat landraces from different geographic regions of Iran were used across 2013-2014 and 2014-2015 to determine the phenotypic diversity and relations between thousand grain weight (TGW), grain morphology and grain quality. A high-throughput method was used to capture grain size and shape. The genotypes were significantly different ( $P < 0.001$ ) for all traits which reflects the high levels of diversity. A moderate to high broad sense heritability was found for all traits and ranged between 0.68 and 0.95 for grain yield and factor from density (FFD), respectively. Significant positive correlations were observed between TGW and grain size (or shape) exception of aspect ratio (AR) and roundness. However, grain quality traits, especially GPC had significant negative correlation with TGW. Based on stepwise regression analysis by taking TGW as dependent variable, grain volume, FFD, width, perimeter and Hardness Index (HI) were recognized as the most important traits and explained more than 99.3% of total variation of TGW. The path analysis revealed that FFD has maximum direct effect on TGW followed by volume, whereas perimeter and width had relatively less direct effect on TGW. According to cluster analysis, landraces separated into 5 clusters, and cluster III and IV had the maximum and minimum average for the most traits, respectively. Our study provides new knowledge on the relations between TGW, grain morphology and grain quality in bread wheat, which may aid the improvement of wheat grain weight trait in further research.

**Keywords:** bread wheat, grain size and shape, grain quality, thousand grain weight, *Triticum aestivum*

## Introduction

Wheat (*Triticum aestivum* L.) as a strategic crop has a vital role in the developing countries economy and known as main food crop in abiotic stress prone areas which have low output productivity such as Iran (Abdipour *et al.*, 2013). With increasing world population, it has been estimated that the global demand for wheat will increase by a further 60% by 2050 (Licker *et al.*, 2010). It is a huge challenge to ensure global food security through sustainable wheat production for the projected population with the increasing adverse impact of climate change (Palm *et al.*, 2010). So, it is necessary to continuously raise production mainly through higher yields. Accordingly, development of high yielding varieties with good end-use quality is a major focus in wheat breeding programs. In the past four decades, improvement of grain yield has come from increased grains per

square meter or larger grain sizes, due to the utilization of *Rht* genes in wheat breeding (Calderini and Reynolds, 2000). However, the breeding gains in wheat yield have substantially slowed in recent years due to the lack of 'breakthrough' germplasm and breeding methodologies (Jia *et al.*, 2013). It has been proven that grain yield is a complex and quantitative trait that controlled by a number of genes with low heritability and it is significantly influenced by the environment, which make it difficult to be manipulated and improved in breeding programs (Koebner and Snape, 1999; Deng *et al.*, 2011). Wheat grain yield can be divided into several direct components: spike number per unit area, grain number per spike, and TGW (Fuller, 2007; Wang *et al.*, 2012). According to low heritability for most of the yield-related traits (Shi *et al.*, 2009), and laborious, time-consuming, and costly to measure yield-related traits, assess all of them at the early stages of a breeding

program is not benefit and practical. However, some components such as TGW are characterized by a higher heritability than grain yield itself (Bezant *et al.*, 1997; Huang *et al.*, 2006; Tsilo *et al.*, 2010; Deng *et al.*, 2011). TGW is positively correlated with agronomic yield (Baril 1992; Fuller, 2007; Maccaferri *et al.*, 2011) and flour yield (Chasten *et al.*, 1995; Breseghello and Sorrells, 2006; Williams *et al.*, 2013). Moreover, the high heritability values (59% to 96%) in most of the cultivars studied so far have proved that this character is phenotypically the most-stable yield component (Giura and Saulescu, 1996; Huang *et al.*, 2006; Sun *et al.*, 2009; Tsilo *et al.*, 2010; Patil *et al.*, 2013). Therefore, together with the number of seeds per square meter, improved TGW is one of the main targets of wheat breeding activities. However, TGW is a complex trait, and is largely controlled by several grain traits, including grain size and shape (Zhang *et al.*, 2014). Grain size is mainly characterized by grain weight and area, whereas shape means a relative proportion of the main growth axes of the grain (Breseghello and Sorrells, 2007; Gegas *et al.*, 2010). While, grain shape is generally estimated by length, width, vertical perimeter, sphericity and horizontal axes proportion (Breseghello and Sorrells, 2007). Many studies have been shown that wheat grain size and shape is positively correlated with TGW and they have affected flour yield, end-use quality and market price (Evers *et al.*, 1990; Breseghello and Sorrells, 2006; Tsilo *et al.*, 2010; Cui *et al.*, 2011; Blanco *et al.*, 2012; Williams *et al.*, 2013; Rasheed *et al.*, 2014). Theoretical models predict that milling yield could be increased by optimizing grain size and shape with large and spherical grains being the optimum grain morphology (Evers *et al.*, 1990). Although grain yield is one of the major determinants of a farmer's income, quality traits such as GPC is very important for bread-making quality. In more study, grain quality traits such as GPC in wheat have negatively correlated with grain yield and any genetic improvement in GPC has been restricted by the negative correlation between productivity and GPC (Kamra 1971; Bhatia 1975; Loffler and Busch, 1982; Blanco *et al.*, 2012). Although grain yield and GPC are often negatively associated, some researchers reported wheat cultivars with high grain yield and high GPC in bread and durum wheat (Stuber *et al.*, 1962; Johnson *et al.*, 1973; Sears, 1998; De Ambrogio and Ranieri, 2002; Oury *et al.*, 2003; De Ambrogio and Ranieri, 2002; Clarke *et al.*, 2005). The primitive wheat species exhibit broad variation in grain size and shape, and quality in contrast to modern wheat varieties, meaning that the modern breeding germplasm has lost grain morphology and quality variation, probably due to selection for more uniform grain shape and a certain quality in the elite varieties (Gegas *et al.*, 2010). In this context, landraces, wild forms and other related wild species can have crucial roles in breeding programs because of their wide variability in terms of phenological, morphological, abiotic, biotic and quality traits (Moragues *et al.*, 2006 and 2007; Peleg *et al.*, 2008). However, accurate characterization of grain size and shape remains a big challenge due to laborious, time consuming techniques (in particular in large sets of samples) and complex nature of wheat grain shape. (Houle *et al.*, 2010; Patil *et al.*, 2013). More recently, a high-throughput method was used to capture grain size and shape variations in multiple mapping populations, elite varieties, and in a broad collection of ancestral wheat species (Gegas *et al.*, 2010; Williams *et al.*, 2013; Okamoto *et al.*, 2013; Williams and Sorrells, 2014; Rasheed *et al.*, 2014). The majority of these studies have been

performed for only some grain morphology traits without quality traits. Therefore, in spite of the importance of these traits, a severe lack of information on the some important grain morphology and quality traits and as well as the association between TGW and grain morphology, and quality traits in bread wheat is evident. So, the evaluation of grain shape and size and the association between TGW and grain morphology can be useful in this case.

For this purpose, a collection of 98 bread wheat landraces from different geographic regions of Iran along with two local bread cultivar as check evaluated for TGW, grain morphology and quality traits. The present study was initiated (i) to determine the level of heritability, phenotypic and genotypic diversity of TGW, grain morphology and quality traits, (ii) identify association between TGW and other traits and develop best model to identify selection indirect indices for TGW (iii) and, the screen the best genotypes group based on studied traits.

## Materials and Methods

### *Plant materials and field trials*

A set of 100 bread wheat genotypes included 98 Iranian bread wheat landraces plus two additional check varieties as the best locally adapted cultivars used as plant materials in this study. This collection of bread wheat landraces were selected from 27 provinces that were classified into five groups based on their geographic origins. These landraces were generally winter hardy with different heading dates and flowering times. The genotype panel was planted under the open-field conditions in during the two growing seasons (2013/2014 and 2014/2015) at the experimental farm of College of Aburairhan, University of Tehran, Tehran (Pakdasht), Iran (53°28' N, 50°58' E; 1180 m above sea level). The soil included clay (32%), loam (39.2%) and sand (29.2%). The experiments followed a 10×10 square lattice design with three replications. Each cultivar was planted in two-row plots with a length of 2 m and 30 cm spacing rows (with 40 seeds per row). The grain yield ha<sup>-1</sup> was calculated based on the plot area (0.6m<sup>2</sup>).

### *Phenotypic evaluation*

Whole plots were harvested on 31 May 2014 and 15 June 2015 and stored at 4 °C until analysis. A minimum of 500 grains sound, intact grains were selected for evaluation. Undamaged, non-shriveled grains which excluded the occasionally extremely large or extremely small grains seen in some threshed samples were included as representative of each line. In this study, grain yield, TGW, nine grain morphology traits including; grain length, width, thickness, roundness, area, FFD, volume, perimeter, AR, and five grain quality traits including; GPC, Zeleny sedimentation (ZS), water absorption (WA), hardness index (HI), falling number (FN) were evaluated. The measurements of TGW, grain length, width, thickness, roundness, area, perimeter and AR were performed directly using the a digital grain analyzer assisted by an automatic digital image analysis suite that allowed high-throughput data collection from a large number of grains and lines. The quality traits was determined on whole-meal flour using near-infrared reflectance spectroscopy. The FFD describes the differences in grain density and the deviation of a shape from a cylindrical form, and was calculated using the formula in Eq. (1): (Giura and Saulescu, 1996):

$$FFD = \frac{\text{grain weight}}{(\text{grain width} \times \text{grain length})} \quad (1)$$

The grain volume was approximated as VOL<sub>xyz</sub> using the formula for volume of an ellipsoid (Eric W. Weisstein, Ellipsoid, from MathWorld: <http://mathworld.wolfram.com/Ellipsoid.html>) based on x, y, and z axes corresponding to grain width, length, and thickness measures (respectively) from grain counter using the formula in Eq.(2):

$$VOL_{xyz} = \left(\frac{4}{3}\right)\pi xyz \quad (2)$$

*Statistical analysis*

The frequency distributions of the phenotypic data were tested for normal distributions (with Kolmogorov–Smirnov test) to estimate the complexity of the genetic control of the traits. Analysis of variance (ANOVA) was performed to test the significance of source of variation and the efficiency of the lattice design compared to a randomized complete block design, using the PROC LATTICE statement in the SAS Version 9.0 (SAS Institute, Inc., Cary, NC). Since the efficiency of the lattice design compared to a randomized complete block design for all traits is less than 105 percent, the ANOVA and other analysis were performed based on the randomized complete block design.

The values of variance obtained from the ANOVA were used to calculate the broad sense heritability ( $h_B^2$ ), using the formula in Eq. (3):

$$h_B^2 = \frac{(\delta^2 e + r\delta^2_{gy} + ry\delta^2_g) - \delta^2 e + r\delta^2_{gy}}{ry} \quad (3)$$

where  $\delta^2_e$  is the error variance,  $\delta^2_{gy}$  is the genotypic and year interaction variance,  $\delta^2_g$  is the genotypic variance,  $r$  is the number of replication and  $y$  is the number of year.

The genotypic covariance and variance among traits were calculated using the PROC GLM and multiple analysis of variance (MANOVA) in SAS. The genotypic correlation coefficients were calculated using the formula in Eq.(4):

$$r_g = \frac{\delta_{gxy}}{\sqrt{(\delta_{gx}^2)(\delta_{gy}^2)}} \quad (4)$$

where  $\delta_{gxy}$  is the genotypic covariance between x and y traits,  $\delta_{gx}^2$  and  $\delta_{gy}^2$  are the genotypic variance for x and y traits, respectively.

In order to determine the best combination of variables that determinate grain weight in bread wheat landraces a stepwise regression analysis was employed by taking TGW as dependent variable and other traits as independent variables, using the formula in Eq.(5):

$$y = a + b_1x_1 + b_2x_2 + b_3x_3 + \dots + b_ix_i \quad (5)$$

where, y is the dependent variable (TGW), the x's are independent variables (measured traits) affecting dependent one, a is the intercept coefficient, and the b's are the related coefficients of independent variables in predicting the dependent variable.

To estimate the contribution of individual characters to grain yield, a path coefficient analysis using TGW as dependent variable and variables that remained in model as independent variables was computed.

In order to classify the genotypes, a hierarchical cluster analysis was carried out based on ward's method (Ward, 1963) and the similarity matrix, and the resulting dendrogram was drawn by IBM SPSS Statistics 22.0.0 software (<http://www.brothersoft.com/ibm-spss-statistics-469577.html>). MANOVA method was used to determine the correct cut-off point of clusters. We used four statistics in MANOVA, including: Wilks' Lambda, Pillai's Trace, Hotelling-Lawley Trace and Roy's Greatest Root statistics by SAS Version 9.0 (SAS Institute, Inc., Cary, NC).

**Results**

*Variation and heritability*

The raw data of combined experiments across two years were checked for normal distributions, and the variation for grain weight, grain morphology and grain quality measures were tested by two-way ANOVA (Table 1).

Four sources of variation were considered: year, block in year, genotype, and genotype × year. There were significant differences between two years for most of the traits with the exception of

Table 1. Combined analysis of variance for bread wheat landraces over two years (2013-2015)

Source of Variation	df	Length	Width	Thickness	TGW	AR	Roundness	Area	FFD	Volume
Year (Y)	1	9.521**	0.461	0.587	1540.67**	0.254	0.124	341.45**	1.89E-06	8245.4
Block (Year)	4	0.925*	0.275**	0.194	154.88***	0.065	0.031	40.93**	4.70E-07	3214.2***
Genotype (G)	99	9.254***	7.670***	6.235***	845.13***	1.123***	0.521***	300.45***	2.70E-05***	21451.2***
G×Y	99	0.511**	0.088	0.142*	37.25**	0.088*	0.024	17.24**	3.20E-07*	745.3*
Error	396	0.329	0.075	0.110	25.15	0.062	0.021	11.24	2.30E-07	543.0

\*, \*\* and \*\*\* Represents significance at P < 0.05, P < 0.01 and P < 0.001 respectively.  
TGW: Thousand grain weight; AR: Aspect ratio; FFD: Factor from density

Table I (continued)

Source of Variation	df	Perimeter	GPC	ZS	HI	WA	FN	BV	Yield
Year (Y)	1	828.21**	284.32**	425.72*	324.56*	1345.02**	45431.3***	19354.3*	2662267**
Block (Year)	4	87.23**	24.18*	62.14*	54.23*	132.42**	3321.5*	2974.2*	305498***
Genotype (G)	99	632.32***	185.67***	421.45***	573.45***	533.41***	18321.9***	20321.9***	920076***
G×Y	99	21.00	14.25**	29.87**	30.21*	43.25**	1587.2**	1354.8*	107569***
Error	396	22.21	9.25	19.48	22.34	32.56	1024.3	978.3	63448

\*, \*\* and \*\*\* Represents significance at P < 0.05, P < 0.01 and P < 0.001 respectively.  
GPC: Grain protein content; ZS: Zeleny sedimentation; HI: Hardness index; WA: Water absorption; FN: Falling number; BV: Bread volume

Table 2. Phenotypic variation for grain weight, grain size (and shape) and grain quality traits over two years (2013-2015)

Variables	Minimum	Maximum	Mean±SD	CV(%)	$\delta^2_G$	$b^2$ (%)
Length	4.85	9.64	7.26±0.57	7.90	1.46	81.58
Width	2.66	4.52	3.35±0.27	8.17	1.26	94.40
Thickness	2.34	4.07	3.19±0.33	10.41	1.02	90.23
TGW	26.2	68.7	50.07±5.01	10.02	134.65	84.26
AR	1	3.22	2.11±0.25	11.82	0.17	73.56
Roundness	0.43	0.99	0.68±0.14	21.27	0.08	79.78
Area	8.1	26.5	17.48±3.35	19.18	47.20	80.77
FFD	1.29E-03	2.89E-03	2.06E-03±4.8E-04	23.29	4.45E-06	95.08
Volume	159.07	458.02	326.53±23.30	7.14	3450.98	86.40
Perimeter	13.71	28.07	22.78±4.71	20.69	101.89	82.10
GPC (%)	13.3	14.7	14.04±3.04	21.66	28.57	75.54
ZS	35	37	35.74±4.41	12.35	65.26	77.01
HI	46	55	51.12±4.73	9.25	90.54	80.21
WA (%)	63.2	66	64.38±5.71	8.86	81.69	70.50
FN	330	663	513±32	6.24	2789.12	73.14
BV	435	554	501±31.27	6.24	4139.14	76.37
Yield	800	9188.89	4027.97±251.89	6.25	135417.83	68.10

SD: Standard deviation; CV: Coefficient of variation;  $\delta^2_G$ : Genotypic variance;  $b^2$ : Broad sense heritability; TGW: Thousand grain weight; AR: Aspect ratio; FFD: Factor from density; GPC: Grain protein content; ZS: Zeleny sedimentation; HI: Hardness index; WA: Water absorption; FN: Falling number; BV: Bread volume

Table 3. Coefficients of genotypic correlation for grain weight, grain size (and shape) and grain quality traits over two years (2013-2015)

Variable	Length	Width	Thickness	TGW	AR	Roundness	Area	FFD	Volume
Width	0.090	1							
Thickness	0.290**	0.456**	1						
TGW	0.694**	0.388**	0.608**	1					
AR	0.646**	-0.585**	-0.079	0.156	1				
Roundness	0.023	0.064	0.225*	0.055	0.149	1			
Area	0.740**	0.681**	0.564**	0.653**	0.119	0.033	1		
FFD	0.157	-0.177	0.270**	0.647**	0.125	0.044	-0.044	1	
Volume	0.665**	0.705**	0.787**	0.753**	0.018	0.142	0.919**	0.063	1
Perimeter	0.969**	0.329**	0.387**	0.753**	0.481**	0.039	0.839**	0.169	0.802**
GPC (%)	0.124	-0.028	-0.256**	-0.210*	0.165	0.303**	-0.032	-0.420**	-0.072
ZS	-0.121	-0.111	-0.108	-0.055	-0.005	0.06	-0.240*	0.098	-0.142
HI	-0.048	0.189	-0.089	-0.105	-0.148	0.004	0.033	-0.231*	0.032
WA (%)	0.127	0.159	-0.205*	-0.167	-0.040	-0.223*	0.120	-0.481	0.031
FN	0.048	-0.255*	-0.174	0.040	0.228*	0.086	-0.080	0.196	-0.180
BV	0.064	-0.172	-0.193	-0.019	0.115	-0.250*	-0.112	0.030	-0.141
Yield	0.212*	0.296**	0.352**	0.448**	-0.150	0.012	0.267**	0.234**	0.375**

\* and \*\* Represents significance at  $P < 0.05$  and  $P < 0.01$  respectively. TGW: Thousand grain weight; AR: Aspect ratio; FFD: Factor from density; GPC: Grain protein content; ZS: Zeleny sedimentation; HI: Hardness index; WA: Water absorption; FN: Falling number; BV: Bread volume

Continued Table 3.

Variable	Perimeter	GPC (%)	ZS	HI	WA (%)	FN	BV	Yield
GPC (%)	0.106	1						
ZS	-0.143	0.325**	1					
HI	-0.005	0.531**	0.200*	1				
WA (%)	0.153	0.524**	0.176	0.477**	1			
FN	-0.014	0.137	-0.020	-0.013	-0.198	1		
BV	0.019	0.062	-0.028	-0.185	0.252*	-0.149	1	
Yield	0.264**	-0.407**	-0.114	-0.172	-0.231*	-0.134	0.03	1

\* and \*\* Represents significance at  $P < 0.05$  and  $P < 0.01$  respectively.

GPC: Grain protein content; ZS: Zeleny sedimentation; HI: Hardness index; WA: Water absorption; FN: Falling number; BV: Bread volume

grain width, thickness, AR, roundness, FFD and volume. However, due to the large number of traits and the large volume of data we used the average of two years for other analysis. Significant differences were observed for all traits with the exception of thickness, AR, roundness and FFD for block (year) effect. However, the genotypes showed very significant differences ( $P < 0.001$ ) for all traits. On the other hand, all traits with the exception of the grain width, roundness and perimeter showed significant differences for year  $\times$  genotype effect. Relative to genotype main effects, the magnitude of year  $\times$  genotype interaction effects was often small.

The phenotypic data for grain weight, grain morphology and grain quality descriptors were averaged from two cropping seasons in 2103-2014 and 2014-2015. For the traits evaluated, the means and ranges, the coefficient of variation, the genotypic variance and heritability estimates presented in Table 2. The high differences between the minimum and maximum of studied traits is a result of high difference between genotypes. This collection of diverse genotypes with such ideal levels for traits can be use as a good source for diversity and mapping studies.

A moderate to high broad sense heritability was found for all traits and ranged between 0.68 and 0.95 for grain yield and FFD,

respectively. The moderate to high heritability values are an index of the weak environmental effect. The all grain size and shape traits with the exception of the AR and roundness had over 80 percent heritability. However, the heritability for quality traits ranged 0.70 and 0.80 for WA and HI, respectively.

#### Genotypic correlation

The coefficients of genotypic correlation were calculated for all traits as means from the two growing seasons (Table 3). Grain length and perimeter showed the maximum positive correlation (0.97), followed by  $r = 0.92$  between grain area and volume. By contrast, grain width and AR showed the maximum negative correlation (-0.59), followed by  $r = -0.42$  between FFD and GPC. Interestingly, the correlations between all grain shape direct measurements and grain weight were positive and very significant. For example, grain length and width were highly correlated with TGW with estimate of  $r = 0.69$  and  $r = 0.39$ , respectively. Similarly, grain thickness was highly correlated with TGW ( $r = 0.61$ ). The other grain morphology traits with the exception of the AR and roundness had highly positive correlation with TGW. The among quality traits, just FN had very low positive association with TGW ( $r = 0.04$ ) and the other traits as negatively correlated with TGW, from -0.09 to -0.210 for HI and GPC, respectively.

#### Stepwise regression analysis and path coefficient analysis

A stepwise regression analysis was computed in order to eliminate no effective variables on grain weight in regression model and study only traits affecting significantly grain weight changes by taking TGW as dependent variable (Table 4). Based on this method, grain volume as the first entered variable in model was the most important character and had the strongest variation in TGW. This model could justify significantly more than 56 percent changes in performance. Four variables, including FFD, width, perimeter and HI respectively were entered to regression model after grain volume. In final step, these variables with grain volume had justified 99.33% of TGW variation (Table 5). Regression coefficients for the accepted variables are shown in Eq.(6). Therefore, based on the final step of stepwise regression analysis, the equation for prediction of TGW was computed as follows:

$$y = -69.16 + 0.00012x_1 + 23326x_2 + 9.81x_3 + 1.81x_4 - 0.057x_5 \quad (6)$$

Where, y is the TGW;  $x_1, x_2, x_3, x_4$  and  $x_5$  are grain volume, FFD, width, perimeter and HI, respectively.

A path coefficient analysis was computed by taking TGW as dependent variable, in order to have a clear understanding of the effect of individual measurement on TKW, and grain volume, FFD, width, perimeter and HI as independent variables (Table 6). FFD exhibited maximum positive direct effect on grain weight followed by volume. Grain perimeter and width had moderate to low positive direct effects on grain weight, respectively. However, protein has a poor negative direct effect on grain weight.

#### Cluster analysis

In order to categorize genotypes that are similar into one group and others into different groups, a cluster analysis used. The most F value for all statistics in MANOVA obtained when the cluster divided into five groups (Table 7). So, the cluster divided into five groups and genotypes separated into groups. Cluster I to V consisted of 35, 39, 2, 2 and 22 genotypes, respectively. Cluster II with 39%, and cluster III and IV with 2% of total genotypes were recognized as the largest and the smallest clusters, respectively. The

average and standard deviation of traits for each cluster from ground mean are shown in Table 8. A diagram of cluster analysis (dendrogram) is given in Fig. 1.

## Discussion

Grain characteristics, particularly grain weight, grain size and shape, and grain protein, are important components of grain yield and quality in wheat due to their significant effect on grain yield, milling yield, end-use quality and market price. In recent years advances in phenotypic measurements have fallen behind progress in high-throughput geno-typing. In order to take full advantage of low-cost genotyping resources, plant breeders are aggressively pursuing more accurate and efficient phenotyping methods (De Souza, 2010; Houle *et al.*, 2010; Montes *et al.*, 2007). Manual measurement methods of grain morphology have limits to the number of data, the quality of measurements, and the variety of shape data that can be gleaned. By contrast, computational methods using digital image technology could enable us to automatically measure robust grain size and shape descriptors (Williams *et*

Table 4. Stepwise regression on the TGW as dependent variable over two years (2013-2015)

Source of variation	df	Mean Square	F
Model	5	1172.62	2430.91***
Error	94	0.4823	
Corrected total	99		

\*\*\* Represents significance at  $P < 0.001$

Table 5. Relative contribution (partial and model R<sup>2</sup>) and F value in predicting TGW by stepwise regression over two years (2013-2015)

Variable in Model	Partial R-Square	Model R-Square	F
Volume	0.5618	0.5618	125.64***
FFD	0.3481	0.9090	374.81***
Width	0.0816	0.9915	925.80***
Perimeter	0.0010	0.9925	12.02***
HI	0.0009	0.9933	12.12***

\*\*\* Represents significance at  $P < 0.001$

FFD: Factor from density; HI: Hardness index

Table 6. Direct (diagonal and bold) and indirect effects of variables remained in stepwise regression model on TGW over two years (2013-2015)

Variable	Volume	FFD	Perimeter	Width	HI
Volume	<b>0.392</b>	0.037	0.239	0.083	-0.001
FFD	0.024	<b>0.592</b>	0.05	-0.021	0
Perimeter	0.315	0.1	<b>0.299</b>	0.038	0
Width	0.276	-0.105	0.098	<b>0.117</b>	-0.001
HI	0.012	-0.137	-0.002	0.022	<b>-0.002</b>
Residual effect	0.223				

FFD: Factor from density; HI: Hardness index

Table 7. MANOVA of bread wheat landraces for 5 clusters

Statistic	Value	F Value
Wilks' Lambda	7.431	32.74***
Pillai's Trace	10.424	21.24***
Hotelling-Lawley Trace	354.625	82.77***
Roy's Greatest Root	276.025	299.11***

\*\*\* Represents significance at  $P < 0.001$

Table 8. Mean and standard deviation of grain weight, grain size (and shape) and grain quality traits over two years (2013-2015)

Variable	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
Length	7.39±0.52	7.59±0.45	7.80±0.20	5.29±0.32	6.59±0.65
Width	3.26±0.22	3.57±0.20	3.72±0.16	3.14±0.05	3.11±0.19
Thickness	3.16±0.14	3.35±0.18	3.70±0.27	2.53±0.08	2.95±0.17
TGW	50.55±6.75	55.59±4.80	53.50±0.17	30.87±0.43	40.95±6.04
AR	2.21±0.31	2.06±0.26	2.12±0.04	1.62±0.11	2.07±0.35
Roundness	0.69±0.12	0.69±0.13	0.81±0.09	0.64±0.04	0.64±0.10
Area	17.12±1.46	19.46±1.29	21.48±0.55	11.15±0.65	14.76±1.31
FFD	2.11E-03±2.69E-04	2.06E-03±2.02E-04	1.85E-03±1.32E-04	1.87E-03±1.14E-04	2.00E-03±2.24E-04
Volume	317.56±15.73	378.34±20.93	448.19±1.63	175.51±7.48	251.61±17.52
Perimeter	23.03±1.63	24.49±1.41	25.62±1.12	15.15±1.24	19.77±2.12
GPC (%)	14.13±0.39	14.02±0.44	14.05±0.35	14.15±0.55	14.00±0.46
ZS	35.83±0.56	35.85±0.58	36.00±0.00	36.00±1.00	35.91±0.60
HI	50.97±2.12	50.62±2.34	53.00±1.00	52.50±2.50	50.86±2.36
WA (%)	64.43±0.68	64.44±0.63	64.45±0.25	64.65±1.05	64.36±0.67
FN	500.14±26.21	500.33±25.43	452±17	500±11	506.68±23.91
BV	500±26	500±25	452±17	500±11	506±24
Yield	3899.11±1389.74	4563.06±1299.54	4455.56±374.07	3388.89±877.78	3303.64±1374.39
Number of genotypes	35	39	2	2	22

TGW: Thousand grain weight; AR: Aspect ratio; FFD: Factor from density; GPC: Grain protein content; ZS: Zeleny sedimentation; HI: Hardness index; WA: Water absorption; FN: Falling number; BV: Bread volume

*et al.*, 2013). Only few studies are available based on digital image analysis of grain size and shape in bread wheat (Williams *et al.*, 2013; Gegas *et al.*, 2010; Williams and Sorrells, 2014; Brescghello and Sorrells, 2007; Xiao *et al.*, 2011; Rasheed *et al.*, 2014; Zhang *et al.*, 2015). Among these studies, Gegas *et al.*, 2010; Williams *et al.* (2013), Williams and Sorrells (2014) and Rasheed *et al.* (2014), used shape variations as targeted traits influencing grain size and weight and results are comparable to our work. However, the most of them just used some grain morphology traits and didn't use any quality traits. The high significant differences ( $P < 0.001$ ) among genotypes for all traits indicating the presence of adequate variability among the bread wheat local landraces which can be used for the improvement of grain weight and quality in order to create high yielding varieties with good end-use quality. Though compare the measures for traits such as grain size and shape in different studies because of different approaches to converting raw images into quantitative data is not completely correct, differences between high and low level for the most traits in this study are more in compared with other studies such as Sun *et al.* (2009), Tsilo *et al.* (2010), Jia *et al.* (2013) and Zhang *et al.* (2015). Due to the high diversity, it seems can find suitable genotypes with high and low limit bounds for two or more traits as versus each other. For example, two genotype 97 and 42 with 9.64 and 7.58 length, and with 3.43 and 4.53 width can used as parents to create segregation populations for quantitative trait loci (QTL) studies. Despite the high variety and acceptable quality of Iranian bread wheat landraces, it seems these genotypes had lower quality compared with some bread populations in Blanco *et al.* (2012) and Kalous *et al.* (2015) studies.

Similar to the results of other researchers (Huang *et al.*, 2006; Sun *et al.*, 2009; Tsilo *et al.*, 2010; Blanco *et al.*, 2012 and Patil *et al.*, 2013), the grain yield with the lowest heritability, more than other traits had influenced by environmental factors. By contrast, other traits were less environmentally sensitive and have higher heritability than

grain yield itself. However, FFD, grain width and thickness with over 0.9 heritability, followed by volume and TGW (0.86 and 0.84, respectively), had the highest heritability. These characters were phenotypically the most-stable yield components and can used as independent descriptors in the breeding programs for grain yield improvement.

Some other grain morphology traits such as volume, perimeter and area also because of larger heritability than grain yield can used as indirect indices in grain yield improvement (Table 2). Grain quality traits with exception of HI after grain yield had the most affectability by environmental factors. By contrast, HI less environmentally sensitive and have higher heritability than other quality traits. Tsilo *et al.* (2010) also were reported larger heritabilities for kernel morphology and quality traits than grain yield. Huang *et al.* (2006), for TGW and quality traits and Blanco *et al.* (2012), for TGW and quality traits similarly were reported larger heritabilities than grain yield. Russo *et al.* (2014), also observed high heritability for TGW, but they had lower heritabilities for grain size and shape. However, heritability estimates for each trait can be different, depending upon the genetic material, environment and the method of computation (Blanco *et al.*, 2012).

As a result of genetic correlations, several kernel size and shape measurements are inherently correlated, like length versus AR, area and perimeter ( $r=0.65$ , 0.74 and 0.97 respectively) and width versus thickness (0.46). According to strong associations among these traits, it is possible with increasing of one of them, the other traits creased. Rasheed *et al.* (2014) and Russo *et al.* (2014), also reported similar correlations for mentioned traits in bread and durum wheat, respectively.

In accordance with the results of Rasheed *et al.* (2014), other important derived measurements like volume and perimeter on the contrary FFD were also positively correlated with length and width. So, with the contemporary increasing of grain length and width, volume and perimeter can creased.

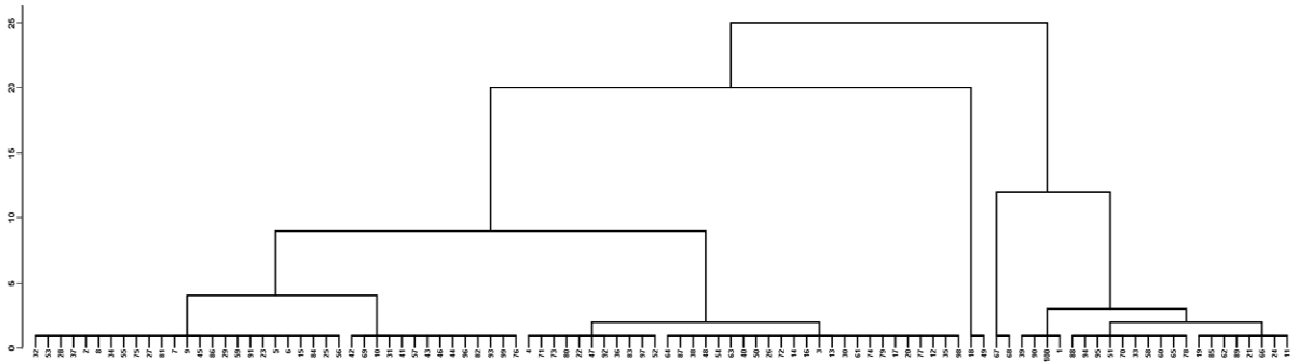


Fig. 1. Dendrogram produced using Ward's minimum variance method based on similarity matrix of bread wheat landraces

Roundness showed significant positive correlation only with thickness. However, this trait had poor positive correlation with other grain morphology traits. So, It seems with the selection of kernel with more thickness, the roundness will be creased. This positive significant association between roundness and thickness before reported by Rasheed *et al.* (2014). However, Russo *et al.* (2014), reported a significant positive association between roundness and length, and a significant negative correlation between roundness and width.

The weak correlation of AR and roundness with TGW with estimate of  $r = 0.16$  and  $r = 0.06$ , respectively, giving the first indication that these traits are independent. However, all grain morphology traits with the exception of AR and roundness showed a strong and positive relationship with TGW. According to correlation result, it would be preferred if grain size and shape were used in selection to increase grain weight. Gegas *et al.* (2010) and Rasheed *et al.* (2014), also reported a such correlations between TGW and above mentioned traits. Despite the strong correlation between TGW and grain morphology traits, GPC showed negative significant association with TGW. Meanwhile, other quality traits with exception of HI had negatively correlation with TGW, range from -0.05 to -0.17 for ZS and WA, respectively. Therefore, by the creasing of grain quality, TGW can be decreased. It can be a result of wheat cultivars with high grain yield and low quality or vice versa. Other researchers also have similar result and reported grain quality traits such as GPC in wheat have negatively correlated with grain yield and any genetic improvement in GPC has been restricted by the negative correlation between productivity and GPC (Kamra 1971; Bhatia 1975; Loffler and Busch, 1982 and Blanco *et al.*, 2012).

In this study, all morphology traits with exception of AR and roundness showed strong and positive correlation with grain yield, meanwhile, GPC and WA had significant negative correlation with grain yield. The efficiency of indirect selection depends on the correlation between a selected trait and a target trait as well as the heritability of the selected trait (Blanco *et al.*, 2012). As a result of our study, all morphology traits with exception of AR and roundness with moderate to high heritability, and high positive correlation with TGW had good efficiency as indirect selection for TGW. Gegas *et al.* (2010), also confirmed that kernel size and shape were largely independent traits in a study of six wheat populations.

Based on stepwise multiple regression analysis as a multiple statistical method that can screen or select the most important variables through a dependent variable such as the grain weight (Draper and Smith, 1966), the two first variable that entered in model i.e. volume and FFD are the most important variables contributing to the grain weight. Three other variables including the perimeter, width and HI that entered in model in next steps are important in addition to the two mentioned variables.

At the end of stepwise regression analysis, a five variables regression model with explain more than 99.33% of TGW variation, was recognize as the best model. The other variables were not included in the analysis due to their low relative contributions. Existence of positive and significant R square (regression coefficient) of volume, FFD, width and perimeter in a successful regression equation indicates the effectiveness of these traits to increase grain weight. Considering the positive and significant regression coefficient, it could be stated that increase in the amount of these characters would increase the grain weight. Furthermore, regarding the negative and significant regression coefficient of HI, it could be said that by increasing the amount of this trait, TGW will be reduced.

The association of grain size and shape, and quality descriptors with TGW was further resolved by path coefficient analysis which depicted the phenotypic model with more precision (Table 6). This revealed that FFD has maximum direct effect on grain weight followed by volume, whereas perimeter and width had relatively less direct effect on grain weight. On the other hand, HI had negative direct effect on grain weight and this should undergo negative selection in order to get superior genotypes with high grain weight. Although, the efficiency of indirect selection depends on the correlation between a selected trait and a target trait as well as the heritability of the selected trait, it seems the magnitude and positive or negative direct effects are also important. Therefore, FFD because of having the maximum direct effect, high correlation with TGW and high heritability as the best indirect indices for TGW was recognized in this study. The grain volume followed by perimeter and width also can be used as a good indirect descriptors for TGW.

According to cluster analysis 100 genotypes of wheat separated into 5 clusters, a range of two to 39 members (Fig. 1). The cluster III and IV had the highest and lowest average for the most traits, and identified as the most valuable and the least significant clusters, respectively.

Though cluster analysis grouped genotypes together with greater morphological similarity, the clusters did not necessarily include all genotypes from same origin.

## Conclusion

Conclusively, we found high diversity for all traits in this collection of Iranian bread wheat landraces, specialty for grain size and shape. The most of descriptors had higher heritability than TGW. FFD and volume had high correlation with TGW and explained the most of TGW variation. These former traits as indirect selection indices can be use for improving grain weight and enhanced our deep understanding on grain weight components in wheat.

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