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Variability and Association Grain Weight with Grain size (and shape) and grain quality, and stepwise Regression analysis on thousand grain weight in Iranian Durum Wheat Landraces

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ABSTRACT: Grain weight and quality in durum wheat, greatly influence by grain morphology and grain protein content (GPC). In order to detection of phenotypic diversity level and relations between thousand grain weight (TGW), grain size (and shape) and grain quality, a collection of 79 durum wheat landraces from different geographic regions of Iran was used across two years (2013-15). A high-throughput method was used to capture grain size and shape. The high level of variation was observed with significant differences (P <0.001) among genotypes for all traits. A moderate to high broad sense heritability was found for all traits and ranged between 0.70 and 0.93 for grain yield and width, respectively. Grain weight significantly correlated with grain morphology (with exception of aspect ratio (AR) and roundness) and GPC. It can be a result of durum wheat landraces with high grain yield and high grain protein. By taking TGW as dependent variable a six variables regression model, including grain volume, factor from density (FFD), width, length, perimeter and GPC with explain more than 99.25% of TGW variation, was recognize as the best model. Based on path analysis, FFD exhibited maximum positive direct effect on grain weight followed by perimeter. Grain length, width and volume had similar positive direct effects. However, GPC has negative direct effect on grain weight. According to cluster analysis, landraces separated into 6 clusters, and cluster V and IV had the maximum and minimum average for the most traits, respectively. This study provides useful information on the relations between TGW, grain size (and shape) and grain quality in durum wheat, that may help to improve grain weight in breeding programs.

Key words: Durum wheat, *Triricum turgidum*, TGW, Grain size and shape, Grain quality

INTRODUCTION

Durum wheat (*Triticum turgidum* L. var. durum) because of features related to responses to abiotic stress has promoted its spread in semiarid environments better than any other cultivated wheat. Now, it as well-adapted crop to the Mediterranean regions is the main source of semolina for the production of pasta, bagel, couscous and other Mediterranean local end-products (Russo *et al*. 2014). Always increasing the yield potential of wheat has been a major focus of most wheat breeding programs around the world. Since the introduction of reduced height (Rht) into wheat varieties in the 1960s, wheat production has experienced a tremendous yield increase. However, the breeding gains in wheat yield have substantially slowed in recent years due to the lack of 'breakthrough' germplasms and breeding methodologies (Jia *et al.* 2013). Basically, grain yield is a complex trait and usually controlled by a number of quantitative trait loci (QTL) with minor effects. It is also influenced by environmental factors, which make it difficult to be manipulated and improved in breeding programs (Deng *et al*. 2011). Wheat grain yield is determined by three key factors, viz. spikes per unit area, grain number per spike and TGW (Fuller 2007; Wang *et al*. 2012). Unfortunately, most of the yield-related traits are controlled by genes with low heritability (Shi *et al*. 2009). However, some of them are less environmentally sensitive and have higher heritability than grain yield itself (Bezant *et al*. 1997; 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). Grain shape is generally estimated by length, width, vertical perimeter, sphericity and horizontal axes proportion (Breseghello and Sorrells 2007). Many studies have been showed that wheat grain size and shape have positively correlation with TGW and have influenced four 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 and sorrels, 2014; 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). 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; Lofler and Busch 1982; Blanco *et al*. 2012). Although grain yield and GPC are often negatively associated, researchers also reported wheat cultivars with high grain yield and high GPC (Stuber *et al*. 1962; Johnson *et al*. 1973). Nonetheless, some selected genotypes in bread wheat (Sears 1998; Oury *et al*. 2003) and durum wheat (De Ambrogio and Ranieri 2002; Clarke *et al*. 2005) did not follow this general relationship, which represented an increases in both grain yield and GPC. According to Sears (1998) it is possible to improve both GPC and grain yield simultaneously when an adequate source of genes increasing GPC is used in wheat breeding. However, the primitive wheat species exhibit broad variation in grain size and shape, and grain quality traits in contrast to modern wheat varieties, meaning that the modern breeding germplasm has lost grain shape 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; Okamoto *et al*. 2013; Rasheed *et al*. 2014).

The majority of these studies have been performed in bread wheat, while there is a lack of such information available for durum wheat (Blanco *et al*. 2001, 2012; Peng *et al*. 2003; Elouafi and Nachit 2004; Golabadi *et al*. 2010; Patil *et al*. 2013). On the other hand, there is not information available about the association between grain morphology and quality traits in durum wheat.

In the present study, 79 durum wheat landrace from different geographic regions of Iran along with two local durum cultivar as check were evaluated for grain morphology and quality traits. The objectives of this study were to: (i) estimate 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 grouping of genotypes based studied traits and screen the best genotypes group. This study provide useful information for increased yield in future wheat breeding programs.

MATERIALS AND METHODS

A. Plant Materials and Field Traits

A panel of 79 Iranian durum wheat landraces plus two additional checks as the best locally adapted cultivars used as plant materials in this study. This collection of durum wheat landraces were selected from 25 provinces that was 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 Aburaihan, University of Tehran, Tehran (Pakdasht), Iran (53°28' N, 50°581' E; 1180 m above sea level). The soil included clay (32%), loam (39.2%) and sand (29.2%). The experiments followed a 8×8 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^{-1} was calculated based on the plot area ($0.6m^2$).

B. 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 four grain quality traits including; GPC, Zeleny sedimentation (ZS), hardness index (HI) and 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).

$$FFD = \frac{grain\,weight}{(grain\,width \times grain\,lenght)} \quad (1)$$

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

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

C. 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 was 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^2b), using the formula in Eq. (3):

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

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 coefficients of genotypic correlation were calculated using the formula in Eq.(4):

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

where δ_{gxy} is the genotypic covariance between *x* and *y* traits, δ^2_{gx} and δ^2_{gy} are the genotypic variance for *x* and *y* traits, respectively.

In order to determine the best combination of variables that determinate grain weight in durum 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):

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

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 grouping of genotypes, a hierarchical cluster analysis was carried out based 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). To determine the correct cut-off point cluster that determine the correct number of clusters, used MANOVA. We used four statistics in MANOVA, including: Wilks' Lambda, Pillai's Trace, Hotelling-Lawley Trace and Roy's Greatest Root statistics.

RESULT AND DISCUSSSION

A. Variation and Heritability

Grain size and shape along with grain quality traits are among the most important agronomic traits due to their significant effect on grain weight, milling yield, end-use quality and market price. 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 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; Breseghello and Sorrells, 2007; Xiao *et al*. 2011). Unfortunately, there is not such studies in durum wheat.

However, there are rare studies such as Russo *et al*. (2014) used shape variations as targeted traits influencing grain size and weight in durum wheat and for some results are comparable to our work. The data of combined experiments were checked for normal distributions, and the variation for grain weight, grain morphology and grain quality measures was tested by two-way ANOVA (Table 1). Four sources of variation were considered: year, block in year, genotype, and genotype × year. The ANOVA revealed significant differences between two years for most of the traits with the exception of thickness, round, FFD and HI. However, due to the large number of traits and the amount of data we used the average of two years for other analysis.

The all traits with the exception of AR, roundness and FFD for block (year) effect had significant differences. However, there were very significant differences (P < 0.001) among genotypes for all parameters. On the other hand, all traits with the exception of the width, perimeter and HI showed significant differences for year × genotype effect. Compared to the main effects of genotype, the magnitude of year × genotype interaction effects was often small. Significant differences among genotypes for all traits, indicating the presence of high level of variability among the durum wheat landraces which can be exploited through selection. However, the high variability for any character is very important in the improvement of crop through breeding. The phenotypic data for grain weight, grain morphology and grain quality descriptors were averaged from two cropping seasons in 213-2014 and 2014-2015, and the basic statistics for them summarized in Table 2.

Table 1: Combined analysis of variance for durum wheat landraces over two years (2013-15).

Source of Variation	df	Length	Width	Thickness	TGW	AR	Roundness	Area	FFD
Year (Y)	1	10.254**	1.965*	0.942	378.23**	0.875*	0.541	234.95**	1.20E-06
Block (Year)	4	1.240*	0.458**	0.287*	38.25*	0.187	0.143	25.36*	4.10E-07
Genotype (G)	80	13.254***	9.454***	7.453***	625.34***	1.680***	1.721***	284.23***	1.20E-05***
G×Y	80	0.578*	0.142	0.152*	24.35**	0.112*	0.081***	14.46**	2.10E-07***
Error	320	0.426	0.124	0.107	15.64	0.082	0.064	9.21	1.80E-07

*, ** 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 1: Combined analysis of variance for durum wheat landraces over two years (2013-15).

Source of Variation	df	Volume	Perimeter	GPC	ZS	HI	FN	Yield
Year (Y)	1	9364.7*	445.37*	221.35**	351.24*	174.35	14204.6**	2585466**
Block (Year)	4	1898.5***	71.35**	21.45*	33.54*	51.21*	1562.9*	317422***
Genotype (G)	80	11641.9***	435.46***	155.64***	321.45***	556.74***	8554.2***	911254***
G×Y	80	586.3**	21.06	11.02**	20.45*	22.78	745.3**	90215**
Error	320	387.2	17.25	7.15	13.24	18.67	472.3	58235

*, ** 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

Table 2: Phenotypic variation for grain weight, grain size (and shape) and grain quality traits over two years (2013-15).

Variables	Minimum	Maximum	Mean±SD	CV(%)	δ^2_G	h^2(%)
Length	5.7	11.7	7.22±0.65	9.05	2.11	0.83
Width	2.22	3.91	3.3±0.35	10.68	1.55	0.93
Thickness	2.2	3.77	3.14±0.33	10.40	1.22	0.92
TGW	32.1	85	48.15±3.95	8.21	66.83	0.81
AR	1.61	3.33	2.12±0.29	13.49	0.26	0.76
Roundness	0.44	0.95	0.66±0.25	38.40	0.27	0.81
Area	10.2	30.01	16.88±3.04	17.98	44.96	0.83
FFD	1.34E-03	2.78E-03	2.03E-03±4.24E-04	20.88	1.97E-06	0.92
Volume	198.25	449.94	314.18±19.68	6.26	1842.59	0.83
Perimeter	15.4	30.99	21.33±4.15	19.48	69.07	0.80
GPC (%)	14.30	14.90	14.67±2.67	18.22	24.10	0.77
ZS	34.00	38.00	36.31±3.64	10.02	50.17	0.79
HI	52.00	62.00	59.73±4.32	7.23	88.99	0.83
FN	195.00	751.00	481.65±21.73	4.51	1301.48	0.73
Yield	511.11	11066.67	3863.43±241.32	6.25	136839.91	0.70

SD: Standard deviation; CV: Coefficient of variation; δ^2_G: Genotypic variance; h^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; FN: Falling number

The high differences between the minimum and maximum of studied traits is a result of high difference between genotypes. This collection of different genotypes with such ideal levels for traits can be use as a good source for diversity and mapping studies.

Though compare the measures for traits such as grain size and shape in different studies because of different methods for evaluation 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 Troccoli *et al*. (2000) and Russo *et al*. (2014). 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 genotypes 44 and 20 with 11.7 and 7.45 length, and with 3.19 and 3.91 width can used as parents to create segregation populations for QTL studies (data not showed). Despite the high variety of quality properties for Iranian durum wheat landraces, it seems these landraces had lower quality compared with some durum populations in Blanco *et al*. (2012); Kalous *et al*. (2015) studies.

Broad sense heritability was found to be moderate to high for all traits and ranged between 0.70 and 0.93 for grain yield and width, respectively. The heritability mid-values are an index of the strong environmental effect. The all grain size and shape traits with the exception of the AR had over 80 percent heritability. However, the heritability for quality traits ranged 0.73 and 0.83 for FN and HI, respectively. As expected, 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, grain width, thickness and FFD with over 0.9 heritability, followed by TGW with over 0.86, had the highest heritability. These characters were phenotypically the most-stable yield components and can use as independent descriptors in the breeding programs for grain yield improvement. Other traits also because of larger heritability than grain yield can used as indirect indices in grain yield improvement. FN after grain yield had the most affectability by environmental factors. By contrast, HI was less environmentally sensitive and had higher heritability than other quality traits. Similar results were reported by Tsilo *et al*. (2010), and for grain morphology and quality traits had 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).

B. Genotypic Correlation

The coefficients of genotypic correlation were calculated for all traits based on the data averaged from two seasons (Table 3). The maximum positive correlation (0.98) was observed between grain length and grain perimeter, followed by r = 0.91 between area and perimeter. The maximum negative correlation (-0.48) was observed between width and AR, followed by r = -0.34 between TGW and FN. The coefficient of correlation between grain shape direct measurements and grain weight was positive and very significant. For example, grain length and width had positive correlation with TGW with estimate of r =0.53 and r = 0.62, respectively. Similarly, grain thickness was highly correlated with TGW (r =0.45). The other traits with the exception of the AR and roundness had highly positive correlation with TGW. The among quality traits, just GPC had positive significant association with TGW and the other traits as negatively correlated with TGW, from -0.07 to -0.335 for HI and FN, respectively. As expected, several grain measurements are inherently correlated, like length versus AR, area and perimeter (r=0.70, 0.84 and 0.98 respectively) and width versus thickness (0.49). Therefore, because of high correlation among above traits, it is possible with creasing of one of them, the other traits creased. Russo *et al*. (2014); Zhang *et al*. (2015) also reported similar correlations for mentioned traits in durum and bread wheat, respectively. Other important derived measurements like volume and perimeter on the contrary FFD were also positively correlated with length and width. So, with the contemporary creasing of grain length and width, volume and perimeter can creased. Roundness was only grain morphology trait that didn't show positive correlation with any other grain morphology traits in this study. Meanwhile, this trait showed significant negative correlation with length, AR and perimeter. So, It seems with the selection of grain with long length, the roundness will be decreased. However, Russo *et al*. (2014) reported a significant positive association between roundness and length, and a significant negative correlation between roundness and width. In this study, TGW showed low correlations with AR and roundness (0.11 and -0.06, respectively), giving the first indication that these traits are independent, while TGW was highly corrected with other traits. However, grain volume, area, perimeter, width, length, FFD and thickness showed a strong and positive relationship with TGW, therefore, it would be preferred if grain size and shape were used in selection to increase TGW. These result are agreement with Gegas *et al*. (2010) and Rasheed *et al*. (2014) reports. GPC showed positive significant association with TGW. So, with the creasing of GPC, TGW can creased. It can be a result of wheat cultivars with high grain yield and high grain protein. Other researchers also reported wheat cultivars with high grain yield and high GPC (Stuber *et al*. 1962; Johnson *et al*. 1973). By contrast, other quality traits had negatively correlation with TGW, range from -0.07 to -0.34 for HI and FN, respectively. It seems with the selection of grain with high HI and FN, the grain weight will be decreased. These result are in agreement with previous studies (Kamra 1971; Bhatia 1975; Lofler and Busch 1982, Blanco *et al*. 2012).

Table 3: Coefficients of genotypic correlation for grain weight, grain size (and shape) and grain quality traits over two years (2013-15).

Variable	Length	Width	Thickness	TGW	AR	Roundness	Area	FFD
Length	1							
Width	0.155	1						
Thickness	-0.077	0.492**	1					
TGW	0.527**	0.616**	0.451**	1				
AR	0.698**	0.481**	0.295**	0.105	1			
Roundness	-0.265*	-0.051	0.094	-0.058	-0.346**	1		
Area	0.843**	0.549**	0.036	0.659**	0.446**	-0.134	1	
FFD	-0.308**	0.045	0.382**	0.508**	-0.186	0.215	-0.207	1
Volume	0.675**	0.757**	0.645**	0.796**	0.111	-0.124	0.838**	-0.005
Perimeter	0.978**	0.398**	0.026	0.627**	0.559**	-0.262*	0.912**	-0.279*
GPC (%)	0.394**	0.106	0.217	0.306**	0.207	-0.090	0.393**	0.090
ZS	-0.032	-0.195	-0.053	-0.184	0.091	-0.021	-0.107	-0.098
HI	-0.118	-0.026	-0.198	-0.071	-0.141	0.119	0.024	0.032
FN	-0.063	-0.090	-0.071	-0.335**	-0.123	0.097	0.041	-0.252*
Yield	0.094	0.301**	-0.037	0.317**	-0.275*	0.007	0.327**	0.132

* 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; FN: Falling number

Table 3: Coefficients of genotypic correlation for grain weight, grain size (and shape) and grain quality traits over two years (2013-15).

Variables	Volume	Perimeter	GPC (%)	ZS	HI	FN	Yield
Volume	1						
Perimeter	0.735**	1					
GPC (%)	0.389**	0.392**	1				
ZS	-0.122	-0.074	0.450**	1			
HI	-0.205	-0.116	0.001	0.202	1		
FN	-0.134	-0.080	-0.001	-0.085	0.251*	1	
Yield	0.193	0.120	0.190	-0.254*	-0.039	-0.021	1

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

GPC: Grain protein content; ZS: Zeleny sedimentation; HI: Hardness index; FN: Falling number

In the final, only grain width, TGW and area showed strong and positive correlation with grain yield, meanwhile, AR and ZS 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). In this study, length, width, thickness, area, FFD, volume, perimeter and GPC with moderate to high heritability, and high positive correlation with TGW had good efficiency as indirect selection for TGW. Gegas *et al*. (2010) confirmed that grain size and shape were largely independent traits in a study of six wheat populations.

C. Stepwise Regression Analysis and Path Coefficient Analysis

In order to eliminate no effective variables on grain weight in regression model and study only traits affecting significantly grain weight changes (Draper and Smith, 1966), a stepwise regression analysis was computed 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 47 percent changes in performance.

After grain volume five variables, including FFD, width, length, perimeter and GPC respectively were entered to regression model. In the final step, these variables along with grain volume had justified 99.25% 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:

$$y = -180.79- 0.0031x_2 + 24883x_2 + 30.088x_3 + 29.82x_4 - 6.327x_5 -0.075x_6 \quad ...(6)$$

Where, y is the TGW; and x_1, x_2, x_3, x_4, x_5 and x_6 are grain volume, FFD, width, length, perimeter and GPC, respectively.

Based on this method, the two first variable that entered in model i.e. grain volume and FFD are the most important variables contributing to the grain weight. In addition to the two mentioned variables four other variables, including the width, length, perimeter and GPC that entered in model in next steps are important. However, a six variables regression model with explain more than 99.25% of TGW variation, was recognize as the best model.

Table 4: Stepwise regression on the TGW as dependent variable over two years (2013-15).

Source of variation	df	Mean Square	F
Model	6	668.53	1915.97***
Error	74	0.3489	
Corrected total	80		

*** Represents significance at $P < 0.001$

Table 5: Relative contribution (partial and model R^2) and F value in predicting TGW by stepwise regression over two years (2013-15).

Variable in Model	Partial R-Square	Model R-Square	F
Volume	0.4723	0.4723	115.10***
FFD	0.2571	0.7294	142.73***
Width	0.1222	0.8516	146.47***
Length	0.1044	0.956	345.27***
Perimeter	0.0351	0.9911	274.07***
GPC	0.0014	0.9925	32.74***

*** Represents significance at $P < 0.001$
FFD: Factor from density; GPC: Grain protein content

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 FFD, width and length in a successful regression equation indicates the effectiveness of these traits to increase grain weight. Considering the positive and significant regression coefficient of mentioned characters, 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 volume, perimeter and GPC, it could be said that increasing the amount of this trait would decrease.

In order to have a clear understanding of the effect of individual measurement on grain weight, path coefficient analysis was computed by taking TGW as dependent variable, and grain volume ,FFD, width, length, perimeter and GPC as independent variables (Table 6). FFD exhibited maximum positive direct effect on grain weight followed by perimeter. Grain volume, width and length had similar positive direct effects. However, GPC has negative direct effect on grain weight, and this should undergo negative selection in order to get superior grain weight genotypes. 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 effect is important. Therefore, FFD because of 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 perimeter followed by length, width and volume also can used as a good indirect descriptors for TGW.

Table 6: Direct (diagonal and bold) and indirect effects of variables remained in stepwise regression model on TGW over two years (2013-15).

Variable	Volume	FFD	Width	Length	Perimeter	GPC
Volume	**0.231**	-0.004	0.187	0.170	0.244	-0.035
FFD	-0.002	**0.679**	0.011	-0.081	-0.093	-0.009
Width	0.175	0.030	**0.247**	0.040	0.132	-0.011
Length	0.152	-0.210	0.038	**0.260**	0.323	-0.039
Perimeter	0.170	-0.190	0.098	0.253	**0.332**	-0.039
GPC	0.083	0.061	0.026	0.102	0.130	**-0.098**
Residual effect	0.045					

FFD: Factor from density; GPC: Grain protein content

D. Cluster Analysis

Cluster analysis is a technique used to categorize genotypes that are similar into one group and others into different groups. The most F value for all statistics in MANOVA obtained when the cluster divided into 6 group (Table 7). So, the cluster divided into 6 groups and genotypes separated into groups. Cluster I to VI consisted of 10, 5, 34, 1, 3 and 28 genotypes, respectively. Clusters III and IV with 41.97% and 1.23% of total genotypes were recognized as the largest and the smallest cluster, 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. The clusters V and IV because of the maximum and minimum average for the most traits identified as the most valuable and the least significant clusters, respectively.

Table 7: MANAOVA of durum wheat landraces for 6 clusters.

Statistic	Value	F Value
Wilks' Lambda	4.032	21.38***
Pillai's Trace	6.452	8.42***
Hotelling-Lawley Trace	244.704	59.10***
Roy's Greatest Root	158.99	321.20***

*** Represents significance at P < 0.001

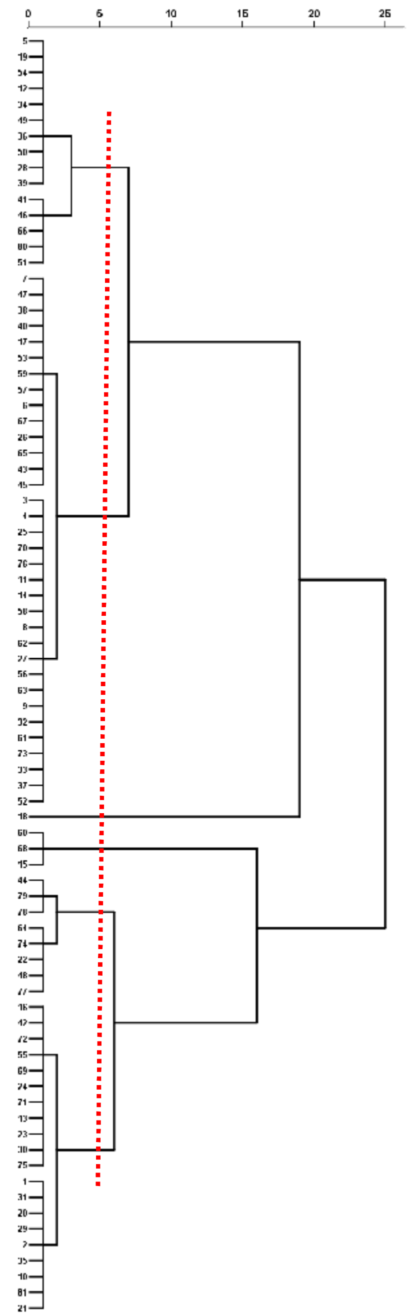

Fig. 1. Dendrogram produced using ward's minimum variance method based on similarity matrix of durum wheat landraces.

Table 8: Mean and standard deviation of grain weight, grain size (and shape) and grain quality traits over two years (2013-15).

Variable	Cluster					
	I	II	III	IV	V	VI
Length	6.74±0.37	6.41±0.54	7.07±0.58	7.34±0	8.28±0.12	7.59±0.58
Width	3.16±0.15	2.99±0.21	3.24±0.15	2.47±0	3.59±0.06	3.46±0.14
Thickness	2.93±0.26	2.93±0.10	3.10±0.19	2.66±0	3.49±0.05	3.29±0.17
TGW	42.07±4.26	40.91±2.50	45.85±3.90	33.77±0	61.13±6.05	53.55±5.53
AR	2.04±0.17	2.05±0.36	2.13±0.21	2.72±0	2.19±0.13	2.13±0.20
Roundness	0.66±0.10	0.73±0.07	0.66±0.09	0.58±0	0.67±0.02	0.65±0.09
Area	15.25±1.85	14.50±1.04	16.13±1.76	12.20±0	20.00±0.63	18.63±1.70
FFD	1.98E-03±1.9E-04	2.16E-03±1.4E-04	2.02E-03±1.7E-04	1.87E-03±0	2.06E-03±2.1E-04	2.05E-03±2.4E-04
Volume	259.52±6.27	233.75±5.99	296.02±12.41	202.01±0	434.08±5.26	361.28±21.50
Perimeter	19.27±1.36	17.68±1.56	20.66±1.98	19.92±0	25.90±0.32	23.07±2.05
GPC (%)	14.59±0.13	14.66±0.08	14.66±0.15	14.80±0	14.80±0.08	14.72±0.12
ZS	36.20±0.75	36.40±0.49	36.26±0.85	35.00±0	36.33±0.47	36.32±0.89
HI	59.60±1.36	60.00±1.10	59.53±1.80	60.00±0	60.00±2.16	59.64±1.37
FN	447±108	522±110	489±108	630±0	399±198	486±121
Yield	3952.59±2515.14	3090.37±1753.43	3555.90±1914.94	3037.04±0	5469.14±1525.04	4200.53±2105.84
Number of genotypes	10	5	34	1	3	28

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

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 durum wheat landraces, specialty for grain size and shape. The most of descriptors had higher heritability than TGW. FFD and perimeter 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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