

Association between Grain Size, Shape and Thousand Kernel Weight in Pakistani Wheat Landraces

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ABSTRACT

Wheat (*Triticum aestivum*. L) grain size is considered to be one of the main criteria of yield constituents by wheat breeders. In order to detect phenotypic diversity and relationship between thousand kernel weight, a collection of 204 landraces from different parts of Pakistan was planted across two years (2012-2014). High throughput method based on seed imaging was used to measure the kernel size and shape. The correlation analysis revealed significant positive correlation between thousand kernel weight (TKW) with perimeter of vertical (PV), thickness (T), area of vertical (AV), area of horizontal (AH), perimeter of horizontal (PH), width (W) and Length (L). Biplot showed that accessions with high seed shape parameters have higher TKW. By taking TKW as a dependent variable multiple regression analysis was performed. Regression summary indicated that 31% of the variations in TKW are explained by the independent variables. It was shown that grain thickness, length and width are most important for predicting TKW. Based on image analysis this study provides useful information about the relationship between TKW, kernel size and shape in Pakistani wheat landraces that may help to improve grain weight in a breeding program.

Keywords: Landraces, Wheat, Seed morphology, thousand kernel weight

INTRODUCTION

In Pakistan wheat remains the major staple food. Average yearly wheat production is almost equal to 24.032 million tons to 9.046 million hectares (Oury and Godin., 2007). Increase in population puts a pressure for demand of increased yield of wheat. The current cultivars lack genetic diversity making them an easy target for many biotic and abiotic stresses (Hussain et al., 2011). In contrast with modern wheat varieties, primitive wheat species exhibit wide

variations in grain size, shape and quality parameters due to uniform selection of these traits in elite wheat cultivars (Gegas et al., 2010). Because of wide variations in phenological, morphological, abiotic, biotic and quality traits landraces and other wild species play a major role in breeding programs (Moragues et al., 2007 ; Moragues et al., 2006).

Important agronomic traits like seed shape and size affect the yield and market price. The seeds of a single plant have very few differences in their shape and size, therefore

large amount of measurements are needed to obtain accurate size data (Tanabata et al., 2012). Digital imaging generates the measure of length, width, perimeter and area which are used to define dimensions. It can also capture seed roughness, asymmetric skewing and thickness that contribute to shape variations (Williams et al., 2013). Studies based on grain morphology can be used to exploit increasingly sophisticated phenotyping methods. In wheat such measurements can be related to yield or milling qualities. These traits are economically significant but expensive to measure. Based on geometric models it is recommended that wheat kernel shape and size might effect flour yield as spherical seeds have the maximum possible endosperm to bran ratio (Marshall et al., 1984). Grain weight by volume is the primary criteria used to grade wheat before milling, and is found to be associated with wheat kernel size and shape (Campbell et al., 1999). Harlan (Harlan., 1975) described wheat grain as a major focus of assortment since the dawn of agriculture. Grain weight is a complex quantitative and polygenic trait, affected by numerous heritable interactions at all stages of its growth and development. Grain weight is a very stable yield component and is positively related with agronomic yield (Golan et al., 2015).

Plant breeders are aggressively following the new quick and accurate methods of phenotyping to benefit from the lower price genotyping processes (de Souza., 2010 ; Houle et al., 2010 ; Montes et al., 2007). Computerized digital imaging is a non-destructive and non-invasive technique that can acquire, process and analyze data attained from images (Richardson et al., 2001; Díaz-Lago et al., 2003; Karcher et al., 2003). Improvement of photometric processes or digital imaging will offer more accurate, cheaper phenotypic information to better explain the individual component of complex traits. The digital imaging process generates quantitative data from

digital images of plant organs (Kwack et al., 2005 ; Dana and Ivo., 2008 ; Diao et al., 1999). Digital imaging has the capacity to determine the dimensions of seed morphology that eventually contribute to grain weight.

Grain size is one of the major components of the domestication syndrome in cereals (Fuller., 2007). Evidences from the fertile crescent indicated that the transition from the diploid einkorn and tetraploid emmer wheat to the domesticated forms was associated with a trend towards large grain size (Fuller., 2007 ; Feldman., 2000). Grain size and shape also effect complex traits like thousand kernel weight (TKW) (Zhang et al., 2014). Kernel shape becomes important in current time due to market preferences and industrial demands (Gegas et al., 2010). Grain size is largely described by grain weight and area, whereas shape means the comparative proportions of the major growth axis of the grains (Breseghello and Sorrells., 2007 ; Gegas et al., 2010). It was observed in many studies that grain size and shape have a positive correlation with TKW and have influenced four yield, end-use quality and market price (Evers et al., 1999 ; Breseghello and Sorrells., 2006 ; Tsilo et al., 2010 ; Cui et al., 2011 ; Blanco et al., 2012 ; Williams and Sorrells., 2014 , Rasheed et al., 2014). TKW is a complex trait and is controlled by many traits like grain shape and size (Zhang et al., 2014). Generally length, width, sphericity and horizontal axes proportions and vertical perimeters determine the grain shape (Breseghello and Sorrells., 2007). Grain weight and area are used to characterize grain size (Gegas et al. 2010, Breseghello and Sorrells., 2007).

Image analysis has been implicated for the grain examination (Davies et al., 2003) damaged kernel identification (Luo et al., 1999) differentiation of grains of different species (Chtioui et al., 1996) and determination of flour milling yield potentials in wheat (Berman et al., 1996).

Plant domestication and the beginning of an agricultural centered economy initiated the most important human cultural development. Highly conscious selection has taken place in the transfer of plants from their wild habitats to a new human managed environment (Abbo et al., 2009, Abbo et al., 2011 ; Abbo et al., 2014). The process of domestication has led to many morphological and physiological changes, most of which are related to domestication process (Olsen and Wendel., 2013). Gegas *et al.* (Gegas et al., 2010) carried out examination of grain weight and shape of ancestral wheat species and elite cultivars and observed that elite varieties with heavy grains had kernels that were wider and shorter than those of wild *Triticum* species. Due to laborious and time consuming techniques the exact characterization of grain size and shape remains a huge challenge (Houle et al., 2010 ; Rasheed et al., 2014; Patil et al., 2013) recently used high throughput methods to capture grain size and shape. In spite of the importance of the relationship between grain morphology and TGW a lack of information is evident especially on primitive cultivars like landraces. This study was undertaken to realize the importance and the need of such study on hexaploid wheat landraces. Therefore the evaluation of grain shape, size and TKW was carried out on a set of 204 landraces collected from different areas of Pakistan.

MATERIALS AND METHODS

Seeds from 204 landraces collected from different parts of Pakistan were used in this study. Seeds were acquired from the gene bank of Plant Genetic Resources Institute (PGRI), National Agricultural Research Center (NARC) Islamabad. The genotypes were planted in field conditions during two growing seasons, i.e. 2012-2013 and 2013-2014 in National Agricultural Research Center (NARC) Islamabad (33° 33' N and 73° 06'E). Seeds were hand threshed and 25 sound seeds from each accession were

selected for imaging. Seeds were positioned horizontally and vertically on a black paper at an equal distance. For horizontal images the major axis of seeds defined grain length and minor axis related to grain width. While in case of vertical images the major axis corresponded to grain width and minor axis to thickness. A standard measuring 1 cm² was placed with seeds while taking photographs. The photographs taken were termed according to the genotype accession number and planting year. *Smart Grain* developed by the National Institute of Agro biological Sciences, Tsukuba, Ibaraki, Japan, was used to measure multiple seed perimeters in addition to the area. *Smart grain* automatically recognizes all the seeds in a digital image and then calculates area of horizontal (AH), area of vertical (AV), perimeter of horizontal (PH), perimeter of vertical (PV), length (L), width (W), thickness (T), length to width ratio (LWR), circularity of horizontal (CSH), circularity of vertical (CSV), distance of center of gravity from the vertical intersection of length and width (DSV) and horizontal intersection of length and width (DSH) Statistical analysis (descriptive stats, PCA and multiple regression analysis) was performed with the software XLSTAT 2010. Multiple regression analysis was performed by the exclusion of the variables in high inter-correlation.

RESULTS

Manual methods of measuring grain morphology have some limitations due to the number of data, quality of measured characteristics and different gleaned shape data. Whereas computational methods like digital imaging help in generating data associated with seed size and shape in a small time interval (Williams et al., 2013). Grain shape and size are important agronomic traits along with grain quality due to their significant influence on grain weight, milling yield, end use quality and market value (Abdipour et al., 2016).

Analysis of variance revealed significant differences among genotypes for AH, AV, PH, PV, L, T and TKW while there was significant difference ($P < 0.001$) between years for all investigated traits. Analysis of

variance showed that there are no significant interaction between genotype and year for any traits which means genotypes respond to year variation in same ways (Table 1).

Table 1: Mixed linear model applied, with genotype as fixed and years as random effects. Mean squares of studied traits of 204 genotypes across two years.

SOV	df	AH	AV	PH	PV	L	W	T
Genotypes (G)	203	5.46*	3.11***	2.00***	1.365*	0.28***	0.06ns	0.08***
Year (Y)	1	1233.7** *	764.9** *	740.35** *	523.03** *	40.39** *	19.7** *	35.01** *
GXY	203	4.29ns	2.07ns	1.45ns	1.02ns	0.18ns	0.06ns	0.05ns
		LWR	CSH	CSV	DSH	DSV	TKW	
Genotypes (G)	203	0.019ns	0.001ns	0.0007ns	0.007ns	0.001ns	97.22***	
Year (Y)	1	0.38***	0.29***	0.2***	0.004ns	0.082** *	7.07** *	
GXY	203	0.016ns	0.0009ns	0.0008ns	0.006ns	0.001ns	0.263ns	

Significance *** = p-value ≤ 0.001 ; * = p-value ≤ 0.05

Digital image analysis provided the information about the shape dimensions. Statistical description (Table 2) showed the pattern of parameters with respect to range, central tendency (mean) and level of dispersion (standard deviation, SD) along with a coefficient of variation percentage. Thousand kernel weight is one of the key yield associated trait. TKW in these landraces ranged from 14.6 to 48.84g in 2013 and 16.12 to 49.4g in 2014. Mueenud-Din *et al.* (Mueen-ud-Din et al., 2007) reported the range of 39.22 to 43.16g in Pakistani wheat varieties. Anjum *et al.* (Anjum et al., 1998) observed thousand kernel weight ranging from 31.4 to 37.2g. Variations in TKW are because of environment and genetic control (Halverson et al., 1988). Grain length ranged from 5.9 to 10.5mm in 2013 and 5.5 to 9.5mm in 2014. Grain width ranged from 3.1 to 4.5mm in 2013 and 2.7 to 4.1mm in 2014. Butt *et al.* (Butt et al., 1997) reported the grain length of 5.06-7.01mm

and width of 5.79-7.12mm width. Maximum and minimum variability were observed in the seed area (horizontal) during the year 2013 and vertical circularity in the year 2014. Dispersion to mean ratio expressed, as coefficient of variation (CV %) was highest in DS horizontal and for vertical seed area during 2013. High range of TKW was observed during both years expanding, coefficient of variation percentage.

Correlation among traits is important in plant breeding, due to its reflection in dependence degree between different traits. Both genetics and environmental factors influence the phenotypic correlation among traits. The correlation analysis revealed significant positive correlation between TKW with PV, T, AV, AH, PH, W and L (Table 3). This suggests that by increasing one of them the other trait is increased. Abdipour (Abdipour et al., 2016) reported that several grain size and shape measure-

Table 2: Statistical description of studied parameters.

Statistic	AH (mm ²)		AV (mm ²)		PH (mm)		PV (mm)	
	2013	2014	2013	2014	2013	2014	2013	2014
Minimum	13.077	11.936	6.944	6.613	16.530	14.785	11.065	10.556
Maximum	29.148	25.046	15.870	13.468	29.088	23.851	18.175	15.266
Range	16.070	13.110	8.926	6.855	12.558	9.066	7.110	4.710
Mean	20.082	16.604	11.867	9.128	20.386	17.692	14.756	12.492
SD	2.337	2.072	1.914	1.233	1.449	1.188	1.314	0.814
CV %	11.638	12.482	16.132	13.502	7.106	6.717	8.904	6.515
	L (mm)		W (mm)		T (mm)		LWR	
Minimum	5.943	5.535	3.106	2.784	2.826	2.643	1.611	1.609
Maximum	10.515	9.551	4.560	4.195	4.356	3.823	2.644	3.009
Range	4.573	4.016	1.454	1.411	1.530	1.179	1.033	1.400
Mean	7.092	6.462	3.844	3.404	3.691	3.105	1.858	1.919
SD	0.484	0.485	0.284	0.227	0.311	0.219	0.140	0.130
CV %	6.827	7.504	7.379	6.671	8.425	7.059	7.511	6.779
	CSH		CSV		DSH (mm)		DSV (mm)	
Minimum	0.435	0.490	0.596	0.626	0.310	0.331	0.286	0.245
Maximum	0.685	0.719	0.749	0.778	0.862	0.786	0.515	0.499
Range	0.250	0.230	0.153	0.151	0.552	0.456	0.229	0.253
Mean	0.608	0.662	0.681	0.726	0.506	0.500	0.379	0.351
SD	0.037	0.026	0.034	0.022	0.095	0.076	0.044	0.043
CV %	6.078	3.899	4.940	3.040	18.743	15.221	11.530	12.229
	TKW (g)							
Minimum	14.6	16.120						
Maximum	48.84	49.420						
Range	34.24	33.300						
Mean	32.25	33.110						
SD	7.02	7.230						
CV %	21.76	22						

ments like length versus aspect ratio, area and perimeter and thickness versus width are inherently correlated. According to a study conducted, (Rasheed et al., 2014) grain length and width were positively correlated with volume and perimeter. Seeds that are larger in size have the ability to produce vigorous seedlings. Seed size is

positively associated with seed strength. Drikvand (Drikvand et al., 2013) and Ramya (Ramya et al., 2010) reported a positive correlation between thousand kernel weight grain length and width.

Principal component analysis (PCA) is a classical technique applied widely for analyzing data, compression and data set's

features visualization. PCA revealed the relationship among studied variables along with their importance towards capturing the variability. Importance of the shape parameters is inversely proportional to the magnitude of angle adapted with PC1 and PC2 respectively. In this regard, width (W),

thickness (T) and length (L) were selected in the first dimension (PC1) and length to width ratio (LWR), CSH (circularity of horizontal) and DS (distance of center of gravity from the intersection of length and width) were selected from the second dimension (PC2).

Table 3: Pearson’s correlation coefficient (r) of studied traits

Traits	AH	AV	PH	PV	L	W	T	LWR	CSH	CSV	DSH	DSV
AV	0.645											
PH	0.910	0.532										
PV	0.636	0.966	0.539									
L	0.881	0.429	0.867	0.444								
W	0.840	0.738	0.714	0.698	0.520							
T	0.621	0.975	0.527	0.948	0.399	0.732						
LWR	0.110	-0.254	0.236	-0.204	0.550	0.420	0.277					
CSH	-0.163	0.057	0.421	-0.006	0.444	0.124	0.060	0.608				
CSV	0.056	0.159	0.002	-0.087	-0.037	0.181	0.164	-0.205	0.232			
DSH	0.225	-0.029	0.336	-0.003	0.378	0.048	0.016	0.460	0.297	-0.062		
DSV	0.318	0.331	0.258	0.352	0.321	0.210	0.263	0.132	-0.077	-0.074	0.169	
TKW	0.459	0.490	0.395	0.492	0.370	0.388	0.492	0.003	-0.014	0.058	0.112	0.265

*.Values in bold are different from 0 with a significance level alpha= 0.0001

Area and perimeter were also found close to X-axis with significantly highest squared cosines on PC1 dimension. Only vertical circularity was found to the third dimension. About 65.56 % of variation was explained within two dimensions (PC1 and PC2). TKW having significant squared cosine along PC1 indicated that it has surely been explained in the same direction as length, width and thickness (Table 4).

Genotypes with serial no. 9 (accession no. 12021: location Mansehra), 120 (accession no. 18873: location Chitral), 119 (accession no. 12233: location Abbottabad), 133 (accession no. 18776: location Abbottabad) and 177 (accession no. 11549: location

Balochistan) made the periphery of the Biplot indicating the extremes. Genotype 9 (accession no. 12021: location Mansehra) and 19 (accession no. 12104 : location Muzaffarabad) were with high PV, W, T and TKW (Figure 1). Genotype 120 (accession no. 18873 : location Chitral) and 119 (accession no. 12233: location Abbottabad) were best in seed length and horizontal parameter. Accessions spread in overall left half of the Biplot were lower in all the shape parameters. Seed roundness (circularity) was best in genotype no. 135 (accession no. 12016: location Abbottabad), 95 (accession no. 18667 : location Swat) and 168 (accession no.

Table 4: Principal component analysis (PCA) illustrating the diversity captured by studied variables.

	PC1	PC2	PC3			
Eigenvalue	5.694	2.829	1.082			
Variability (%)	43.799	21.761	8.325			
Cumulative %	43.799	65.560	73.885	PC1	PC2	PC3
	Eigenvectors			Squared cosines		
AH	0.384	0.091	0.206	0.839	0.023	0.046
AV	0.364	-0.218	-0.118	0.755	0.135	0.015
PH	0.359	0.246	0.154	0.734	0.171	0.026
PV	0.362	-0.170	-0.306	0.747	0.081	0.101
L	0.325	0.324	0.178	0.602	0.298	0.034
W	0.350	-0.199	0.205	0.698	0.112	0.045
T	0.356	-0.228	-0.097	0.721	0.148	0.010
LWR	0.005	0.540	-0.006	0.000	0.823	0.000
CSH	-0.074	-0.447	0.064	0.031	0.565	0.004
CSV	0.026	-0.194	0.750	0.004	0.106	0.609
DSH	0.077	0.349	0.091	0.034	0.344	0.009
DSV	0.178	0.080	-0.396	0.181	0.018	0.170
TKW	0.248	-0.034	-0.108	0.350	0.003	0.013

11229 : location Balochistan) and the same genotypes are lowest in LWR (Figure 1). In the Biplot, genotypes in the right half from the extreme right are good in all shape parameters. The Biplot explained that the accessions with high values of seed shape parameters also possess high TKW (Figure 1) for example, genotype no. 9 (accession no. 12021: location Mansehra), 19 (accession no. 12104: location Muzaffarabad), 120 (accession no. 18873: location Chitral), 20 (accession no. 12114: location Balochistan), 12 (accession no. 11931: location Northern Areas), 106 (accession no. 11766: Skardu), 8 (accession no. 18842: location Dir), 6 (accession no. 12012: location Abbottabad). TKW in various studies was observed to be positively correlated with agronomic yield (Fuller., 2007 ; Cui et al., 2011 ; Maccaferri

et al., 2010) and flour yield (Williams and Sorrells., 2014 ; Breseghello and Sorrells., 2006 ; Chastain et al., 1995).

Multiple regression analysis was performed by taking TKW as dependent Y-variable. Model ANOVA (Table 5-A) showed the significance of the hypothesis. Ratio of model mean square with error mean square indicated that the predictors are appropriate to depict the values of TKW. Significant prediction of TKW has been assessed by the ANOVA table regarding independent estimates used in the model. Durbin-Watson d-value (1.9) is rejecting any possibility of autocorrelation among the predictors. Moreover the value of serial correlation (0.04) highlights that there is no auto-correlation between variables. This indicated that the most effective variables

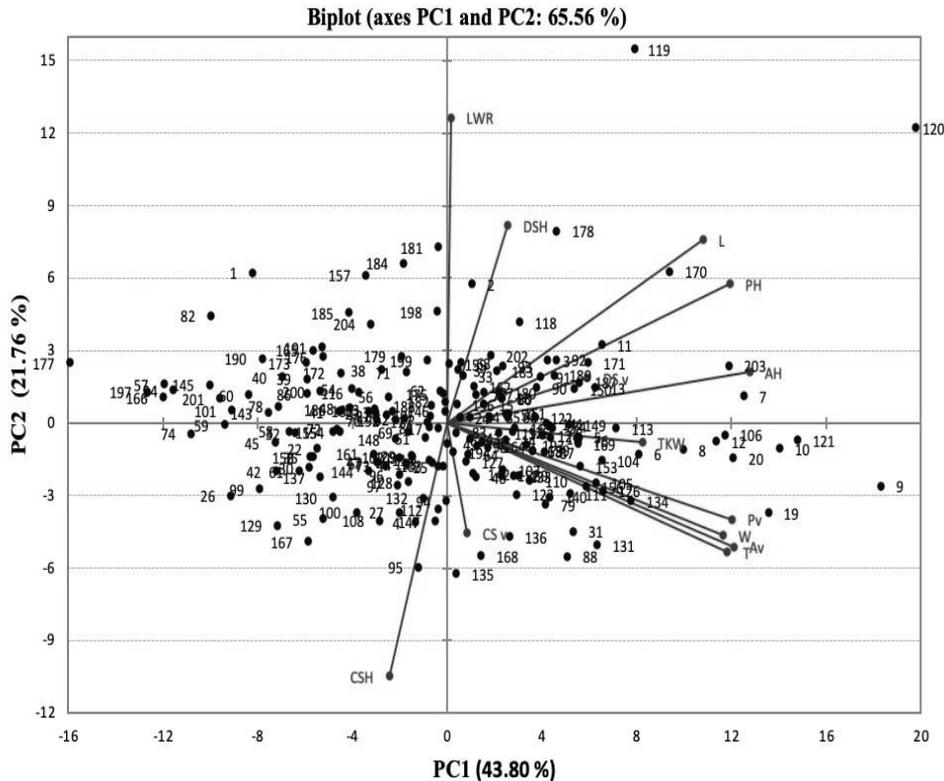


Figure 1: Biplot of studied variables along with the spread of 204 genotypes.

have been chosen for the prediction of TKW. Among 12 studied shape parameters, dependent six variables of the model were selected with the help of PCA and multicollinearity diagnostics. To avoid the multicollinearity, area and perimeters were not selected in the model because these were the function of length and width (included in the model). The selected six variables were the seed shape parameters with significant cosines at PC1 and PC2, included as the predictors of TKW (Table 5-B). Regression summary indicated that 31% of the TKW variation has been explained by the six independent variables. Regression equation of the model can be written as:

$$TKW = 87.549 + 24.718(L) - 41.86(W) + 15.5(T) - 74.04(LWR) + 19.69(CSH) + 7.133(DSH)$$

Relationship showed that thickness is the most important predictor to assess TKW (Table 5-C). Length and width are also important for the TKW prediction. Four out of six shape parameters significantly predicted TKW. Except few values, both (+0.95% and -0.95%) sides of confidence interval has been observed, hence revealing the prediction confidence for TKW with the help of shape parameters (Figure 2).

DISCUSSION

Image analysis is an important technique that can have many applications in identification of varieties and seed certification. For the crop improvement seed size and shape are important due to their influence on yield and quality. One of the major approaches to increase wheat yield is through the improvement of kernel weight. The variability in seed size and shape parameters provides initial

information to breeders to produce combinations in their programs for enhancing grain size in the new cultivars.

The correlation between seed parameters helps in suggesting that this correlation is related to uniformity and smoothness of kernel to grain weight ratio. The association of major seed dimensions also reveals that thickness of grain has maximum direct effect on grain weight which is followed by vertical area. It can also be revealed that horizontal area of seed has less impact on grain weight. Overall, this high throughput phenomic characterization identifies the underlying genetic mechanisms for grain size and shape at much more high

resolution than that expected by conventional and manual seed measurement techniques. In a study by Gegas and colleagues (Gegas et al., 2010) on six wheat populations, it was confirmed that seed size and shape are highly independent traits. The genetic correlations which are estimated and measured here are consistent with previous observations in winter wheat (Dholakia et al., 2003).

It is observed in previous studies that length of seed is set earlier in developmental process whereas width of seed requires time to be influenced by environmental conditions during seed filling period (Sardras and Egli., 2008).

Table 5: Regression analysis of shape parameters as independent X-variables and TKW as dependent Y-variable.

A	SOV	DF	MS	F	Pr > F			
	Model	6	501.716	14.138	< 0.0001			
	Error	197	35.487					
	Total	203	(Computed against model Y = Mean (Y))					
B	SOV	DF	MS (I)	MS(III)	F (I)	F (III)	Pr > F (I)	Pr > F (III)
	L	1	1373.187	241.846	38.696	6.815	< 0.0001	0.010
	W	1	526.964	178.364	14.850	5.026	0.000	0.026
	T	1	874.107	964.346	24.632	27.175	< 0.0001	< 0.0001
	LWR	1	178.528	160.818	5.031	4.532	0.026	0.035
	CSH	1	26.336	25.409	0.742	0.716	0.390	0.398
	DSH	1	31.177	31.177	0.879	0.879	0.350	0.350
C	Variable s	Beta	SE	T	Pr > t	-95%	95%	
	Intercept	87.549	72.466	1.208	0.228	-55.359	230.457	
	L	24.718	9.468	2.611	0.010	6.046	43.390	
	W	-41.865	18.673	-2.242	0.026	-78.690	-5.039	
	T	15.500	2.973	5.213	< 0.0001	9.637	21.364	
	LWR	-74.044	34.782	-2.129	0.035	-142.637	-5.451	
	CSH	19.697	23.278	0.846	0.398	-26.209	65.603	
DSH	7.133	7.610	0.937	0.350	-7.874	22.140		

- a) Over all variance analysis of the model of multiple linear regression.
- b) Analysis of variance (Type I and III) for dependent variables.
- c) Model parameters indicating Beta and t-values along with their probability.

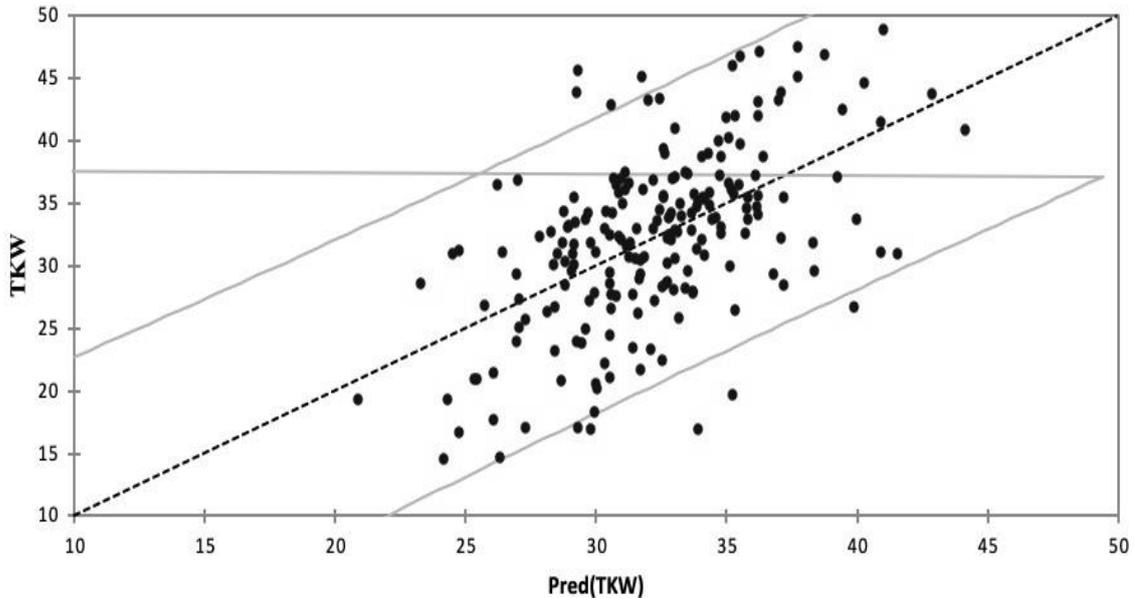


Figure 2: Scatterplot of TKW against predicted scores of TKW.

Our results have been in correlation with Rasheed (Rasheed et al., 2014), who reported a strong correlation between kernel and seed weight and size. Studies have shown that kernel weight has positive correlation with grain yield and kernel growth rate are less correlated with grain yield across environments (Ramya et al., 2010).

Knowledge of seed shape and morphological characters is required for direct manipulation of seed morphology to improve quality and yield in wheat. Low correlations and negative correlations for seed characters show that these characters should be manipulated independently. Similarly, positive correlating characters should be manipulated together (Abdipour et al., 2016).

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