



**Full Length Article**

# High-Throughput Phenomic Characterization of Wheat Grain Architecture and Diversity for Conventional Morpho-Physiological Traits

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## Abstract

Grain morphology affects the weight of grain which ultimately affects grain yield in wheat. Several morpho-physiological traits influence grain morphology. To assess the diversity for morpho-physiological traits and to characterize wheat for grain morphology, a collection comprising of 60 wheat varieties were explored. The ANOVA showed significant differences between varieties for all the parameters except grain thickness, peduncle length, and plant height. Descriptive statistics indicated that the collection of germplasm contained enough variability for the traits under consideration. Grain architectural traits showed positive significant correlations with most of the metric traits suggesting several criteria for indirect selection of traits like grain yield. A positive significant correlation of WUE was observed with grain morphology traits viz: grain width, grain size, grain thickness and grain volume. While 1000 grain weight, water use efficiency, grain length, grain width, grain size, grain thickness and grain volume showed positive significant correlation with grain yield. Principal component analysis extracted seven significant PCs having a cumulative variance of 78.87%. This variation was quite encouraging to initiate a breeding program to improve grain morphology and morpho-physiological traits. The PC1 indicted that grain width, unproductive water use, grain area size, and grain volume were the most diverse traits. In PC2, the maximum positive contribution towards variation was shown by the grain area, grain length and grain volume. The cluster analysis grouped varieties into seven clusters of high, medium, and low performance based on morphology and grain architecture traits. This classification might help in the selection of high and low performing varieties and could be used as parents in hybridization program. © 2021 Friends Science Publishers

**Keywords:** Genepool; Grain Morphology; Varieties; Wheat; WUE

## Introduction

Morphological features like grain volume and shape in wheat have profound effects on grain weight and flour yield (Williams *et al.* 2013). Improvement in these traits is the main objective of plant breeders from marketing point of view (Gegas *et al.* 2010). The milling yield could be improved by modifying grain morphology (Evers *et al.* 1990). A significant positive relationship has been observed in grain size and milling yield. This relationship is quite variable among the genotypes indicating scope for the improvement through selection. The density of seed also influences the milling yield. The crease of seed is critical in milling quality and mechanical work. It is considered as cleavage point of wheat grain. The size and depth of crease is also important for breeders. Grain size depends on the factors like test weight, size of the embryo, grain cross-section and smooth surface area (Marshall *et al.* 1986).

The grain size, shape and color variation have economic implications in bread wheat. These are the

primary physical features that determine the market value of grain (Tańska *et al.* 2018). The grain size is positively correlated with specific grain weight, a trait related to the efficiency of wheat transport and storage of photosynthates (Clarke *et al.* 2004). A high proportion of small grains is commercially disliked by the milling industry because the small grains are lost during the cleaning process prior to milling. In addition, a high percentage of small grains are indicative of a poor flour yield (Nuttall *et al.* 2017). The digital system is a useful tool for capturing 3-D analysis of grains image dimensions. Phenomic characterization through digital imaging (DI) can capture the three-dimensional variation in wheat grain size and shape using different image orientations (Ali *et al.* 2020).

From agronomic perspectives, wheat grain yield is the most important trait. Grain yield in bread wheat is usually analyzed through various yield components: the number of spikes  $m^{-2}$ , the number of grains spike $^{-1}$ , the number of grains  $m^{-2}$  and the thousand-kernel weight (TKW) (Ahmad *et al.* 2019). Correlation of yield traits with morpho-

physiological traits has been reported for efficient selection in wheat. Strong negative relationship between these yield components have already been found for both environmental and genetic sources of variation between (i) the number of spikes  $m^{-2}$  and the number of grains  $spike^{-1}$  and (ii) the number of grains  $m^{-2}$  and 1000 grains weight (Beral *et al.* 2020). Improvement in grain weight is considered a promising approach to improve wheat yield potential and is regarded as an important area of wheat genetic and breeding studies. Similarly, water use efficiency and the dry matter are related to volume of wheat grain. If water content is high during grain formation, the seed volume will also be increased. The water use enhances the values of all seed parameters which increases crop yield. An increase in length, width, thickness, and volume indicates that there is more space for photosynthates during the grain filling period (Hasan *et al.* 2011).

Knowledge of genetic diversity for grain morphology and water use efficiency found in the Pakistani wheat varieties is still incomplete. Keeping in view the importance of cited issue, the present research was conducted with the objective to determine the intra-cultivar variation of image features for grain shape, size and to determine its association with grain weight using high-throughput digital imaging phenotyping and to assess the diversity among wheat varieties for traits like grain morphology, WUE and grain yield.

## Materials and Methods

This research work was conducted at the Department of Plant Breeding and Molecular Genetics, Faculty of Agriculture, University of Poonch Rawalakot, Azad Kashmir. The seed material included sixty hexaploid spring wheat varieties were acquired from National Agriculture Research Center (NARC) Islamabad, Pakistan (Table 1). The seed of each variety were sown in 02 rows during Rabi seasons of 2014–2015 and 2015–2016 in an augmented block design with three blocks. The check varieties *i.e.*, NARC-2009, NARC-2011 and Ufaq-2002 were replicated after every 20 entries. The row to row spacing was kept 30 cm. The row length was kept 3 m long.

## Data collection

The data were collected from ten plants for parameters namely, flag leaf area ( $cm^2$ ), number of tillers  $plant^{-1}$ , peduncle length (cm), number of spikelets  $spike^{-1}$ , 1000 grain weight (g) and grain yield  $plant^{-1}$ . The physiological traits included water use (WU), water use efficiency (WUE), potential yield ( $kg\ ha^{-1}$ ), percentage of potential yield achieved, transpiration and unproductive water-use. The summer fallow and growing season rainfall data were acquired from Pakistan Meteorological Department (<http://www.pmd.gov.pk>) and the water use was calculated by assuming that 25% of rainfall received during the

summer fallow is stored for crop use (Hunt and Kirkegaard 2013). It also assumes that there is no water carrying over from the previous season. It was calculated by using the formula:

$$\text{Water use} = 0.25 \times (\text{summer fallow rainfall} + \text{growing season rainfall})$$

Water use efficiency was estimated by dividing grain yield with water use. The potential yield was estimated by following French and Schultz (1984).

$$\text{Potential yield (kg/ha)} = 22 \times (\text{wateruse} - 60)$$

The percentage of potential yield achieved was obtained as a ratio of actual crop yield to potential yield, while the transpiration was estimated by the following formula:

$$\text{Transpiration (mm)} = \frac{\text{total dry matter (kg/ha)}}{55}$$

The unproductive water-use was obtained by computing the difference between water-use and transpiration (Hunt and Kirkegaard 2013).

## Phenotyping for grain morphology

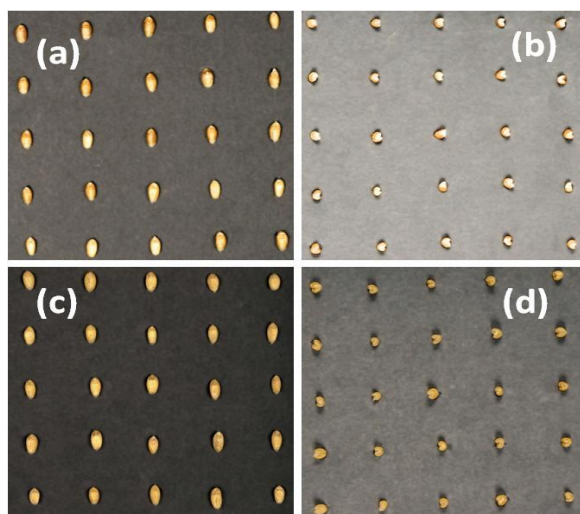
Phenotyping based on digital imaging (DI) of grain size and various orientations was conducted as proposed by Rasheed *et al.* (2014). Twenty-five grains were photographed with the help of a digital camera (Sony Alpha NEX5 digital). A sample of sound and healthy seeds of the varieties was selected by visual observations. To provide color contrast seeds were placed in a horizontal and vertical position with equal distances (Fig. 1). Two photographs were taken from each unit. After assigning names all images were cropped using Adobe Photoshop ([www.adobe.com/products/photoshop](http://www.adobe.com/products/photoshop)). Image contrast and brightness were also improved to minimize the edge detection error.

## Grain analysis

Smart Grain Software ([www.nias.affrc.go.jp/qtl/SmartGrain/](http://www.nias.affrc.go.jp/qtl/SmartGrain/)) is developed for high-throughput measurement of seed shape. This software uses a new image analysis method to minimize the time taken in the preparation of seeds and image capture. Outlines of seeds are automatically recognized from digital images. Several shape parameters, such as grain length, grain width, grain size, grain thickness, and grain volume were calculated. High-throughput measurements with Smart Grain lessen the sampling error and made it possible to distinguish between lines with small differences in seed shape. Smart Grains Software can accurately recognize grains of wheat (Rasheed *et al.* 2014). Twenty-five healthy grains were selected for pictorial representation. Horizontal and vertical images of wheat grains were taken (Fig. 1). For horizontal image, the grains were placed at equidistance while their germ ends were pointing to the same side and crease of the grains was facing downward direction. For vertical image, the brushy

**Table 1:** Wheat varieties utilized to screen water-use efficiency, grain morphology, grain yield and yield related traits

Sr. No	Varieties	Sr. No	Varieties	Sr. No	Varieties	Sr. No	Varieties
1	Kohistan-97	16	Pavon-76	31	Johar	46	Tatara
2	Noshehra-96	17	Zardana	32	Janbaz	47	NIA-Amber
3	Parwaz-94	18	Paster	33	AARI	48	NARC-2009
4	Kiran-95	19	PBW-373	34	Anmol-91	49	Takbeer
5	Fakhar-e-Sarhad	20	Manthar-03	35	NIA-Sunari	50	Ufaq-2002
6	Suleman-96	21	MH-97	36	Pasban-90	51	NIFA Barsat-09
7	Khayber-87	22	Blue Silver	37	Zam-04	52	Millet-2011
8	Saleem-2000	23	Marvi-2000	38	Nasir-2k	53	Pirsabak-2013
9	Yacora-70	24	Bahwalpur-97	39	Imdad-1	54	KT-2000
10	Bhitai	25	Pirsabaq-2005	40	Tijbans	55	SKD-1
11	Chakwal-97	26	SH-2002	41	Darabi-2011	56	Gomal-08
12	Margalla-99	27	Punjab-85	42	Tandojam	57	Pirsabak-2008
13	Fareed-2006	28	Kaghan-93	43	Shaheen-94	58	TD-1
14	Bakhtawar-93	29	Moomal-2002	44	Benezir	59	Pak-81
15	Mehran-87	30	Abadgar-93	45	NARC-2011	60	Sarsabz

**Fig. 1:** Horizontal and vertical grain arrangements for digital imaging in varieties Manthar-03 (a & b) and Gomal-08 (c & d)

ends were facing upwards direction and germ ends were facing downward direction. The digital images were analyzed by the software “Smart Grain”. The parameters like grain length, width, grain area size and perimeter length were obtained. The images were selected for the measurements, and then seed color and background color were selected to measure the parameters. From horizontal images length, width, and grain area size of 25 grains were obtained. For vertical images, the length of the seeds was considered the thickness of the grain. It gave the average values of parameters for each variety. Horizontal area and vertical thickness provided the volume of the grains. Smart Grain provided values for the parameters like grain width, grain size, grain volume, and grain thickness.

### Statistical Analysis

Analysis of variance was done by using the software Agri-STAT. Pearson’s correlation coefficients between morpho-physiological and grain architecture traits were assessed following the method previously reported by Snedecor

(1956). Principal component (PCA) and cluster analyses were used to assess the diversity among wheat varieties for the grain yield and related parameters, water use efficiency and grain morphology. As scales of measurement of different traits were different therefore, the means were standardized as Hair *et al.* (2006), followed by the construction of dendrogram utilizing Ward’s method (Kumar *et al.* 2009) using software Past Ver. 3 (Hammer *et al.* 2001). All measurements regarding the assessment of grain morphology were recorded by using Smart Grain package v. 1.3 (<http://phenotyping.image.coocon.jp>).

## Results

### Analysis of variance and correlation

Analysis of variance showed non-significant differences among varieties for grain thickness, peduncle length, and plant height (Table 2). To analyze whether grain morphology was linked to average yield components, Pearson’s correlations were calculated between grain characters and each yield component (Table 3). The traits *viz.*, flag leaf area, plant height, number of tillers per plant, spike length, number of spikelets spike<sup>-1</sup>, biological yield, 1000 grain weight, water use efficiency, grain width, grain size, grain thickness and grain volume showed positive significant correlation with grain yield. A positive significant correlation of WUE was observed with grain morphology traits *viz.*, grain width, grain size, grain thickness and grain volume. In general, the grain morphology showed positive significant correlations with most of the morpho-physiological traits, showing multiple directional models for the selection of complex traits. By using grain characters that are easy to assay and simple to score, a broad range of complex traits can be selected indirectly.

### Principal Component Analysis

The PCs with Eigen value greater than Joliffe’s cut off value of 0.75 were considered significant. Seven significant

**Table 2:** Mean squares for morpho-physiological and grain architecture traits in 60 spring wheat varieties

Source	Blocks	Varieties	Checks	Test Entries	Check x Test
Df	2	59	2	56	112
FLA	1.92**	14.85**	41.66**	13.77**	22.89**
PH	12.79 <sup>NS</sup>	7.06 <sup>NS</sup>	13.7 <sup>NS</sup>	7.13 <sup>NS</sup>	4.3 <sup>NS</sup>
NOT	0.68*	1.08**	2.65**	1.02**	1.14*
SL	0.44**	0.72**	7.34**	0.49**	0.54**
PL	0.05 <sup>NS</sup>	1.05 <sup>NS</sup>	0.09 <sup>NS</sup>	1.10 <sup>NS</sup>	0.06 <sup>NS</sup>
NSL	4.70**	3.58**	3.56**	3.79**	8.42**
BY	4542743.29*	3324562.54 <sup>NS</sup>	6312872.56*	3280638.12*	148365.91 <sup>NS</sup>
GY	360360**	132308**	84266**	144981**	494009**
1000 GW	4.89**	32.60**	86.04**	31.32**	1.41 <sup>NS</sup>
WUE	0.39**	0.12*	0.11*	0.13*	0.64**
% of PY	38.32*	11.21 <sup>NS</sup>	9.14 <sup>NS</sup>	12.55**	60.61**
Trans	107.40 <sup>NS</sup>	2118.88*	5363.95**	2035.56*	377.74 <sup>NS</sup>
UPWU	2674.94*	4229.72**	4108.66**	4396.53**	5035.99**
GL	0.21*	0.11*	0.02 <sup>NS</sup>	0.11*	0.23*
GW	0.18**	0.09**	0.00 <sup>NS</sup>	0.10**	0.07**
GS	2.18**	2.01**	1.62**	2.13**	4.14**
GT	0.09 <sup>NS</sup>	0.04 <sup>NS</sup>	0.02 <sup>NS</sup>	0.04 <sup>NS</sup>	0.04 <sup>NS</sup>
GV	91.90**	44.79**	6.28 <sup>NS</sup>	49.90**	169.11**

Where, Df = Degree of Freedom, \* = Significant at 5% level, \*\* = Significant at 1% level, NS = Non-significant, FLA = Flag Leaf Area, PH = Plant Height, NOT = Number of Tillers, PL = Peduncle Length, SL = Spike Length, NSL = Number of Spikelets, BY = Biological Yield, GY = Grain Yield, GW = 1000 Grain Weight, WUE = Water Use Efficiency, % of PY = Percentage of Potential Yield, Trans = Transpiration, UPWU = Unproductive Water Use, GL = Grain Length, GW = Grain Width, GT = Grain Thickness, GS = Grain Size, GV = Grain Volume

**Table 3:** Pearson's correlation coefficients among morpho-physiological and grain architecture traits

	FLA	PH	TILLER	SL	PL	NS	BY	GY	GWT	PY	TRANS	UPWU	WUE	GL	GW	GS	GT
PH	.281*																
TILLER	.434**	.356**															
SL	.241	.380**	.237														
PL	.138	.271*	.182	.349**													
NS	.504**	.347**	.377**	.398**	.189												
B_Y	.270*	.211	.327*	.283*	.137	.144											
GY	.286*	.290*	.420**	.358**	.124	.298*	.358**										
GWT	.409**	.249	.253	.342**	.319*	.221	.247	.266*									
PY	.504**	.259*	.351**	.118	.178	.164	.467**	.220	.243								
TRANS	-.248	.160	-.175	.040	.168	-.029	.095	-.068	-.118	.034							
UPWU	.510**	.245	.452**	.346**	.225	.243	.408**	.423**	.563**	.291*	-.493**						
WUE	.286*	.290*	.420**	.358**	.124	.298*	.358**	.940**	.266*	.220	-.068	.423**					
GL	.377**	.146	.212	.226	.310*	.408**	.292*	.218	.368**	.260*	.045	.403**	.218				
GW	.448**	.469**	.415**	.440**	.252	.342**	.250	.395**	.369**	.384**	-.051	.426**	.395**	.464**			
GS	.363**	.193	.208	.271*	.211	.313*	.214	.270*	.349**	.273*	-.008	.409**	.270*	.740**	.816**		
GT	.271*	.348**	.320*	.375**	.341**	.265*	.253	.262*	.363**	.300*	.050	.326*	.262*	.365**	.553**	.434**	
GV	.374**	.258*	.284*	.327*	.293*	.325*	.244	.303*	.420**	.292*	-.030	.450**	.303*	.692**	.822**	.912**	.726**

\*. Correlation is significant at the 0.05 level, \*\*. Correlation is significant at the 0.01 level

FLA = Flag Leaf Area, PH = Plant Height, TILLER = Number of Tillers, SL = Spike Length, PL = Peduncle Length, NS = Number of Spikelets, BY = Biological Yield, GY = Grain Yield, GWT = 1000 Grain Weight, PY = % of Potential Yield, TRANS = Transpiration, UPWU = Unproductive Water Use, WUE = Water Use Efficiency, GL = Grain Length, GW = Grain Width, GS = Grain Size, GT = Grain Thickness, GV = Grain Volume

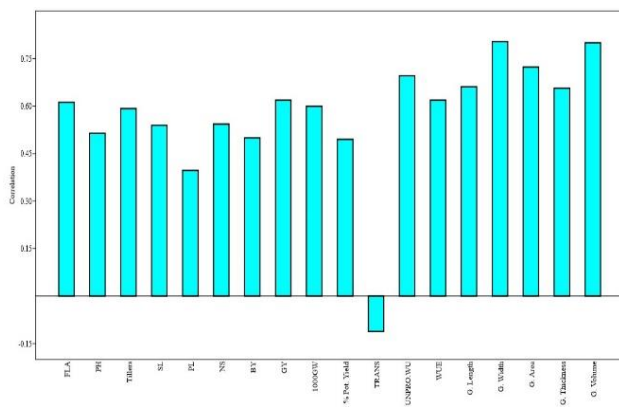
PCs were extracted having a cumulative variance of 78.87% (Table 4). Similarly, Beheshtizadeh *et al.* (2013) indicated four significant components accounting for almost 76% of the total diversity between the traits in wheat varieties. The first principal component showed the highest contribution (35.48%) towards the total variation. The PCA loadings indicated that grain width contributed maximum to the variation followed by unproductive water use, grain area size and grain volume. The contribution of all the traits was positive except transpiration which contributed negatively (Fig. 2). The second PC contributed 10.54% to the total variation. The maximum positive contribution in PC2 was observed for grain area followed by grain length and grain volume. A negative contribution was found for number of tillers per plant, grain yield, and water use efficiency (Fig.

3). The maximum positive contribution of the traits to PC3 was found in transpiration followed by plant height (Fig. 4). The maximum positive contribution in PC4 was exhibited by percentage of potential yield followed by flag leaf area and biological yield (Fig. 5). While PC5 contributed 6.4% to the total diversity having highest positive contribution of spike length and peduncle length followed by plant height and 1000 grain weight. The maximum positive contribution to PC6 was contributed by biological yield followed by peduncle length, 1000 grain weight and unproductive water use. The last significant PC contributed 4.63% to the total variation with positive contribution of characters like grain length, number of spikelets spike<sup>-1</sup> while negative contribution was found in grain thickness and plant height. Biplot of the first two PCs

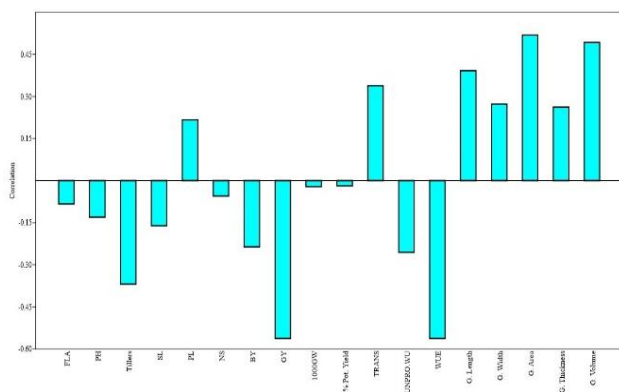
**Table 4:** Principal component analysis for morpho-physiological and grain architecture traits in 60 spring wheat varieties

PC	Eigen value	Variance (%)	Cumulative variance
1	6.39	35.48	35.48
2	1.90	10.54	46.02
3	1.50	8.31	54.33
4	1.36	7.57	61.09
5	1.17	6.48	68.38
6	1.05	5.86	74.24
7	0.83	4.63	78.87

Jolliffe's cut off value = 0.75

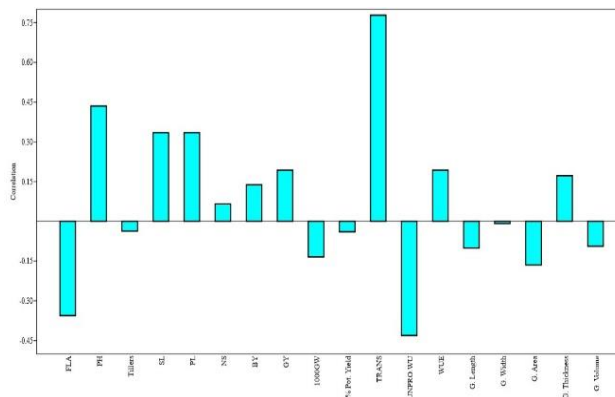


**Fig. 2:** Loadings for PC 1 obtained from morpho-physiological and grain architecture traits in 60 varieties of wheat

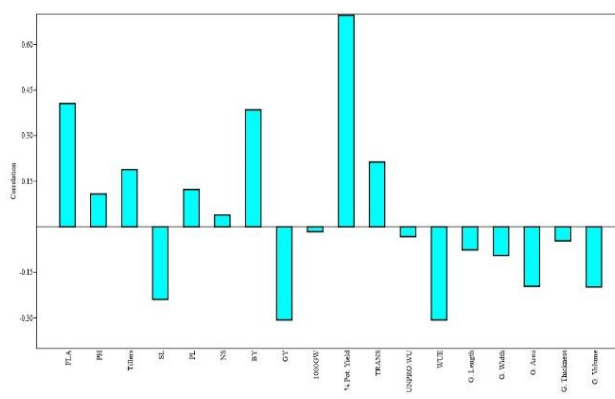


**Fig. 3:** Loadings for PC 2 obtained from morpho-physiological and grain architecture traits in 60 varieties of wheat

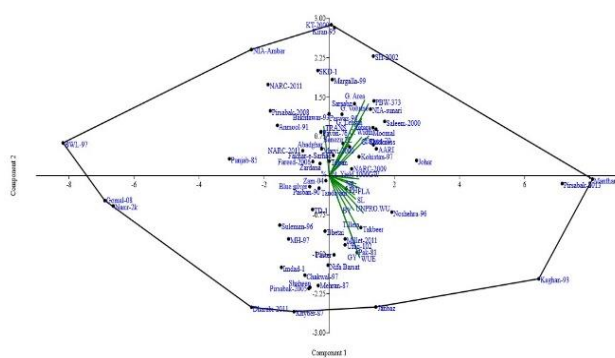
revealed that WUE, grain yield, number of tillers, grain area size and grain volume had maximum contribution towards the total variance (Fig. 6). The varieties Manthar-03, Kaghan-93, Pirsabak-2013, KT-2000, NIA-Amber, Gomal-08, Janbaz, Kiran-85, SH-2002, Bahawalpur-97, Nasir-2K, Khyber-81, and Dharabi-2011 were outliers showing diversity. The varieties Pak-81, Ufaq-2002, Millet-2011, Takbeer and Fakhar-e-Sarhad were the high yielding so lie very close to the grain yield vector. While varieties like Manthar-03, Pirsabak-2013 and Kaghan-93 showed the highest while BWL-97, Gomal-08 and Nasir-2K showed the lowest values for grain morphology, grain yield and water use efficiency.



**Fig. 4:** Loadings for PC 3 obtained from morpho-physiological and grain architecture traits in 60 varieties of wheat



**Fig. 5:** Loadings for PC 4 obtained from morpho-physiological and grain architecture traits in 60 varieties of wheat

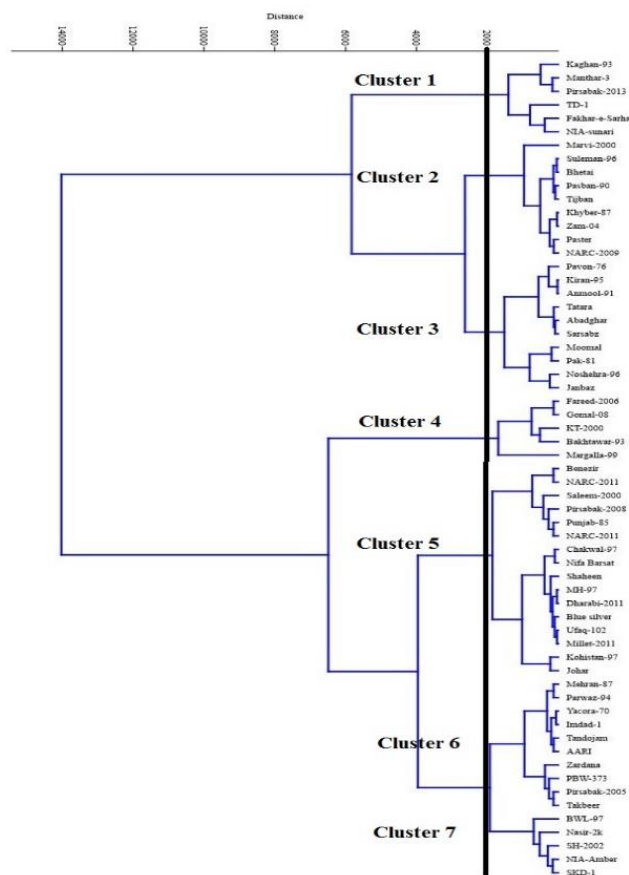


**Fig. 6:** Biplot diagram obtained from morpho-physiological and grain architecture traits in 60 varieties of wheat

**Cluster analysis**

The dendrogram based on various morpho-physiological traits of wheat varieties indicated seven logical clusters based on similarities and differences (Fig. 7). The 06 varieties included in cluster 1 were high in grain morphology, grain yield, and water use efficiency. While the cluster 2 included 09 varieties, moderate in grain yield





**Fig. 7:** Dendrogram of 60 wheat varieties based on water use efficiency, grain morphology, grain yield and yield related traits

and water use efficiency and low in grain morphology. Similarly, the cluster 03 contained 10 varieties which were moderate to low in grain yield, water use efficiency and high in grain morphology traits. The cluster 04 comprised of 05 varieties, low in grain yield, water use efficiency and low to moderate in grain morphology. The cluster 05 contained 16 varieties which were moderate in grain yield, water use efficiency and grain morphology traits. However, the cluster 06 consisted of 10 varieties characterized by moderate in grain yield, water use efficiency and moderate to high in grain morphology. The cluster 07 contained 05 varieties which were lowest in grain yield, water use efficiency and grain morphology traits.

## Discussion

The use of less water to achieve higher yield is a major objective of the modern agriculture (Araus 2004). As world cereal demand is increasing, producing more grains per unit of water during the crop cycle through higher WUE may have strong impact at local and regional level (Tambussi *et al.* 2007). The increase in individual grain weight and size has the potential to improve wheat yield (Molero *et al.*

2019). Genetic manipulation of grain morphology through selection can lead to increase in yield potential (Calderini *et al.* 2021). Manual measurement methods of grain parameters have some limitations, like limited data and low-quality measurements. Therefore, high-throughput grain phenotyping method is needed to validate the genetic analysis and selection for seed shape in plant breeding (Williams *et al.* 2013).

Analysis of variance (ANOVA) is a statistical test for detecting differences in group means when there is one parametric dependent variable and one or more independent variables (Sawyer 2009). To test the significant difference among the varieties for various parameters, the ANOVA was computed. ANOVA and descriptive statistics represented that the varieties have higher variability for the target traits, like water use efficiency and grain morphology to initiate a breeding program (Table 2). Awan *et al.* (2015) also indicated variability in 176 wheat varieties to initiate a breeding program. Correlation coefficient analysis assesses the joint association between varieties of plant traits. It also finds out the component traits on which we can base selection for genetic enhancement in yield (Barman *et al.* 2020). To find the degree and direction of association of yield with the traits contributing to yield and inter-relationship among them, analysis of correlation coefficient was executed. The correlation coefficient showed a positive relationship of WUE with grain morphology. Hasan *et al.* (2011) indicated a positive relation among WUE and wheat grain. In addition, positive correlations were observed among 1000 grain weight, grain yield, grain morphology traits and water use efficiency. The traits like 1000 grain weight, WUE and grain architectural traits are suggested as the simplest indirect way to improve grain yield. Similarly, Kanwal *et al.* (2019) observed significant positive correlation of grain size for grain yield, days to anthesis, days to maturity, spikelets spike<sup>-1</sup>, plant height and flag leaf area. While, Khokhar *et al.* (2021) indicated negative association among plant height and grain yield per plant.

Analysis of crop genetic diversity and structure provides valuable information needed to broaden the narrow genetic base as well as to devise the breeding and conservation strategies of crops (El-Esawi *et al.* 2018). Cluster analysis and PCA are effective bio-statistical tools for the identification of genetic diversity, selection of parents, center of origin and diversity, tracking evolutionary pathway of the crops, and to study interaction with the environments (Khodadadi *et al.* 2011). Principal component analysis can transform several possibly correlated variables into a smaller number of variables called principal components. It has been argued that principal component analysis should be conducted before cluster analysis (Mujaju and Chakuya 2008). Several authors have suggested the use of cluster and principal component analyses to study the genetic diversity and relationships of wheat genotypes (Lysenko 2011; Beheshtizadeh *et al.* 2013; Adilova *et al.* 2020; Tariq *et al.* 2020). The PCA indicated

seven significant PCs. In PC1, grain width contributed maximum to the variation followed by unproductive water use, grain area size and grain volume (Fig. 2). The grain width, unproductive water use, grain area size, and grain volume showed maximum load for this PC indicating that these were the most variable traits. However, Tańska *et al.* (2018) pointed out that the seed dimensions and shape were constant among the cultivars with highest variation shown by grain thickness and grain volume. The highest values for grain width, unproductive water use, grain size and grain volume indicated a positive correlation among these traits. Yoshioka *et al.* (2019) also reported a positive correlation among grain morphology traits and concluded that bread wheat had the largest diversity in grain morphology. The PC1 also indicated a negative correlation between grain morphology traits and transpiration, endorsing the results obtained from Pearson's correlation showing negative but non-significant relationship. The PC2 indicated a negative relationship among the grain characteristics like grain area, grain length, grain volume and morpho-physiological traits like number of tillers plant<sup>-1</sup>, grain yield, and water use efficiency (Fig. 3). Gegas *et al.* (2010) reported the traits like grain shape and size is under the influence of distinct genetic components in wheat. Yoshioka *et al.* (2019) speculated that grain characters are the outcome of epistatic interactions of multiple genetic factors. Rest of the significant PCs *i.e.*, from PC3 to PC7 contributed 32.85% to the total diversity. The relative contribution to total diversity decreased with the succeeding PCs indicating decreased contribution of traits residing in those PCs to total diversity. The loadings from PCA describe the strength of the relationship between the parameter and the component; these are linear weights that account for the full variance of the parameters (Byrne 2005). The traits with highest load in the initial PCs are more variable than those found in later PCs. The highest positive load of the traits to PC3 was observed in transpiration followed by plant height (Fig. 4). While in PC4 maximum positive load was exerted by percentage of potential yield followed by flag leaf area and biological yield (Fig. 5). Awan *et al.* (2015) reported grain yield/plant was closely related to flag leaf area.

The biplot was introduced by Gabriel in 1971. It is a plot of two kinds of information displayed together (Gower *et al.* 2015). This paper deals with biplot obtained from quantitative data generated by the first two principal components. The biplot revealed the interaction of traits and varieties (Fig. 6). It was observed that WUE, grain yield, number of tillers, grain area size and grain volume had maximum contribution towards the total variance, as indicated by the longest vector length. Similarly, the varieties lying near to and in the same quadrants of their respective vectors showed higher values for the development of those traits. The vectors for spike length, peduncle length lie very close to the origin hence their role could not be predicted. The varieties showed variation, as they scattered around the quadrants of biplot. The varieties

Manthar-03, Kaghan-93, Pirsabak-2013, KT-2000, NIA-Amber, Gomal-08, Janbaz, Kiran-85, SH-2002, Bahawalpur-97, Nasir-2K, Khyber-81, and Darabi-2011 were outliers and considered most diverse. The varieties found in the same quadrant and lying near to the grain yield and grain quality vectors were found high yielding with better grain quality and vice versa. The varieties Pak-81, Ufaq-2002, Millet-2011, Takbeer and Fakhare-Sarhad showed the highest grain yield as they lie very close to the grain yield vector. The varieties showing the lowest yield were found furthest from vector and in the opposite quadrant. The varieties like Manthar-03, Pirsabak-2013 and Kaghan-93 showed the highest values while BWL-97, Gomal-08 and Nasir-2K showed the lowest values for grain morphology, grain yield and water use efficiency. These varieties may be selected for further hybridization and estimation of genetic components. Analysis of genetic diversity and population structure is an essential step in their conservation, utilization and breeding (Iqbal *et al.* 2018; Holasou *et al.* 2019). The genetic variability based on morphological characters especially those of economic interest could also be used to select appropriate materials in breeding programs for crop improvement (Farooq *et al.* 2014; Xhulaj *et al.* 2019; UI-Allah *et al.* 2019). The cluster analysis indicated 07 clades based on morpho-physiological traits in 60 wheat varieties while Drikvand *et al.* (2013) grouped 92 wheat cultivars into six clusters.

Based on cluster diagram (Fig. 7) two sets of varieties were identified *i.e.*, those showing highest and lowest performance for target traits like grain yield, water use efficiency and grain morphology traits. Therefore, the varieties grouped in clusters 01, 04 and 07 may be selected for further studies. The varieties included in the high-performance set of target traits were Kaghan-93, Manthar-03 and Pirsabak-2013 while those of low performance set of target traits were Bahawalpur-97, Nasir-2K and Gomal-08. These results confirmed the observations depicted in the biplot diagram. These varieties can be exploited as parents for transgressive breeding and could be used to stack favorable alleles into elite breeding lines through convergent crossing.

## Conclusion

The germplasm showed high variation for grain morphology, WUE and morpho-physiological traits. This variation was quite encouraging to initiate a breeding program targeting to improve these traits. The traits related to grain morphology could be recommended for the indirect selection of grain yield and WUE. Cluster analysis grouped varieties into seven clusters of high, medium and low performance for target traits. This classification may help in the selection of high and low performing varieties and can be used as parents in hybridization program. The varieties like Kaghan-93, Manthar-03 and Pirsabak-2013 were recommended as highest performing varieties, while

Bahawarpur-97, Nasir-2K, and Gomal-08 were lowest performing varieties for the grain yield, grain morphology traits and water use efficiency. These varieties can be exploited as parents to produce variability in succeeding generations and to combine the alleles from parents into a single progeny through convergent crossing.

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## Author Contributions

Shafiq-Ur-Rehman executed the experiment and wrote the initial draft of the manuscript as a Ph.D. scholar. Sardar Ali Khan finalized the draft of the manuscript. Shahid Iqbal Awan planned the experiment and guided the scholar. Muhammad Ilyas contributed to the analysis of data.

## Conflict of Interest

The authors declare that they have no conflict of interest.

## Data Availability

Data are available from the authors on request.

## Ethics Approval

The research work was conducted after approval from the Human & Animal Ethics Committee of University and no humans and animals were investigated.

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