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DIGITAL IMAGING-BASED PHENOTYPING OF WHEAT KERNELS UNDER DROUGHT STRESS

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ABSTRACT

Drought stress significantly impairs wheat growth and productivity, primarily by affecting kernel development. Given their strong association with kernel yield and quality, kernel traits offer reliable means to assess genotypic responses to drought stress conditions. In this study, 70 diverse wheat (*Triticum aestivum* L.) genotypes were evaluated under two moisture regimes: well-watered (four irrigations) and drought stress (irrigation withheld after the first watering). At maturity, the following agronomic traits were recorded: number of days to 50% heading, number of days to 50% physiological maturity, number of kernels per spike, number of spikelets per spike, thousand kernel weight, and kernel yield per spike. Nine kernels from each genotype were photographed in horizontal and vertical orientations using a 3 cm scale. Kernel traits were measured using Image-J software and included: horizontal area, vertical area, horizontal perimeter, vertical perimeter, horizontal length, horizontal roundness, horizontal width, vertical thickness, vertical roundness, factor from density, aspect ratio, kernel volume, horizontal deviation from ellipse, and vertical deviation from ellipse. Analysis of variance (ANOVA) showed significant differences among genotypes for all traits. Principal component analysis (PCA) highlighted kernel volume and horizontal area as the most variable traits. Genotype G17 had the highest thousand kernel weight under drought, while G30 and G41 performed best under normal irrigation. Biplot analysis showed that kernel yield per spike, number of spikelets per spike and number of kernels per spike were positively associated with horizontal kernel traits (horizontal area, horizontal length, and horizontal deviation from ellipse). In contrast, thousand kernel weight was positively associated with vertical kernel traits (vertical area, vertical perimeter, vertical thickness, vertical roundness, and vertical deviation from ellipse). In conclusion, digital imaging effectively captures variation in kernel morphology. The identified relationships between kernel traits and yield components can help breeders select drought-tolerant genotypes using both conventional and image-based traits.

Keywords: Kernel morphology, morphometric traits, biplot, high throughput phenotyping, multivariate analysis.

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INTRODUCTION

Wheat (*Triticum aestivum* L.) is a major cereal crop of global importance, providing calories and essential nutrients to billions of people across the globe. Wheat plays a crucial role in agricultural economies and food security due to its large-scale cultivation. However, wheat production is challenged by various abiotic stresses, and climate change has further exacerbated these challenges. Among abiotic stresses, drought is a serious threat to food security in major wheat growing areas (Farooq *et al.,* 2019; Ullah *et al.,* 2023). Drought affects various biochemical and physiological processes of plants and results in low yield and stunted growth (Itam *et al.,* 2020). Therefore, the development of drought tolerant wheat cultivars is urgently needed to ensure food security for future generations. These cultivars not only depict resistance against water deficit conditions but also have potential to meet global wheat requirements under changing climatic conditions (Zheng *et al.,* 2021).

Drought affects kernel shape and size, which ultimately results in compromised yield and quality (Mehraban *et al.,* 2018). Due to the unavailability of sufficient water, wheat kernels become shriveled and deformed (Pouri *et al.,* 2019). Shriveled kernel not only affects kernel quality but also the nutritional profile, resulting in less accumulation of starch and protein content (Rakszegi *et al.,* 2019). The nature, severity, duration of drought stress, and growth stage of plant as well as genotype, determine final yield losses from drought (Farooq *et al.,* 2014; Duvnjak *et al.,* 2023; Pantha *et al.,* 2024).

The introduction of high throughput phenotyping technologies (high resolution photography, 3D scanning and multispectral imaging) has created new opportunities for plant breeders for real time assessment of plant's responses to environmental conditions (Rasheed *et al.,* 2014). Different studies have revealed that digital imaging systems provide efficient, rapid and robust phenotyping coverage of large datasets of crop plants (Sankaran *et al.,* 2015; Kovar *et al.,* 2024). Digital imaging system has also proven very efficient in assessment of wheat kernel traits including perimeter, length, width and area of kernel (Ali *et al.,* 2020). Conventionally, kernel traits were measured manually that was time taking, labor intensive and imperfect. Additionally, old databases cannot accurately fit in large sets of morphological variables (Ain *et al.,* 2015; Fernandez-Campos *et al.,* 2021; Tahir *et al.,* 2023).

Digital imaging is tremendously useful for abiotic stresses, particularly drought, as it can detect minute phenotypic differences which are difficult to measure manually or visually. High sensitivity of image-based phenotyping enhances precision in assessing plant performance under drought stress. To develop drought tolerant varieties, plant breeders depend on methods which are accurate, cost effective, concise and prompt. Hence, due to throughput phenotyping particularly digital imaging, germplasm screening for drought tolerance has become more feasible.

In this experiment a large collection of wheat germplasm was characterized by high- throughput technique under both normal and drought conditions. In previous studies, digital imaging was applied and restricted to a single environment and limited number of genotypes. Here, we have addressed this limitation by executing digital imaging in a large collection of wheat germplasm and characterized the germplasm under both normal and drought conditions. The study explains that digital imaging provides robust, rapid, reliable and accurate methods for evaluating kernel traits under drought stress, enabling identification of superior genotypes.

MATERIALS AND METHODS

The following research was conducted during the year 2019-20 at the experimental farm of the Department of Plant Breeding and Genetics, Bahauddin Zakariya University, Multan. A total of 367 wheat genotypes, including locally adapted cultivars, exotic germplasm, and synthetic derivatives were initially phenotyped under well-watered and water limited conditions. The genotypes were sown on 17 November. From this set, 70 genotypes were subsequently selected for high throughput digital imaging based on thousand kernel weight in both environments. The experiment was laid out in a randomized complete block design (RCBD) with a two-factor factorial arrangement and replicated twice. Despite the large number of treatments, RCBD was appropriate due to the uniform field conditions and careful randomization within blocks to minimize environmental variation. Seed of each genotype was sown in 3 m rows using a handheld drill, with an inter row spacing of 22.5 cm.

Two irrigation treatments were applied (i) a well-watered control receiving four irrigations from sowing to harvest and (ii) a drought-stress treatment in which irrigation was withheld after the initial post-emergence watering. Passport information for the 70-genotype panel is provided in Table 1.

For data collection, five plants were randomly tagged within each row. The following traits were recorded: days to 50% heading (DTH), days to 50% physiological maturity (DPM), number of spikelets per spike (NOS), kernels per spike (KS), thousand-kernel weight (TKW), and kernel yield per spike (YPS). DTH was noted when 50% of the spikes in a row had fully emerged from the boot, while DPM was recorded when 50% of the plants had turned from green to yellow. NOS was counted on the main tiller of each tagged plant, and all tagged spikes were harvested individually to determine KS. TKW and YPS were subsequently measured using a digital balance.

Digital image based phenotyping of wheat Kernel: A DSLR Canon 750D camera was used to capture images of nine selected seeds from each genotype under both normal and drought stress conditions. For each genotype, images of the nine seeds were taken in both horizontal and vertical orientations, using controlled lighting to minimize shadows. The seeds were placed at equal distances on a black sheet to ensure a uniform background (Figure 1). Images were captured from a distance of 60 cm and subsequently adjusted for brightness and contrast using Adobe Photoshop.

Application of Image-J: Kernel images were analyzed using Image-J software, and a known scale of 3 cm was set for calibration. After applying a color threshold, objects were isolated, and various kernel-related traits were measured (Rasheed *et al.,* 2014). These included kernel horizontal area (HA), horizontal length (HL), horizontal perimeter (HP), horizontal round (HR), vertical perimeter (VP), horizontal width (HW), vertical area (VA), vertical thickness (VT), aspect ratio (AR) and vertical round (VR).

Image J is a computational software commonly used for estimating two-dimensional measurements from individual objects directly extracted from image files. In this study, seed images were uploaded into the software, and a known scale of 3 cm was set. After applying color thresholding, individual objects (kernels) were identified and processed for analysis. Various kernel-related traits were measured, including horizontal area (HA), horizontal.

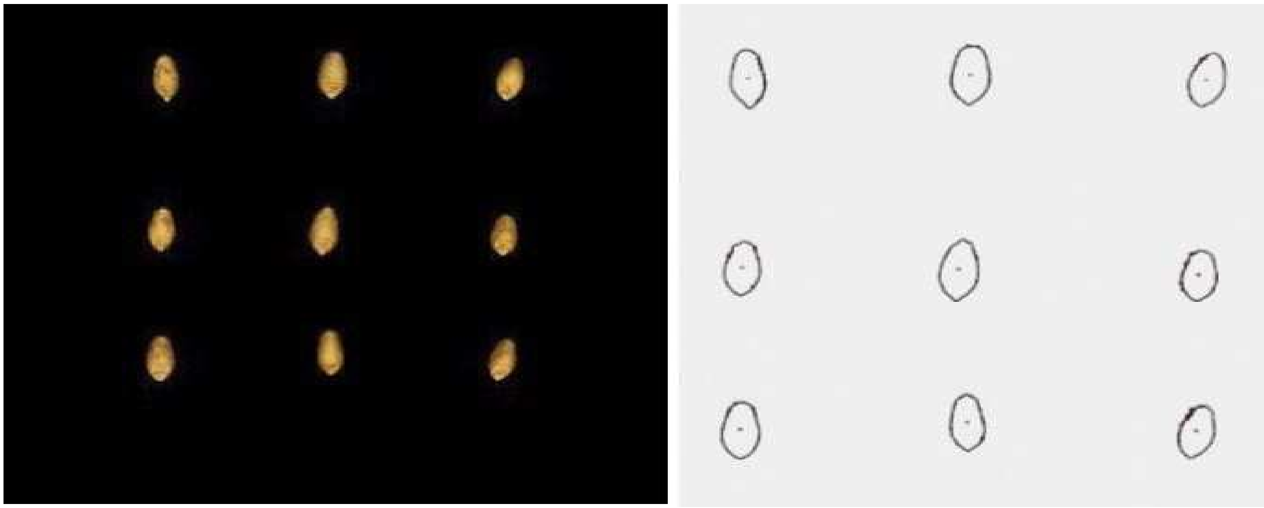

Fig. 1. High throughput imaging of wheat kernel

perimeter (HP), horizontal length (HL), horizontal roundness (HR), horizontal width (HW), vertical area (VA), vertical perimeter (VP), vertical thickness (VT), vertical roundness (VR), aspect ratio (AR), factor from density (FFD), volume (V), horizontal deviation from ellipse (HDFE), and vertical deviation from ellipse (VDFE). The software was used to assess kernel traits such as horizontal and vertical area, perimeter, length, and roundness. Vertical thickness was also recorded. Additionally, several derived traits were calculated using formulas described by Rasheed *et al.,* (2014).

$$\text{Factor from density} = \text{Individual kernel weight}/(\text{length} \times \text{width})$$

$$\text{Volume of seeds} = \left(\frac{4}{3}\right)\pi(\text{length})(\text{width})(\text{thickness})$$

$$\text{Aspect ratio} = \frac{\text{Kernel length}}{\text{Width}}$$

$$\text{Horizontal deviation from optimal ellipses} = \left|(p - \text{hperim}/\text{hperim}\right|$$

Where,

$$p = \pi[3(\text{length} + \text{width}) - \sqrt{(3 \times \text{length} + \text{width}) \times (\text{length} + 3 \times \text{width})}$$

$$\text{Vertical deviation from optimal ellipses} = \left|(p - \text{vperim}/\text{vperim}\right|$$

Where,

$$p = \pi[3(\text{thick} + \text{width}) - \sqrt{(3 \times \text{thick} + \text{width}) \times (\text{thick} + 3 \times \text{width})}$$

Statistical analysis: The recorded data were subjected to two-way factorial Analysis of Variance (ANOVA) following the procedure described by Steel *et al.,* (1997) to assess variation among genotypes for the studied traits ($p \leq 0.05$). Principal Component Analysis (PCA) was conducted to identify patterns of variation and identify key traits contributing to genetic variability among the wheat genotypes (Mohammadi and Prasanna, 2003). In addition, correlation analysis was performed to examine the relationships among various agronomic and kernel-related traits (Steel *et al.,* 1997). All statistical analyses were carried out using the XLSTAT software package.

Table 1. Passport information of the studied genotypes of wheat

Serial No.	Parentage/pedigree
G1	WBLL1*2/KURUKU/4/BABAX/LR42//BABAX*2/3/KURUKU
G2	BLOUK #1/MUNAL
G3	ROLF07*2/DIAMONDBIRD
G4	MUNAL #1/FRANCOLIN #1
G5	BLOUK #1//TACUPETO F2001*2/KIRITATI
G6	KACHU#1/3/T.DICOCCONPI94624/AE.SQUARROSA(409)//BCN/4/2*KACHU
G7	MUNAL #1
G8	BECARD/FRNCLN
G9	FRNCLN*2/TECUE #1
G10	QUAIU #3//TACUPETO F2001*2/KIRITATI
G11	SUP152/BAJ #1
G12	Chakwal 50

G13	FRNCLN/3/GUAM92//PSN/BOW/4/PAURAQ
G14	SUP152/BAJ #1
G15	FRANCOLIN #1*2/KIRITATI
G16	FRANCOLIN #1/WBLL1
G17	ND643//2*ATTILA*2/PASWBLL1*2/KURUKU/4/ *2/BR
G18	QUAIU #3//TACUPETO F2001*2/KIRITATI
G19	WAXWING*2/4/BOW//CBRD
G20	NAC/TH.AC//3* /BUC/4/2*PASTOR/5/
G21	WBLL1*CNDO/R143//ENTE/ SQUARROSA(TAUS)/4 /5/2*JANZ/7/ *2/KURUKU
G22	MILLIT 2011
G23	BAJ #1/3/KIRITATI//ATTILA*2/PASTOR
G24	BABAX/LR42//BABAX*2/3/KUKUNA/4/TINKIO #1
G25	REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA(213)//PGO/4/HUITES/5/T.DICOCCONPI94624/AE. SQUARROSA(409)//BCN/6/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA(213)//PGO/4/HUITES/ 7/MUTUS
G26	SAUAL #1/KACHU
G27	TECUE #1/2*WAXWING
G28	BLOUK #1/5/FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ
G29	CNO79//MUS/3//BAV92*2/5/HAR311
G30	ATTILA*2/PBW65*2//TOBA97/PASTOR
G31	BAV92//IRENA/KAUZ/3/HUITES/4/2*ROLF07
G32	KACHU 1/3*BATAVIA//2*/4/
G33	ATTILA*2/ *2/4/CBRD/3/CD
G34	MILAN/S87230//AKURI
G35	REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA(213) //PGO/4/HUITES/5/T.DICOCCONPI94624/AE.SQUARROSA(409)//BCN/6/REH/HARE
G36	TAM200/PASTOR//TOBA97/3/HEILO/4/PAURAQ
G37	FRET2/KUKUNA//FRET2/3/HEILO/4/BLOUK #1
G38	BAJ #1/AKURI
G39	NAC/TH.AC//3*PVN/3/MIRLO/BUC/4/2*PASTOR/5/KACHU/6/KACHU
G40	BAJ #1*2/HUIRIVIS #1
G41	BECARD/CHYAK
G42	WBLL1*2/BRAMBLING//SUP152
G43	ATTILA*2/PBW65*2//MURGA
G44	CHIBIA//PRLII/CM65531/3/FISCAL/4/ND643/2*WBLL1
G45	SUP152//KIRITATI/2*TRCH
G46	CACUKE #1
G47	WHEAR/KIRITATI/3/C80.1/3*BATAVIA//2*WBLL1/4/WHEAR/SOKOL
G48	TACUPETO F2001*2/KIRITATI/2*TRCH
G49	WHEAR/VIVITSI//WHEAR/3/WHEAR/SOKOLL
G50	VILLA JUAREZ F2009/DANPHE #1
G51	PRL/2*PASTOR//WHEAR/SOKOLL
G52	CHONTE//KIRITATI/2*TRCH
G53	HUW234+LR34/PRINIA//PBW343*2/KUKUNA/3/ROLF07/4/WHEAR/SOKOLL
G54	DANPHE/CHONTE
G55	WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1*2/4/NIINI #1
G56	PRL/2*PASTOR/4/CHOIX/STAR/3/HE1/3*CNO79//2*SERI/5/KIRITATI/2*TRCH/6/PRL/2*PASTOR/4/ CHOIX/STAR/3/HE1/3*CNO79//2*SERI
G57	ND643/2*WBLL1//KACHU
G58	KRONSTAD F2004/WBLL1/3/WHEAR/F2004
G59	PRL/2*PASTOR/3/PFAU/WEAVER*2//CHAPIO
G60	PAURAQ//KIRITATI/2*TRCH/3/PAURAQUE #1
G61	CHIBIA//PRLII/CM65531/3/FISCAL*2/4/TAM200/TURACO
G62	SUP152//ND643/2*WBLL1
G63	WAXWING*2/TUKURU//KISKADEE #1/3/FRNCLN
G64	FRNCLN/NIINI #1//FRANCOLIN #1

G65	SWSR22T.B./2*BLOUK #1//WBLL1*2/KURUKU
G66	DANPHE #1//ND643/2*WBLL1/3/DANPHE
G67	PFAU/MILAN/4/VEE/TRAP#1//ANGRA/3/PASTOR/5/PRL/2*PASTOR/4/CHOIX/STAR/3/HE1/3*CNO79//2*SERI
G68	MUNAL/5/KIRITATI/4/2*SERI.1B*2/3/KAUZ*2/BOW//KAUZ
G69	FRNCLN*2//TAM200/TUI
G70	KACHU//KIRITATI/2*TRCH

RESULTS

Effect of Genotypes and Treatment: The individual effects of genotypes and drought were observed to be highly significant ($p \leq 0.01$) for the majority of the evaluated traits which includes days to 50% heading, days to 50% physiological maturity , number of kernels per spike, thousand kernel weight, yield per spike, horizontal area, vertical area, horizontal perimeter, vertical perimeter, horizontal length, vertical thickness, horizontal roundness, vertical roundness, horizontal width, aspect ratio, factor from density, kernel volume, horizontal deviation from ellipse, and vertical deviation from ellipse (Table 2).

However, certain traits exhibited non-significant variation due to treatment effect, such as horizontal width, vertical perimeter, aspect ratio, and horizontal deviation from the ellipse. Furthermore, the interaction between genotype and treatment was found to be non-significant for several traits, including vertical area, vertical perimeter, vertical thickness, vertical roundness, aspect ratio, and vertical deviation from ellipse (Table 2).

Principal Component Analysis (PCA): Principal Component Analysis was conducted to identify the traits contributing most to phenotypic variation among genotypes. Under both irrigation regimes, the first five principal components (PC1–PC5) accounted for the majority of total variability. In the well-watered treatment, these components together explained 79.5% of the variance. Traits such as kernel volume, horizontal area, and horizontal deviation from ellipse were considered the most discriminating based on their high loading values on PC1 and their distinct positioning in the PCA biplot (Table 3). Under drought stress, kernel volume, horizontal area, and vertical deviation from ellipse showed the highest contributions to variation, while horizontal roundness and aspect ratio had strong loadings on PC2 and remained informative across both environments.

Trait Associations based on Biplot: The Biplot for the well-watered treatment (Fig. 2-A) revealed that kernels per spike displayed a strong positive association with yield per spike, horizontal area, horizontal length, aspect ratio, horizontal deviation from ellipse, and horizontal perimeter. Conversely, factor from density and horizontal roundness exhibited negative relationships with both yield per plant and kernels per spike. Under drought stress, kernels per spike maintained significant positive correlations with yield per spike and thousand-kernel weight; notably, all yield components clustered closely together, indicating their concerted response to water limitation (Fig. 2-B).

Genotypic Performance Based on Biplot Analysis: Biplot analysis displayed that, of the 70 genotypes evaluated, G62, G30, G11, and G65 appeared as the most diverse entries. Under well-watered conditions, genotype G2 recorded the highest aspect ratio, whereas G48 produced the greatest number of spikelets per spike and the highest yield per spike. Genotypes G36, G49, and G55 exhibited higher mean values for horizontal width, horizontal area, and vertical thickness. Genotypes G30 and G41 achieved the highest thousand kernel weight, while G20, G29, and G42 showed the greatest horizontal roundness. Genotypes G11 and G65 displayed extended durations to heading and physiological maturity (Fig. 2-A). Under drought stress, genotypes G61, G69, and G66 produced the most spikelets per spike and took the highest days to physiological maturity. Genotypes G26, G47, G32, and G46 recorded the highest horizontal length and perimeter, whereas G35, G25, G27, G34, G28, G42, and G36 attained the maximum aspect ratio (Fig. 2-B).

Correlation Analysis: Under the normal irrigation regime, the correlation matrix (Table 4) revealed that kernels per spike displayed significant positive associations with yield per spike, horizontal area, horizontal length, aspect ratio, and horizontal deviation from ellipse, while showing significant negative correlations with both horizontal and vertical roundness and with factor from density. Days to 50 % heading was positively correlated only with days to 50 % physiological maturity, and negatively correlated with horizontal area, horizontal width, kernel volume, and horizontal deviation from ellipse; its relationships with all remaining traits were non-significant. Likewise, days to physiological maturity exhibited significant negative correlations solely with horizontal perimeter and vertical deviation from ellipse, while showing non-significant associations with other variables. Yield per spike was significantly and positively correlated with horizontal area, horizontal length, kernel volume, and horizontal deviation from ellipse, whereas its correlations with the remaining traits were not significant.

Table 2: Mean square values of all the studied traits of wheat genotypes under normal and drought conditions

SOV	DF	DTH	DPM	NOS	KS	TKW	YPS	HA	HP	HL	HR
Trt	1	17304.94**	4750.28**	2441.75**	19660.21**	4142.81**	13.59**	0.494**	21.87**	1.121**	0.139**
G	69	17.53**	38.73**	29.79**	359.99**	111.54**	0.517**	0.00348**	0.279**	0.0166**	0.0079**
Trt x G	69	20.11**	20.73**	9.96**	99.36**	13.58**	0.192**	0.000928**	0.248**	0.0055**	0.0045**
E	140	2.51	0.75	0.96	1.60	2.99	0.0044	0.000168	0.030	0.00089	0.00072
SOV	**DF**	**HW**	**VA**	**VP**	**VT**	**VR**	**AR**	**FFD**	**V**	**HDFE**	**VDFE**
Trt	1	0.000004	0.000545**	0.00407	0.00366**	0.01153**	0.0039	0.00078**	0.00352**	0.00754	0.0632**
G	69	0.00026*	0.000583**	0.0605**	0.00561**	0.0339**	0.0389**	0.0007**	0.00607**	0.00815*	0.0577**
Trt x G	69	0.00025*	0.0000532	0.00749	0.000239	0.0021	0.00931	0.000384**	0.000828*	0.01730*	0.00729
E	140	0.00025	0.000067	0.0109	0.00037	0.00248	0.0123	0.00028	0.00088	0.01562	0.00872

**=highly significant at ≤0.01 *=Significant at ≤0.05,

SOV= Source of variation, Trt = treatment, DF= degree of freedom, G = genotypes, E = error, DTH = days to 50% heading, DPM = days to 50% physiological maturity, NOS = number of spikelets per spike, KS = kernel per spike, TKW = thousand kernel weight, YPS = kernel yield per spike, HA = horizontal area, VA = vertical area, HP = horizontal perimeter, VP = vertical perimeter, HL = horizontal length, VT = vertical thickness, HR = horizontal round, VR = vertical round, HW = horizontal width, V = volume, AR = aspect ratio, FFD = factor from density, VDFE = vertical deviation from ellips, HDFE = horizontal deviation from ellipse,

Table 3. Principal component analysis values under normal conditions

	PC1	PC2	PC3	PC4	PC5
Eigenvalue	7.083	3.778	2.117	1.662	1.270
Variability (%)	35.415	18.894	10.588	8.312	6.354
Cumulative (%)	35.415	54.310	64.898	73.211	79.565
Principal component analysis values under drought conditions					
Eigenvalue	7.798	2.983	1.905	1.823	1.175
Variability (%)	38.994	14.915	9.528	9.117	5.877
Cumulative (%)	38.994	53.909	63.437	72.555	78.433

Under water-limited conditions, days to 50 % heading maintained significant positive correlations with days to 50 % physiological maturity and aspect ratio but showed a significant negative correlation with horizontal roundness. All other correlations involving days to heading were non-significant. Both days to physiological maturity and spikelets per spike exhibited non-significant correlations with any of the measured traits. Kernels per spike showed a highly significant positive correlation with yield per spike, underscoring their joint response to drought stress. Thousand-kernel weight correlated positively with vertical roundness and factor from density. The full correlation matrix for traits measured under drought conditions is provided in Table 4.

In addition to association of digital imaging traits with agronomic traits, several inter-correlations between digital image derived traits under normal and drought conditions were also observed. Under normal irrigation, horizontal area was significantly and positively correlated with horizontal length, horizontal perimeter and kernel volume. Vertical area also showed strong positive correlations with vertical perimeter and vertical thickness, suggesting coordinated variation in vertical kernel dimensions. Similar patterns were observed under drought conditions, where the horizontal area maintained significant positive correlations with horizontal length, horizontal perimeter, and volume of the kernel, while the vertical area remained strongly associated with vertical perimeter and vertical thickness. Moreover, both horizontal and vertical deviations from ellipse were consistently correlated with volume and other shape descriptors across both water regimes. The full correlation matrix for rest of the traits under well-watered and drought condition is provided in Table 4.

Table 4. Correlation matrix under normal and Drought conditions

Variables	DTH	DPM	NOS	KS	TKW	YPS	HA	HP	HL	HW	HR	VA	VP	VT	VR	AR	FFD	V	HDFE	VDFE
DTH	1	0.29*	----	----	----	----	----	----	----	----	-0.286	----	----	----	----	0.236	----	----	----	----
DPM	0.26*	1	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----
NOS	----	----	1	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----
KS	----	----	0.28*	1	----	0.92**	----	----	----	----	----	----	----	----	----	----	----	----	----	----
TKW	----	----	----		1	----	----	----	----	----	----	----	----	----	0.23*	----	0.40*	----	----	----
YPS	----	----	----	0.76**	----	1	----	----	----	----	----	----	----	----	----	-0.28*	----	----	----	----
HA	-0.31*	----	0.36*	0.34*	----	0.35*	1	0.66**	*	0.55**		0.78**	0.63**	0.62**	0.33*	----	0.82**	0.85**	0.84**	0.79**
HP	----	-0.23*	----	----	----	----	----	1	0.66**	0.40*		0.59**	0.71**	0.47*	0.25*	----	0.62**	0.62**	0.67**	0.61**
HL	----	----	0.45*	0.42*	----	0.35*	0.88*	0.41*	1	0.33*	-0.40*	0.62**	0.53**	0.52**	----	0.31*	0.73**	0.71**	0.88**	0.64*
HW	-0.34*	----	----		----	----	0.77**	----	0.37**	1	0.37*	0.38*	0.28**	0.27**	-0.26*	-0.7**	0.69**	0.83**	0.73**	0.39*
HR	----	----	-0.35*	-0.27*	----	----	-0.25*	-0.49*	-0.7**	0.42*	1	----	----	----	----	-0.8**	----	----	----	----
VA	----	----	----	----	----	----	0.59**	0.39*	0.41*	0.59**	----	1	0.79**	0.92**	----	----	-0.6**	0.77**	0.62**	0.99**
VP	----	----	----	----	----	----	0.34*	0.33*	----	0.44*	----	0.70**	1	0.67**	----	----	-0.53*	0.58**	0.51**	0.81**
VT	----	----	----	----	----	----	0.47*	0.29*	0.32*	0.50**	----	0.94**	0.67**	1	0.30*	----	-0.43*	0.68**	0.50*	0.90**
VR	----	----	----	-0.25*	----	----	----	----	----	----	----	0.35*	0.28*	0.64**	1	----	0.37*	----	-0.27*	----
AR	----	----	0.37*	0.31*	----	----	0.28*	0.51**	0.70**	-0.39*	-0.9**	----	----	----	----	1	0.27*	-0.4**	----	----
FFD	----	----	-0.27*	-0.24*	0.55**	----	-0.6**	----	-0.6**	-0.42*	----	----	----	----	----	----	1	-0.8**	-0.86**	-0.62**
V	-0.30*	----	0.29*	----	----	0.24*	0.91**	0.28*	0.76**	0.76**	----	0.84**	0.55**	0.79**	0.30*	----	-0.48*	1	0.92**	0.77**
HDFE	-0.26*	----	0.42*	0.40*	----	0.36*	0.96**	0.34*	0.97**	0.57**	-0.50*	0.51**	0.27*	0.40*	----	0.53**	-0.6**	0.85**	1	0.64**
VDFE	----	-0.23*	----	----	----	----	0.60**	0.41*	0.42*	0.59**	----	0.99**	0.71**	0.92**	0.31*	----	-0.23*	0.84**	0.52**	1

Upper diagonal represents drought conditions and lower diagonal represents normal conditions,

*Significant at $p \leq 0.05$; **Significant at $p \leq 0.01$

DTH = days to 50% heading, DPM = days to 50% physiological maturity, NOS = number of spikelets per spike, KS = kernel per spike, TKW = thousand kernel weight, YPS = kernel yield per spike, HA = horizontal area, VA = vertical area, HP = horizontal perimeter, VP = vertical perimeter, HL = horizontal length, VT = vertical thickness, HR = horizontal round, VR = vertical round, HW = horizontal width, AR = aspect ratio, V = volume, FFD = factor from density, VDFE = vertical deviation from ellips, HDFE = horizontal deviation from ellipse,

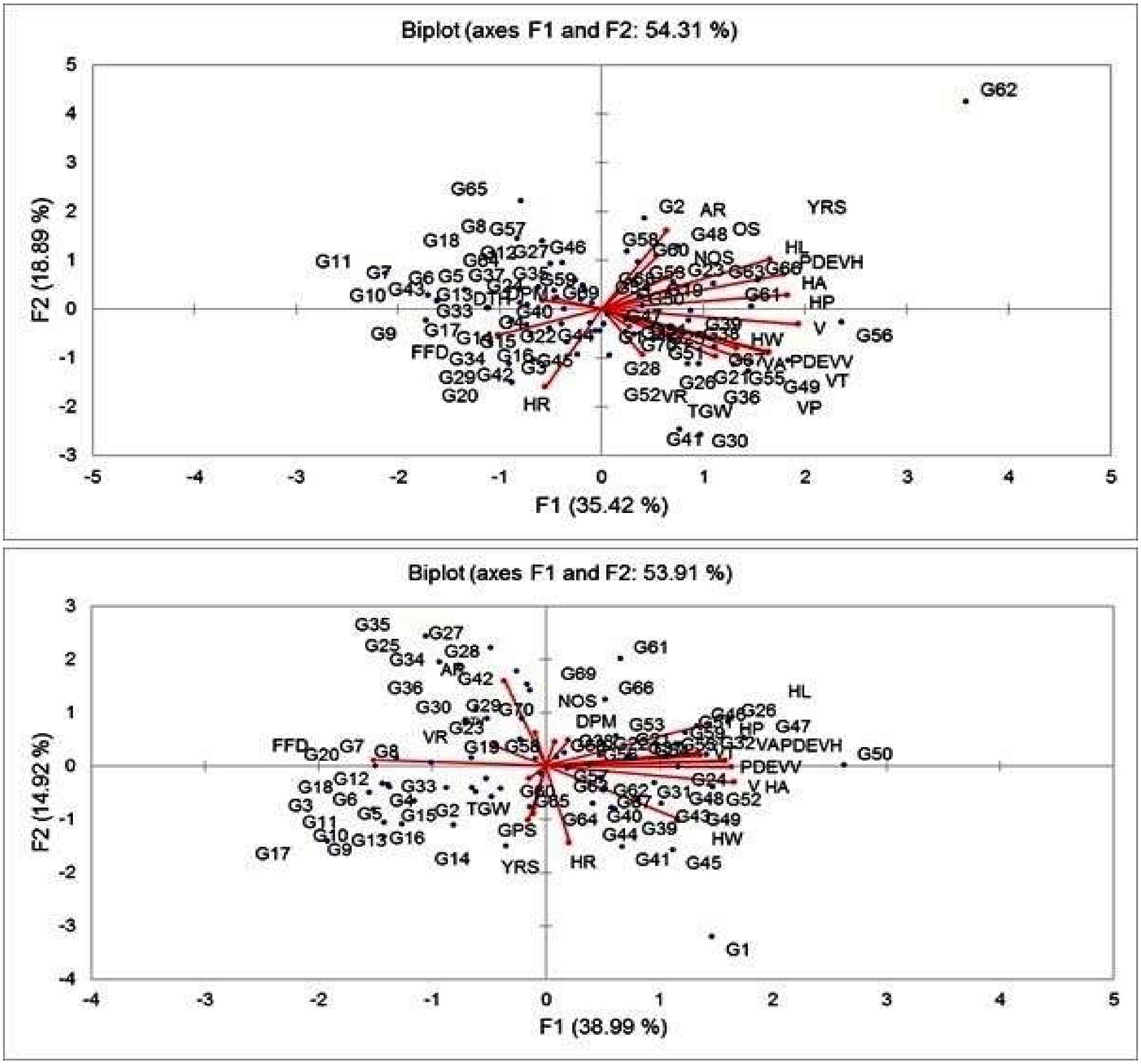

Fig. 2 Biplot analysis under normal (A) and drought (B) conditions

DTH = days to 50% heading, DPM = days to 50% physiological maturity, NOS = number of spikelets per spike, KS = kernel per spike, TKW = thousand kernel weight, YPS = kernel yield per spike, HA = horizontal area, VA = vertical area, HP = horizontal perimeter, VP = vertical perimeter, HL = horizontal length, VT = vertical thickness, HR = horizontal round, VR = vertical round, HW = horizontal width, AR = aspect ratio, V = volume, FFD = factor from density, VDFE = vertical deviation from ellips, HDFE = horizontal deviation from ellipse.

DISCUSSION

The development of high-yielding, climate-resilient wheat cultivars remains a central objective of modern breeding programs. Recent studies confirm that phenotyping diverse germplasm under both natural and managed drought scenarios is an effective strategy for identifying superior parental lines for crossing schemes aimed at enhancing drought tolerance (Guizani *et al.,* 2023; Soares *et al.,* 2025). In the present study, the germplasm panel displayed highly significant genotypic variation for all measured traits, indicating a rich reservoir of allelic diversity that aligns with reports of broad genetic variation identified via multidimensional digital phenotyping of wheat accessions (Wu *et al.,* 2023). This diversity can be exploited to produce transgressive segregants for drought resilience. Selection based on high-yielding traits, particularly kernel-associated traits under both drought and optimal conditions, enables breeders to develop resilient genotypes with sustainable performance across environments. This is supported by previous studies that emphasize the importance of phenotyping complex yield-related traits under drought (Foulkes *et al*., 2007; Soares *et al*., 2025).

Principal component analysis was executed to determine the contribution of individual traits to overall diversity. PC1, as being the most diverse component, explained kernel volume, horizontal deviation from ellipse, and horizontal area as the strongest contributors to diversity. The same traits appeared to be the strongest contributors when studied under drought stress. Digital imaging also reinforces the significance of kernel volume and shape related traits under both conditions (Wu *et al*., 2023; Zhang *et al*., 2024). Strong loading values of kernel volume and horizontal area represents a direct association between these traits which aligned with the previous findings that large surface area of kernel has direct positive association with overall yield and kernel volume (Ali *et al*., 2020).

Although the correlation matrix revealed several significant pairwise associations such as the strong positive relationship between thousand-kernel weight and vertical roundness under drought these traits did not necessarily dominate the principal components. This apparent discrepancy is expected: correlation analysis quantifies pairwise linear relationships, whereas PCA identifies the variables that explain the largest share of total multivariate variance. Consequently, the two approaches are complementary rather than contradictory, which is also highlighted in the recent investigations (Muhammad *et al.,* 2020; Wang *et al.,* 2022)

A genotype x trait (biplot) analysis provided an integrated visual assessment of genotype performance and trait inter-relationships. Genotype G17 exhibited the highest TKW under drought, whereas G30 and G41 were superior under well-watered conditions. As anticipated, genotypes projected farthest from the biplot origin and aligned with trait vectors showed above average performance, while those clustering near the origin showed comparatively poor means, a pattern consistent with earlier findings (Ahmad *et al.,* 2019).

The biplot also indicated that components of yield including yield per spike, number of kernels per spike, and spikelets per spike were clustered with horizontal kernel descriptors (horizontal area, horizontal length, horizontal deviation from ellipse). Selection for these horizontal traits may therefore deliver simultaneous gains in multiple yield attributes through pleiotropy or tight genetic linkage (Ali *et al.,* 2020; Deng *et al.,* 2011; Kumar *et al.,* 2016). Conversely, TKW aligned more closely with vertical kernel metrics (vertical area, perimeter, thickness, roundness, and vertical deviation from ellipse), with the exception of horizontal width.

These positive associations imply that genetic improvement in one trait is likely to be accompanied by gains in its correlated counterparts. Previously, increase in yield due to increase in TKW has also been reported by some researchers (Ahmad *et al.,* 2020; Muhammad *et al.,* 2020). Similarly, Akram *et al.,* (2008) reported increase in yield due to increased number of kernels per spike. Sometimes this increase in yield is compromised due to changing climatic conditions. To meet the challenge of climate change and developing drought tolerant genotypes extensive phenotyping of large germplasm collection should be undertaken (Guizani *et al.,* 2023; Soares *et al.,* 2025). The present study demonstrated that for phenotyping complex traits which are difficult to measure manually, digital imaging platform is rapid and cost effective method (Walter *et al.,* 2019). It has made possible to genotype large collections with short time frame.

Conclusion: The traits horizontal area, kernel volume and deviation from the ellipse showed strong association with yield traits and also appeared as most diverse traits. Notable genotypes which consistently performed under both normal and drought condition include G17, G41 and G30. The study highlighted that, for phenotyping of wheat kernels digital image based platforms are very effective and precise. But efficacy of these methods depends upon several factors including kernel orientation and photography systems.

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