



## Genetic diversity based on principal component and cluster analysis for various characters in spring wheat (*Triticum aestivum* L.) genotypes under drought condition

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### ABSTRACT

Genetic diversity plays an important function in the improvement of germplasm which has a direct association with the crop productivity. A number of statistical methods have been employed to investigate genetic diversity among the genotypes of various crops. Approaches like principal component and cluster analysis are useful and most frequently used for identifying plant characters individually and assisting breeders in genetically enhancing attributes in wheat genotypes. This research was carried out at the experimental field of On-farm Research Division (OFRD), Bangladesh Agricultural Research Institute (BARI), Shyampur, Rajshahi, Bangladesh, to study the genetic diversity and selection of high yielding wheat genotypes with their important agronomic and physiological traits among studied genotypes in drought condition by using principal component and cluster analyses. A total of 70 bread wheat genotypes were evaluated in  $7 \times 10$  alpha lattice design in non-irrigated drought conditions during 2018-2019 cropping season. The first four principal components (PCs) with eigen values greater than 1.0 accounting for 82.81% of the total observed variation among genotypes. Traits with maximum values in PC1 were spikes  $m^{-2}$  (SPM), thousand grain weight (TGW), ground coverage (GC), normalized difference vegetation index (NDVI), grain yield (GY), biomass (BM), and harvest index (HI) while PC2 comprised heading days (HD) and BM. The major contributors to PC3 were grains spike<sup>-1</sup> (GPS) and GC, whereas the maximum value of trait in PC4 was in relative leaf water content (RWC). The principal component biplot selected 21 high yielding genotypes than the average yield as they were distributed on the positive side of the PC1. The cluster analysis grouped 70 genotypes into six diverse clusters. Cluster II containing same 21 genotypes previously selected by principal component biplot provided the highest SPM (257.4), GPS (42.2), TGW (40.51 g), GC (0.27), NDVI (0.73), SPAD (44.24), RWC (88.33%), grain yield (3216 kg ha<sup>-1</sup>), BM (8535 kg ha<sup>-1</sup>) and HI (0.37) belonging to the lowest canopy temperature at vegetative stage (16.14 °C) and canopy temperature at grain filling stage (24.64 °C) and moderate HD (71.65 days). Based on the results of the current study the best genotypes can be used as important breeding materials in upcoming breeding schemes for drought tolerance.

**Keywords:** Wheat, cluster analysis, principal component analysis, canopy temperature, eigen value, grain yield



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## 1 Introduction

Wheat is the most extensively cultivated crop of the world, with yearly production in excess of 600 million tons throughout the Europe, Asia, Americas, Africa and Australia (Sansaloni et al., 2020). Twenty percent of the total calories and protein for human nutrition are provided by Wheat (Goel et al., 2018) and provides above 40% of the dietary consumption of vital micronutrients, as well as iron, zinc, magnesium, manganese and vitamins E and B complex for millions of people, who depend on wheat-based foods (Velu et al., 2017). It is a vital source of energy for farm animals too (Heuzé et al., 2015) and is processed for other numerous uses together with fuel (Talebniya et al., 2010). Around 95% of the world's wheat crop is hexaploid (Genomic constitution, AABBDD) bread wheat (*Triticum aestivum* L. *aestivum*), while the rest is tetraploid (AABB) durum wheat (*Triticum turgidum* L. *durum*) and other types of minor economically important wheat (Peng et al., 2011).

According to a number of publications, the genetic diversity and associations of wheat genotypes have been studied using principal component and cluster analyses. (Devesh et al., 2019; Beheshtizadeh et al., 2013; Lysenko, 2011). The benefit of cluster analysis (CA) is that samples or varieties are categorized according to complex features rather than a single characteristic (Brown-Guedira et al., 2000). Principal component analysis (PCA), which can reduce the number of potentially associated variables to a smaller set of variables termed principal components, should be carried out prior to cluster analysis (CA) (Mujaju and Chakauya, 2008). Mustafa et al. (2015) used PCA and CA to examine maize genotypes under drought stress circumstances and established that PCA makes it easier to choose potential parents for hybridization programs. Ahmad et al. (2019) used factor and cluster analysis to assess the association between bread wheat (*Triticum aestivum* L.) yields and its yield contributing traits. By employing cluster analysis, six groups were identified for various regions according to Narouee (2006) investigation of the genetic diversity of wheat landraces in western Iran. Fang and Xiong (1996) grouped 120 durum wheat genotypes into five clusters on the basis of plant height, maturity date, length of spike, seeds per spike, thousand seed weight and seed yield of spike. Adilova et al. (2020) investigated genetic diversity of wheat genotypes using principal component (PCA) and multivariate cluster analyses of some yield contributing characters of wheat, such as grain number, thousand seed weight, grain yield and plant height. Cluster and Principal Component Analysis among bread Wwheat (*Triticum Aestivum* L) genotypes in Mid Rift Valley of Oromia, Ethiopia (2022) studied principal component and Cluster analysis methods to test the extent of bread wheat genotypes clustering and to specify the essential characteristics that distin-

guish the genotypes. To evaluate genetic variation among plant genotypes and identify high-yielding genotypes, cluster analysis based on genetic diversity of yield variables can be utilized. This can be employed successfully in plant breeding by utilizing important genotypes identified from several clusters (Mostafa et al., 2011). Numerous studies have done preliminary selection of high-performing genotypes to see how well cluster analysis can be used to assess particular *T. aestivum* lines for useful economic attributes and adaptability characteristics (Hailegiorgis et al., 2011; Chekalin et al., 2008). For effective selection of improved genotypes, it is crucial to determine the correlation between yield and other associated variables. Also, by utilizing Euclidean distance in a cluster analysis based on agro-morphic parameters, similarity between the wheat genotypes was assessed. Cluster analysis has also been utilized by other researchers to examine the morphological similarities between the genotypes (Awan et al., 2014; Yadav et al., 2015).

Morphological, physiological, and yield traits are frequently used to assess genetic diversity to breed newer cultivars (Fufa et al., 2005; Cluster and Principal Component Analysis among bread Wwheat (*Triticum Aestivum* L) genotypes in Mid Rift Valley of Oromia, Ethiopia, 2022). Analysis of genotypic stability and the formation of groups with unique features are made possible by multivariate statistical methods (Lin et al., 1986). The aim of this research was to investigate the genetic diversity among studied wheat genotypes with the help of principal component and cluster analyses. Future wheat breeding programs can take use of this variability to develop new wheat cultivars with higher yields and improved grain quality.

## 2 Materials and Methods

### 2.1 Description of experimental sites

The present study was carried out at the experimental field of On-farm Research Division (OFRD), Bangladesh Agricultural Research Institute (BARI), Shyampur, Rajshahi during 2018-19. The experimental site of OFRD, BARI Shyampur, Rajshahi is located between 24.368688° N latitude and 88.662078° E longitude with elevation of about 19 m above sea level. The site belongs to the Agro Ecological Zone of High Ganges River Floodplain (AEZ- 11). In Bangladesh, rainfall pattern is uneven round the year and rainfall mostly concentrated in summer (April- September) and winter (October-March) have minimum rain. The wheat growing season (November-March) is almost rainless. Negligible amount (8.5 mm) of rainfall occurred after seeding to harvest of the trial period. Average monthly maximum temperature of wheat growing season is ranges from 25.46 °C in January

to 32.8 °C in March, while average monthly minimum temperature ranges from 10.23° C in January to 17.45 °C in March. The monthly maximum, minimum temperature and rainfall including the wheat growing season is presented in Table 1. Soil moisture data was collected at ten days interval from both trial fields starting from seeding to maturity. In this trial, drought spell started from 40 days after seeding (DAS). The field capacity of the soil of experimental field was 38%. The moisture percent (%) at different depth (0-15cm and 16-30 cm) are presented in Table 2.

## 2.2 Breeding history of planting material

In the experiment 70 genotypes were evaluated in non-irrigated drought conditions consisting of 15 genotypes from 33rd Semi-Arid Wheat Screening Nursery (33rd SAWSN), 7 genotypes from 23rd Semi-Arid Wheat Yield Trial (23rd SAWYT), 22 genotypes from 24th Semi-Arid Wheat Yield Trial (24th SAWYT), 2 genotypes from 6th Harvest Plus Yield Trial (6th HPYT), 1 genotype from 5th Stress Adaptive Trait Yield Nursery (5th SATYN) of CIMMYT and 11 advanced lines of BARI and 12 BARI released popular new and old varieties. The pedigrees of these genotypes are presented in Table 3.

## 2.3 Experimental design and procedure

Two ploughings and one cross-ploughing were used to cultivate the land. Recommended doses of fertilizers and manures were applied @ 100-27-50-20-1-4.5-5000 kg ha<sup>-1</sup> as N-P-K-S-B-Zn-cow dung, respectively. Before starting of land preparation, the total organic manures (cow dung) was applied and at the time of the last round of land preparation, all inorganic fertilizers including two thirds of urea were used as a basal dose. At 20 days (after the first irrigation) following sowing, the remaining one third of the urea was used as a top dressing. The field experiment was conducted using an alpha lattice design with two replications. The plot size was 5 m × 1 m with 5 rows. The row length was 5 m long and 20 cm distances from rows and rows, respectively. The seed rate was 12 g m<sup>-2</sup> and was sown continuously in rows. The trial was watered one time at 20 days for better establishment of crops and then allowed the crops to grow under non-irrigated drought stressed condition. Hand weeding was used to keep the research fields free of weeds and no pest control measures were taken due to absence of pest incidence.

## 2.4 Measurement of traits

### 2.4.1 Agronomic traits

The number of days between the date of planting and the stage at which half of the shoots in a plot

had fully formed spikes was known as the heading days (HD). Similar to that, maturity days (MD) were calculated as the number of days between the date of planting and the stage at which 80% of the plants had reached physiological maturity. Before harvesting, five randomly chosen plants from each plot were assessed for plant height (PH) in centimeters (cm) from the ground to the tips of the spikes at physiological maturity. The number of spikes m<sup>-2</sup> (SPM) was counted at physiological maturity of crops. Five central spikes were selected, and the average spike length (SL), excluding awns, was measured in centimeters from the base of the first spike to the top of the last spikelet and after the central spikes of five randomly chosen plants were harvested, the number of grains per spike (GPS) was counted. Thousand grains were counted randomly from bulk sample and weighed using a sensitive balance to determine thousand grain weight (TGW). Three middle rows from each plot were collected at maturity and weighed to determine the biomass. Plants were threshed to collect grain yield data after several days of sun drying. Grain moisture was taken by grain moisture metre and adjusted the yield at 12% moisture content. Grain yield divided by biomass was used to compute harvest index.

### 2.4.2 Physiological traits

**Canopy temperature:** A hand-held infrared thermometer (IRT) (Model 8866, JQA Instrument, Inc., Tokyo, Japan) was used to measure the temperature of the canopy of each genotype at a height of around 50 cm. At the vegetative and grain filling stages, the canopy temperature (°C) was monitored twice, five days apart, at noon in bright sunlight with little breeze. Mean of the two data for each stage were used for statistical analysis.

**Chlorophyll content (SPAD)** With the SPAD chlorophyll meter, measurements can be made at any time, in any weather, and at any stage of a plant's development with no particular environmental requirements. Measurements are taken from five flag leaves. A Minolta SPAD-502 chlorophyll meter was used to measure the amount of chlorophyll and express the results in SPAD units at 14 days following anthesis.

**Relative Water Content (RWC)** Fourteen days after anthesis, the RWC in the flag leaves was calculated. In laboratory, 5 cm mid-section of collected six fully expanded flag leaves from each genotypes were taken by cutting the top and bottom of all six leaves and the leaves were immediately inserted in tubes filled with distilled water after a fresh weight (FW) was taken. After allowing leaves to soak in water for around 24 hours at 4 °C in the refrigerator, turgid weight (TW) was determined. The leaf samples were oven dried

**Table 1.** Monthly maximum, minimum temperature and Rainfall (mm) in experimental sites during wheat season of June 2018- May 2019

Months	Temperature (°C)		Rainfall (mm)
	Maximum	Minimum	
Jun-18	35.79	26.22	137.9
Jul-18	33.97	26.7	239.3
Aug-18	34.58	26.97	85.3
Sep-18	34.32	26.12	117.3
Oct-18	32.11	21.84	85.8
Nov-18	29.99	16.73	0
Dec-18	26.27	11.64	8.5
Jan-19	25.46	10.23	0
Feb-19	27.62	13.17	0
Mar-19	32.8	17.45	2
Apr-19	34.52	22.95	113.9
May-19	36.14	25.74	145.8

**Table 2.** Soil moisture of experimental fields at seeding to harvest during 2018-19

Soil sampling (DAS)	MC (%) at 0-15 cm depth	MC (%) at 16-30 cm depth
0 DAS	24.84	27.65
10 DAS	23.56	25.4
20 DAS	22.07	24.24
30 DAS	27.8	29.68
40 DAS	22.42	25.92
50 DAS	20.22	23.53
60 DAS	18.2	22.69
70 DAS	17.39	20.3
80 DAS	16.33	18.62
90 DAS	15.88	17.46
100 DAS	15.02	17
110 DAS	14.07	16.82
120 DAS	13.64	15.12

MC = moisture content, DAS = Days after seeding

**Table 3.** List of wheat genotypes used in the study

Code	Pedigree/Variety	Origin
G1	BARI Gom 26	BARI
G2	BARI Gom 28	BARI
G3	BARI Gom 30	BARI
G4	SUP152/BAJ #1	33rd SAWSN, CIMMYT
G5	HUHW1/KINGBIRD #1	33rd SAWSN, CIMMYT
G6	KIRITATI/4/2*SERI.1B*2/3/KAUZ*2/BOW//KAUZ/5/HUW234+LR34/PRINIA//PBW343*2/KUKUNA/3/ROLF07	33rd SAWSN, CIMMYT
G7	KACHU/2*MUNAL #1	33rd SAWSN, CIMMYT
G8	KACHU*2/PANDORA	33rd SAWSN, CIMMYT
G9	BJY/COC//PRL/BOW/3/FRTL*2/4/QUAIU #1	33rd SAWSN, CIMMYT
G10	BAVIS/4/TC870344/GUI//TEMPORALERA M 87/AGR/3/2*WBL1	33rd SAWSN, CIMMYT
G11	BAVIS/4/SOKOLL/3/PASTOR//HXL7573/2*BAU	33rd SAWSN, CIMMYT
G12	PICAFLOP#1/5/FRET2/KUKUNA//FRET2/3/YANAC/4/FRET2/KIRITATI	33rd SAWSN, CIMMYT
G13	KACHU/6/YAR/AE.SQUARROSA (783)/4/GOV/AZ//MUS/3/SARA/5/MYNA/VUL//JUN	33rd SAWSN, CIMMYT
G14	BAJ#1/8/NG8201/KAUZ/4/SHA7//PRL/VEE#6/3/FASAN/5/MILAN/KAUZ/6/ACHYUTA/7/PBW343*2/KUKUNA	33rd SAWSN, CIMMYT
G15	BAJ#1/6/WBL1*2/4/YACO/PBW65/3/KAUZ*2/TRAP//KAUZ/5/KACHU #1	33rd SAWSN, CIMMYT
G16	PAURAQUE#1/8/NG8201/KAUZ/4/SHA7//PRL/VEE#6/3/FASAN/5/MILAN/KAUZ/6/ACHYUTA/7/PBW343*2/KUKUNA	33rd SAWSN, CIMMYT
G17	FRANCOLIN #1/4/WBL1/KUKUNA//TACUPETO F2001/3/BAJ #1	33rd SAWSN, CIMMYT
G18	PAURAQ/6/TRAP#1/BOW/3/VEE/PJN//2*TUI/4/BAV92/RAYON/5/KACHU #1	33rd SAWSN, CIMMYT
G19	FRNCLN*2/KINGBIRD #1	33rd SAWSN, CIMMYT
G20	SUP152*2/PFUNEY #1	33rd SAWSN, CIMMYT
G21	BAJ#1/4/MARCHOUC*4/SAADA/3/2*FRET2/KUKUNA//FRET2	33rd SAWSN, CIMMYT
G22	ATTILA*2/HUITES//FINSI/3/ATTILA*2/PBW65/4/TRCH/SRTU//KACHU	33rd SAWSN, CIMMYT
G23	FRET2/KIRITATI/5/NAC/TH.AC//3*PVN/3/MIRLO/BUC/4/2*PASTOR*2/6/PVN	33rd SAWSN, CIMMYT
G24	TRAP#1/BOW/3/VEE/PJN//2*TUI/4/BAV92/RAYON/5/KACHU #1*2/6/KINGBIRD #1	33rd SAWSN, CIMMYT
G25	FRANCOLIN #1*2/5/GAN/AE.SQUARROSA (236)//CETA/AE.SQUARROSA (895)/3/MAIZ/4/2*INQALAB 91	33rd SAWSN, CIMMYT
G26	PBW65/2*PASTOR	23rd SAWYT, CIMMYT
G27	KENYA SUNBIRD/2*KACHU	23rd SAWYT, CIMMYT
G28	CHIBIA//PRLII/CM65531/3/MISR 2*2/4/HUW234+LR34/PRINIA//PBW343*2/KUKUNA/3/ROLF07	23rd SAWYT, CIMMYT
G29	MILAN/KAUZ//PRINIA/3/BAV92/4/BAVIS	23rd SAWYT, CIMMYT
G30	NAVJ07/SHORTENED SR26 TRANSLOCATION/3/ATTILA/BAV92//PASTOR	23rd SAWYT, CIMMYT
G31	W15.92/4/PASTOR//HXL7573/2*BAU/3/WBL1*2/5/WHEAR/SOKOLL	23rd SAWYT, CIMMYT
G32	TRCH/SRTU//KACHU*2/3/PVN	23rd SAWYT, CIMMYT
G33	SOKOLL/3/PASTOR//HXL7573/2*BAU/4/PARUS/PASTOR	5th SATYN, CIMMYT
G34	DANPHE #1*2/SOLALA/3/TACUPETO F2001/BRAMBLING*2//KACHU	6th HPT, CIMMYT
G35	KVZ/PPR47.89C//FRANCOLIN #1/3/2*PAURAQ/5/BAV92//IRENA/KAUZ/3/HUITES*2/4/MURGA	6th HPT, CIMMYT
G36	FRANCOLIN #1/3/PBW343*2/KUKUNA*2//YANAC	24th SAWYT, CIMMYT
G37	SUP152/3/TRCH/SRTU//KACHU	24th SAWYT, CIMMYT
G38	ROLF07/4/WBL1/KUKUNA//TACUPETO F2001/3/UP2338*2/VIVITSI/5/SAUAL/MUTUS	24th SAWYT, CIMMYT
G39	HUW234+LR34/PRINIA*2//SNLG/3/BOKOTA	24th SAWYT, CIMMYT
G40	COPIO/5/UP2338*2/SHAMA/3/MILAN/KAUZ//CHIL/CHUM18/4/UP2338*2/SHAMA	24th SAWYT, CIMMYT
G41	SAUAL/YANAC//SAUAL/5/UP2338*2/SHAMA/3/MILAN/KAUZ//CHIL/CHUM18/4/UP2338*2/SHAMA	24th SAWYT, CIMMYT
G42	PRL/2*PASTOR//SUNSTATE/4/2*ATTILA*2/PBW65//PIHA/3/ATTILA/2*PASTOR	24th SAWYT, CIMMYT
G43	SAUAL/MUTUS//KINGBIRD #1/3/SAUAL/MUTUS	24th SAWYT, CIMMYT
G44	ROLF07*2/KIRITATI*2//PICAFLOP #1	24th SAWYT, CIMMYT
G45	FRET2*2/SHAMA//PARUS/3/FRET2*2/KUKUNA*2/4/TRCH/SRTU//KACHU	24th SAWYT, CIMMYT
G46	CROC_1/AE.SQUARROSA (205)//BORL95/3/PRL/SARA//TSI/VEE#5/4/FRET2/6/MTRWA92.161/PRINIA/5/SERI*3//RL6010/4*YR/3/PASTOR/4/BAV92	24th SAWYT, CIMMYT
G47	PASTOR//HXL7573/2*BAU/3/WBL1/6/MTRWA92.161/PRINIA/5/SERI*3//RL6010/4*YR/3/PASTOR/4/BAV92	24th SAWYT, CIMMYT
G48	GLADIUS/5/2*W15.92/4/PASTOR//HXL7573/2*BAU/3/WBL1	24th SAWYT, CIMMYT
G49	D67.2/PARANA 66.270//AE.SQUARROSA (320)/3/CUNNING-HAM/4/PASTOR/SLVS/5/SUNCO/2*PASTOR//EXCALIBUR/6/MTRWA92.161/PRINIA/5/SERI*3//RL6010/4*YR/3/PASTOR/4/BAV92	24th SAWYT, CIMMYT
G50	LIVINGSTON/5/2*W15.92/4/PASTOR//HXL7573/2*BAU/3/WBL1	24th SAWYT, CIMMYT
G51	Kanchan	BARI
G52	Protiva	BARI
G53	Sourav	BARI
G54	Gourab	BARI
G55	Shatabdi	BARI
G56	Bijoy	BARI
G57	Prodip	BARI
G58	Kalyansona	BARI
G59	BARI Gom 33(BAW-1260)	BARI
G60	KANCHAN/ BAW 1035	AYT, BARI
G61	BARI Gom 21/BAW 1027	AYT, BARI
G62	SUFI/BAW1035	AYT, BARI
G63	BARI Gom 24/ SW 89. 5422// BAW 1051	AYT, BARI
G64	BARI Gom 21/ NIAW-34	AYT, BARI
G65	BARI Gom 25/CY8801	AYT, BARI
G66	BAJ #1*2/TECUE #1	AYT, BARI
G67	ND643/2*WBL1//2*BAJ#1	AYT, BARI
G68	SUPER 152	AYT, BARI
G69	SUP152/AKURI//SUP152	AYT, BARI
G70	SW89-5124*2/FASAN	AYT, BARI



at 70 °C for 72 hours to get dry weight (DW). The following formula was used to calculate the RWC using the fresh, turgid, and dry weight values for the flag leaves.

#### Normalized Difference Vegetation Index (NDVI)

The NDVI is frequently used to assess the greenness of vegetation and the area of the canopy's photosynthetic surface. The Ntech "Greenseeker" NDVI meter, a field-portable NDVI sensor, offers quick measurements of crops at ground level with sufficient precision to characterize the canopy for biomass, nutrients (such as nitrogen), green area index (GAI), and leaf area index (LAI). Data can be used to predict yield, assess biomass accumulation, growth rate, early vigor, ground cover, estimates of senescence pattern, and the detection of abiotic and biotic stress. Crop ground coverage (GC) measurements were taken at 20 DAS and for discriminate sensitive and tolerant genotypes measurement was taken in pre-grain filling period. One measurement approximately for 5 seconds was taken per genotype. The NDVI can be calculated using the following formula:

$$NDVI = \frac{(NIR - R)}{(NIR + R)} \quad (1)$$

where *NIR* = Near infrared light, and *R* = red light

#### 2.4.3 Statistical analysis

Principal component analysis was produced by using Statistical Software for Social Science (SPSS, 2017). Cluster analysis and cluster mean were performed using STAR (Statistical tools for Agricultural Research) software to classify the cultivars regarding their tolerance to drought. The biplot diagram and principal component score were analysed to graphically identify the high yielding drought withstand wheat genotypes by using GenStat® version 17.2.0, VSN, International.

## 3 Results

The experimental data were collected and analysed using principal component analysis to identify very much influential traits for selection and principal component biplot also graphically showed association among traits and separated suitable superior genotypes from others. Moreover, using cluster analysis, the genotypes were clustered in several groups to identify high yielding superior cluster for further advancement.

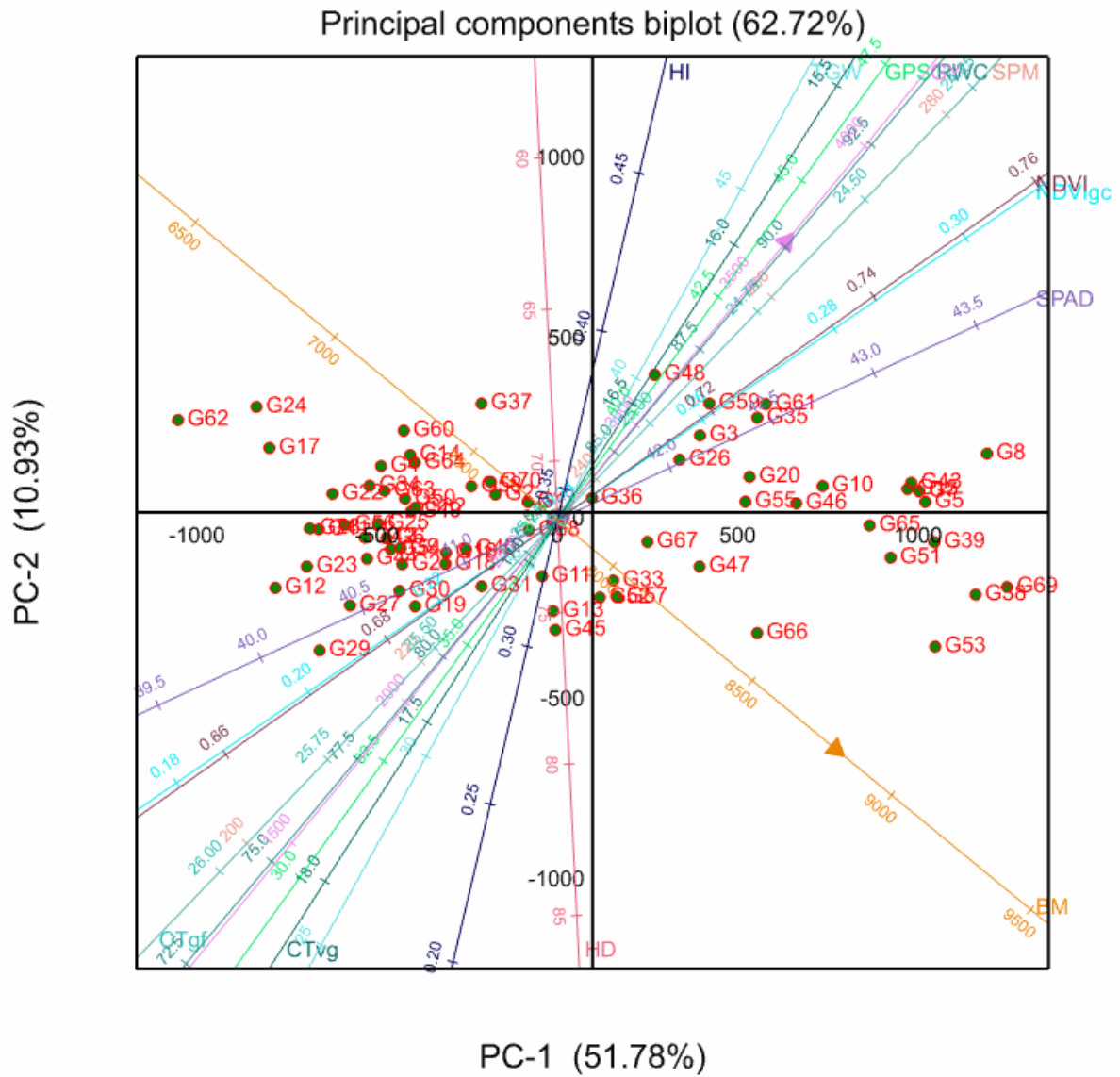
### 3.1 Principal component analysis

The proportion of total variance explained by different principal components, cumulative variance, Eigen

values and the correlations of principal components with agronomic and physiological traits are shown in the component matrix table (Table 4). The first four principal components were significant, with eigen values greater than 1.0 and accounted for 82.81% of the observed variation. The first and second principal components (PC1 and PC2), which together accounted for 51.78% and 10.93% of the total variation, were the most significant, contributing a total of 62.72% to the overall variation. Variables SPM, TGW, GC, NDVI, GY, BM and HI (with more than 50% contribution each) and GPS (more than 30% each) had large positive loadings into the first principal component while the second principal component revealed a substantial positive loading for HD and BM. The third principal component (PC3) exhibited high positive loading for GPS and GC, whereas the fourth principal component (PC4) had high positive loading for RWC. CTvg and CTgf had significant negative loading into the first principal component (PC1). The principal component biplot (Fig. 1) for the drought stress situation also illustrates the relationships between the various traits and genotypes with their corresponding principal components. Smaller angles between dimension vectors pointing in the same direction suggested a high degree of significant correlation of the different characters in terms of discriminating genotypes. The genotypes G3, G5, G7, G8, G10, G15, G20, G26, G33, G35, G38, G39, G43, G46, G47, G48, G51, G52, G53, G55, G57, G59, G61, G65, G66, G67 and G69 were distributed on the first principal component's positive side producing higher yield than the average yield of studied genotypes which was mostly contributed by GPS, SPM and TGW, as well as optimum values of other associated agronomic and physiological traits. These genotypes are more inclined in the direction of the dimension vectors of GPS, SPM, TGW, GC, NDVI, RWC, SPAD, GY, HI and also BM. The dimension vectors of GPS, SPM, TGW, GC, NDVI, RWC, SPAD, GY and HI produced a smaller angle with each other and their directions were also the same.

### 3.2 Non-hierarchical clustering and cluster mean

Regarding grain yield as well as several agronomic and physiological parameters, the genotypes significantly differed from one another. Seventy wheat genotypes were divided into six distinct clusters using Mahalanobis D<sup>2</sup> statistics and Tocher's non-hierarchical clustering approach. Table 5 reveals the pattern of genotype distribution into various clusters. The distribution pattern revealed that cluster II included the greatest number of genotypes (21), followed by cluster I (19), cluster III (15), cluster V (8), cluster IV (4), and cluster VI (3). The cluster means for 13 characters are shown in Table 6. The majority of the characters had differentiating potentiality, indicating their



**Figure 1.** Principal component biplot showing grouping of genotypes under drought stress condition. DH= heading days; GPS= Grains per spike; SPM= spikes per m<sup>2</sup> ; TGW= Thousand grain weight (g); CT<sub>vg</sub> = Canopy temperature at vegetative stage, CT<sub>gf</sub> = Canopy temperature at grain filling stage, SPAD= Chlorophyll content; GC= ground coverage; NDVI= Normalized difference vegetation index; RWC (%) = Relative water content of leaf; GY= grain yield (kg ha<sup>-1</sup>); BM=Biomass ; HI= Harvest Index

proper clustering capabilities. Cluster II provided the highest SPM (257.4), GPS (42.2), TGW (40.51 g), GC (0.27), NDVI (0.73), SPAD (44.24), RWC (88.33%), grain yield (3216 kg ha<sup>-1</sup>), BM (8535 kg ha<sup>-1</sup>) and HI (0.37) belonging to the lowest canopy temperature at vegetative stage (16.14 °C) and canopy temperature at grain filling stage (24.64 °C) and moderate heading days (71.65 days). Cluster V provided the second highest SPM (234.24), TGW (35.26 g), GC (0.24), NDVI(0.69), SPAD (41.72), grain yield (2708 kg ha<sup>-1</sup>), BM (7947 Kg ha<sup>-1</sup>) and HI (0.34) with the second lowest canopy temperature at vegetative stage (16.69 °C) and canopy temperature at grain filling stage (25.37 °C) and moderate heading days (71.83 days). The cluster I showed second highest HD (69.76 days) and exhibited the lowest GC (0.22). The cluster VI provided the lowest HD (68.57 days), SPAD (38.65) and RWC (68.67%). The cluster III exhibited the highest canopy temperature at grain filling stage (25.59 °C) and rest of the traits provided intermediate values. Cluster IV provided the lowest SPM (220.2), GPS (33.8), TGW (31.37 g), GC (0.19), NDVI (0.66), grain yield (2092 kg ha<sup>-1</sup>) and HI (0.29) with the highest HD (75.9 days) and canopy temperature at vegetative stage (17.89 °C). The yield rank of the clusters is cluster II > cluster V > cluster III > cluster I > cluster VI > cluster IV. The cluster II containing 21 genotypes (G3, G5, G7, G8, G10, G15, G20, G26, G35, G38, G39, G43, G46, G48, G51, G53, G55, G59, G61, G65, G69) with high performance in terms of grain yield and other associated characters are selected under drought stress condition for further drought tolerance evaluation.

## 4 Discussion

### 4.1 Principal component analysis

Numerous associated variables can be reduced to a smaller set of variables termed principal components using principal component analysis (Mujaju and Chakauya, 2008). Principal component analyses have been recommended by a number of authors to investigate the degree of divergence and relationships among wheat genotypes (Beheshtizadeh et al., 2013; Devesh et al., 2019; Lysenko, 2011), and this method aids in the selection of promising parents for hybridization programs (Mustafa et al., 2015).

The first four principal components, out of a total of thirteen, were significant, with eigen values larger than 1.0 describing 82.81% of the total variation. The first and second principal components (PC1 and PC2), which together contributed for 51.78% and 10.93% of the variance explained, were the most significant, with a cumulative participation to the overall variation of 62.72%. According to the principal component analysis, under drought stress, SPM, TGW, GC, NDVI, GY, BM, and HI (each contributing more than 50%) and GPS (each contributing more

than 30%) had large positive loadings into the first principal component, which had a significant impact on selection, while HD and BM had a positive significant loading into the second principal component, which could also be selected jointly. This highlights the significance of identifying genotypes based on physiological and agronomic traits even more. Doing so could lead to the simultaneous selection of complementing genes that increase yield. A higher survival rate could be attained at the sacrifice of grain yield if only a few key genes are prioritized (Passioura, 2012). Many researchers used principal component analysis and described that the first two components contribute higher and have much influence during selection (Beheshtizadeh et al., 2013; Ali et al., 2021; Devesh et al., 2019). The principal component biplot for the drought stress situation also illustrates the relationships between the various traits and genotypes and their associated principal components. The genotypes G3, G5, G7, G8, G10, G15, G20, G26, G33, G35, G38, G39, G43, G46, G47, G48, G51, G52, G53, G55, G57, G59, G61, G65, G66, G67 and G69 were distributed on the first principal component's positive side producing higher yield than the average yield of studied genotypes which was mostly contributed by GPS, SPM and TGW, as well as optimum values of other associated agronomic and physiological traits. The dimension vectors of GPS, SPM, TGW, GC, NDVI, RWC, SPAD, GY and HI produced smaller angle with each other and their direction is also same. Biplot analysis has been used in several studies to identify drought-tolerant genotypes in a variety of crop species based on the first two principal components. Golabadi et al. (2006); Mohammadi et al. (2011); Farshadfar et al. (2012); Rahimi et al. (2013); Mohammadi et al. (2012); Aliakbari et al. (2014).

### 4.2 Non-hierarchical clustering and cluster mean

For various physiological and agronomic parameters as well as grain yield, the genotypes differed significantly from one another. Seventy wheat genotypes were divided into six groups using Mahalanobis D<sup>2</sup> statistics and Tocher's non-hierarchical clustering approach. This analysis revealed that cluster II had the most genotypes (21) followed by cluster I (19), cluster III (15), cluster V (8), cluster IV (4), and cluster VI (3). The major objective of the cluster analysis is to separate the top yielding genotypes under drought condition for inclusion in further drought tolerance study. Clustering these genotypes can also be helpful for identification of genotypes with same characters, which can be suitable for breeding programs. In a crop development program, it is crucial that genotypes from the appropriate cluster can be employed for the appropriate attributes. Additionally, it was noted that genotypes with high levels of diversity



**Table 4.** Principal component analysis of different agronomic and physiological traits of 70 wheat genotypes evaluated under drought-stressed conditions

Traits	PC1	PC2	PC3	PC4
HD	-0.013	0.945	0.01	-0.026
GPS	0.405	-0.008	0.889	0.16
SPM	0.873	-0.099	0.196	0.123
TGW	0.764	-0.19	0.261	0.217
GC	0.846	-0.031	0.338	-0.011
CTgf	-0.699	0.118	-0.193	-0.3
CTvg	-0.824	0.175	-0.073	-0.284
NDVI	0.788	-0.073	0.257	0.153
SPAD	0.768	0.183	0.146	0.251
RWC	0.274	-0.04	0.128	0.935
GY	0.914	-0.117	0.25	0.192
BM	0.848	0.336	0.153	0.149
HI	0.705	-0.519	0.259	0.153
Proportion of variance (%)	51.78	10.93	10.08	10.01
Cumulative variance (%)	51.78	62.72	72.8	82.81
Explained variance (Eigen values)	6.73	1.42	1.31	1.3

DH= heading days; GPS= Grains per spike; SPM= spikes per m<sup>2</sup>; TGW= Thousand grain weight (g); CTvg= Canopy temperature at vegetative stage, CTgf= Canopy temperature at grain filling stage, SPAD= Chlorophyll content; GC= ground coverage; NDVI= Normalized difference vegetation index; RWC (%) = Relative water content of leaf; GY= grain yield (kg ha<sup>-1</sup>); BM=Biomass; HI= Harvest Index

**Table 5.** Distribution of 70 spring wheat genotypes in different cluster based on Mahalanobis' D<sup>2</sup> values

Cluster	No. of genotypes	% of total gen.	Cluster member
I	19	27.14	G1, G2, G4, G6, G14, G17, G19, G22, G23, G24, G25, G28, G30, G34, G41, G44, G60, G63, G64
II	21	30	G3, G5, G7, G8, G10, G15, G20, G26, G35, G38, G39, G43, G46, G48, G51, G53, G55, G59, G61, G65, G69
III	15	21.43	G9, G11, G13, G16, G18, G21, G31, G32, G33, G40, G45, G49, G50, G57, G58
IV	4	5.71	G12, G27, G29, G42
V	8	11.43	G36, G37, G47, G52, G66, G67, G68, G70
VI	3	4.29	G54, G56, G62

**Table 6.** Cluster means for thirteen characters of 70 spring wheat genotypes

Traits	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
HD	69.76	71.65	74.87	75.9	71.83	68.57
GPS	35.68	42.2	36.66	33.8	34.34	38.1
SPM	225.7	257.4	224.8	220.2	234.24	222.9
TGW	34.66	40.51	34.35	31.37	35.26	35.04
GC	0.22	0.27	0.23	0.19	0.24	0.23
CTgf	25.39	24.64	25.59	25.57	25.37	25.43
CTvg	17.25	16.14	17.06	17.89	16.69	17.59
NDVI	0.69	0.73	0.69	0.66	0.69	0.69
SPAD	39.16	44.24	41.33	39.73	41.72	38.65
RWC	83.15	88.33	84.06	78.54	77.23	68.67
GY	2371	3216	2445	2092	2708	2260
BM	7246	8535	7672	7312	7947	7035
HI	0.33	0.37	0.32	0.29	0.34	0.32

DH= heading days; GPS= Grains per spike; SPM= spikes per m<sup>2</sup>; TGW= Thousand grain weight (g); CTvg= Canopy temperature at vegetative stage, CTgf= Canopy temperature at grain filling stage, SPAD= Chlorophyll content; GC= ground coverage; NDVI= Normalized difference vegetation index; RWC (%) =Relative water content of leaf; GY= grain yield (kg ha<sup>-1</sup>); BM=Biomass; HI= Harvest Index

within clusters would result in more breeding resources expected to produce high levels of genetic advancement (Singh et al., 2010), and high diversity in the parents suggested a greater possibility of obtaining higher levels of heterosis (Zaman et al., 2005). Crossing genotypes from other clusters may increase the probability of transgressive segregation because there is a greater possibility that unrelated genotypes will provide distinctive expected alleles at several loci (Beer et al., 1993). The yield rank of the clusters is cluster II > cluster V > cluster III > cluster I > cluster VI > cluster IV. The clustering with the performance of genotypes under drought stress condition is further presented by the dendrogram created based on all studied traits using agglomerative clustering algorithm. The cluster II containing 21 genotypes (G3, G5, G7, G8, G10, G15, G20, G26, G35, G38, G39, G43, G46, G48, G51, G53, G55, G59, G61, G65, G69) with high performance in terms of grain yield and other associated characters are selected under drought stress condition for further drought tolerance evaluation.

## 5 Conclusion

The results of the principal component analysis showed that the major components took part greatly to evaluate the superior genotypes in under drought stress condition. Hence, the variables SPM, TGW, GC, NDVI, GY, BM, HI and GPS had large positive loading into the first principal component while the second principal component revealed a substantial positive loading for HD and BM. The third principal component (PC3) exhibited high positive loading for GPS and GC, whereas the fourth principal component (PC4) had positive high loading for RWC. The

principal component biplot separated high yielding and superior genotypes than average yield as they are distributed on the first principal component's positive side. The cluster analysis revealed that cluster II had the most genotypes (21) followed by cluster I (19), cluster III (15), cluster V(8), cluster IV(4), and cluster VI (3). The yield rank of the clusters is cluster II > cluster V > cluster III > cluster I > cluster VI > cluster IV. The cluster II containing 21 genotypes with high performance in terms of grain yield and its associated characters were selected under drought stress condition for further drought tolerance breeding to obtain drought tolerant high yielding genotypes.

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## Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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