



## GENETIC DIVERSITY AND GENOTYPE BY TRAIT ANALYSIS FOR AGRO-MORPHOLOGICAL AND PHYSIOLOGICAL TRAITS OF WHEAT (*Triticum aestivum* L.)

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### SUMMARY

Assessment of genetic diversity and its application for wheat breeding results in enhanced and sustainable production and productivity. Twenty-three wheat genotypes along with 2 checks were evaluated for 9 agro-morphological and physiological traits viz., early vigor, leaf firing, days to heading, plant height, number of tillers/m, chlorophyll content Index (CCI) at 3 stages and grain yield. The data was further subjected to PCA (principal component analysis) and genotype by trait biplot analysis. The first 5 principal components accounted for 90.5% of total variation. The cluster analysis shows that there is significant genetic variability among tested wheat genotypes that indicates the presence of excellent opportunity to bring about improvement through hybridizing the selected genotypes present in distant clusters. Across the 25 tested wheat genotypes grain yield was positively associated with tillers/m and early vigor while negatively associated with leaf firing. CCI (Chlorophyll Content Index) measured at 3 stages, were positively associated with each other. The vector traits yield made a 180 degree angle with leaf firing indicating they were opposite in genotype ranking.

**Keywords:** Genotype by trait biplot, principal component analysis, wheat

**Short summary statement:** Winter wheat gene pool is a rich reservoir of genetic diversity for various traits of economic importance. It thus becomes imperative to assess the variability for different traits and utilize it in the wheat breeding programs. This study has identified the winter wheat derived genotypes for agro-morphological and physiological traits and these genotypes can be used in breeding programs.

Manuscript received: August 8, 2014; Decision on manuscript: September 12, 2014; Manuscript accepted: October 3, 2014.

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Communicating Editor: Sam Periyannan

### INTRODUCTION

Wheat is one of the world's most important agricultural crops; with annual global grain production estimated around 710 million tons (IGC, 2014) and it provides 20% of the calories and protein for the world's population (Braun *et.al*, 2010). The value and need to further

increase the production of wheat is recognized widely.

Plant breeding, in principle involves creation and exploitation of the genetic variation for different traits of economic importance. However, a century-long bread wheat breeding effort for developing high yielding varieties resulted in reduction of genetic diversity, thus

emphasizing the need for creation of variability in wheat breeding programs (Fu and Somers, 2009).

The winter wheat gene pool possesses huge diversity for various agronomic traits, can be exploited for widening the genetic base and improving the yield and other agro-morphological traits of spring wheat varieties. Winter wheat being hardier for most of the biotic and abiotic stress tolerance, presents themselves as a source for these traits. Also since there is no crossability barriers it becomes imperative to use these as donors for the improvement of spring wheat. One of the important approaches of wheat breeding is hybridization followed by selection. Choice of suitable parents is the basic need of any crop improvement program. Precise information on the nature and degree of genetic diversity helps plant breeders in selecting the parents for targeted hybridization (Samsuddin, 1985). Genetic divergence analysis estimates the extent of diversity existing among selected genotypes. The cluster analysis is an appropriate method for determining family relationships i.e. to determine the extent of genetic affinity or distance of genotypes from each other (Mellingers, 1972).

In order to benefit from transgressive segregation, genetic distance between parents is necessary (Joshi *et al.*, 2004) that can be estimated by Euclidean distance (Hoque and Rahman, 2006). Principal component analysis (PCA) allows natural grouping of the genotypes and is precise indicator of differences among genotypes. The main advantage of using PCA over cluster analysis is that each genotype can be assigned to one group only (Mohammadi, 2002).

Relationships among traits of economic importance impact the breeding strategies along with selection procedure. If all breeding objectives were positively correlated, selection would not be difficult than selecting for a single trait. If all breeding objectives were either positively correlated or independently inherited, selection would not be too difficult either (Mohammadi or Amri, 2011). Strong negative genetic correlations between breeding traits often exist, which make breeding very challenging (Yan and Wallace., 1995; Lewis, 2006). The genotype-by-trait (GT) biplot is a statistical tool for evaluating cultivars based on multiple traits

and for identifying lines that are superior. Entries identified for agro-morphological and physiological traits hence could be candidates for use as parents in a breeding program (Yan and Rajcan, 2002). The GT biplot analysis allows visualization of genetic correlation among traits (Ma *et al.*, 2004; Yan and Fregeau-Reid, 2008) and also helps in studying genotype by trait relationships.

The objectives of this study were to (i) estimate the diversity among tested genotypes for agronomic and physiological traits and (ii) to identify the genotype and trait relationship by GT biplot.

## MATERIALS AND METHODS

### Plant Material

The plant material comprised 11 advance lines bulked from winter x spring crosses and 12 lines selected from International Nurseries supplied from CIMMYT, Mexico (Table 1). These 23 wheat strains (WS) numbered WS1 to WS23 along with 2 checks HD2967 and GW322 were evaluated in a 5 X 5 simple lattice design with 2 replications during crop season of 2012-13 at the Directorate of Wheat Research Karnal farm ((Latitude 29° 43' N, longitude 76° 58' E and altitude 245 m). These genotypes were sown in plots of 6 rows of 5 meter length with a row spacing of 20 cm. A pre-emergence spray of Penidmethalin (Stomp) 30EC was applied at the rate of 1 kg AI/Hectare for control of weeds. The recommended dose of fertilizer (N:P:K:: 150:60::40 Kg/ha) for North Western Plains Zone of India was applied as: ½ dose of Nitrogen and full dose of phosphorus and potash as basal and remaining nitrogen was top dressed in 2 splits at the time of first and second irrigation. During the crop season 4 irrigations were applied at CRI (Crown Root Initiation), first node (45DAS), jointing (65 DAS) and milking (105 DAS) stages.

The observations were recorded on the following parameters:

Early vigour (EV): The early vigour was assessed visually at the 6-7-leaf stage (1 to 5, with 5 being the best).

**Table 1.** Tested genotypes of wheat with their pedigree.

Wheat Strain #	Pedigree	Source
WS 1	UP 2572/WUGENG 8025	Winter X Spring derivatives
WS2	UP2425/SPARTANKA-KAK-HORI-DOLI//PHR 1010	Winter X Spring derivatives
WS3	UP2425/Centruk//PHR1010	Winter X Spring derivatives
WS4	UP2425/Centruk//PHR1010	Winter X Spring derivatives
WS5	UP2425/Centruk//PHR1010	Winter X Spring derivatives
WS6	UP2425/Centruk//PHR1010	Winter X Spring derivatives
WS7	UP2425/Centruk//PHR1010	Winter X Spring derivatives
WS8	90Zhong65/UP 2572	Winter X Spring derivatives
WS9	90Zhong65/UP 2572	Winter X Spring derivatives
WS10	UP2572/F35.70	Winter X Spring derivatives
WS11	HD 2590/Amigo//UP2572	Winter X Spring derivatives
WS12	SOKOLL/3/PASTOR//HXL7573/2*BAU	28 <sup>th</sup> SWASN 3013 (2010-11)
WS13	KLDR/PEWIT1//MILAN/DUCULA	28 <sup>th</sup> SAWSN 3111 (10-11)
WS14	FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ*2/5/KIRITATI	5 <sup>TH</sup> STEMRRSN 6056 (2010-11)
WS15	SERI82/SHUHA'S//PASTOR2	CWANA 11 <sup>th</sup> SBWON 69 (2010-11)
WS16	IBWSN34/QAFZAH24//SUNBRI	CAWAN 11 <sup>th</sup> SBWON 134 (2010-11)
WS17	Grackle	I CSISA HTEM 10218 (2009-10)
WS18	PAURAQUE	I CSISA HTEM 10236 (2009-10)
WS19	CNO79//PF70354/MUS/3/PASTOR/4/BAV92/5/FRET2/KUKUNA//FRET2/6/MILAN/KAUZ//PRINIA/3/BAV92	3CSISA DRYT 5221 (2010-11)
WS20	WAXWING*2/CIRCUS	ICSISA SB6737
WS21	GOUBARA-1/2*SOKOLL	19 <sup>th</sup> SAWYT 340 (2011-12)
WS22	SOKOLL//FRTL/2*PIFED	19 <sup>th</sup> SAWYT 331 (2011-12)
WS23	GK ARON/AG SECO 7846//2180/4/2*MILAN/KAUZ//PRINIA/3/BAV92	19 <sup>th</sup> SAWYT 326 (2011-12)
HD 2967	ALD/CUC//URES/HD2160M/HD2278	Check
GW 322	PBW173/GW196	Check

Leaf firing (FI): Yellowing of leaf tips at 65 days after sowing was recorded for presence (1) and absence (0).

Days to heading (DTH): It was calculated as days taken from sowing to emergence of 50% spikes in a plot.

Plant height (PtHT): Measured at time of maturity in centimeters from the ground level up to terminal spikelet, excluding awns.

Number of tillers/m (Tillers): Productive tillers were counted from 3<sup>rd</sup> and 4<sup>th</sup> row in one meter row length of the plot and averaged.

Chlorophyll content Index: The chlorophyll content Index (CCI) of flag leaf was measured from the middle part of the leaf by OPTI-SCIENCES CCM 200 plus chlorophyll meter at 3 stages:

CHL I: Chlorophyll Concentration Index at time of when the crop was fully flowered.

CHL II: Chlorophyll Concentration Index during grain filling duration (10 days after CHL I)

CHL III: Chlorophyll Concentration Index during at physiological maturity

Grain yield (Yld): The grain yield was recorded in grams from the gross plot area (6.0 m<sup>2</sup>.)

### Statistical analysis

The Euclidean distances were calculated by the Wards method and dendrogram was constructed to examine the relationships among different genotypes (Karon' ski and Calin' ski, 1973; Sokal and Rohlf, 2003). Principal component analysis, cluster analysis and Genotype by trait Biplot was constructed from Paleontological Statistics 3.01 Software Package (University of Oslo, Norway) (Hammer *et al.*, 2001).

### RESULTS AND DISCUSSION

Results obtained from the experiment are described and discussed under the following heads.

#### Principal Component Analysis

Principal component analysis (PCA) reveals the significance of the major contributor to the total variation at each axis of differentiation. The eigen values helps in determining the number of factors to be retained. Therefore, in this analysis the first factor retains the information contained in 3.788 of the original variables (Table 2).

**Table 2.** Vector loadings and percentage explained variation by the first 5 PCs.

Traits	PC1	PC2	PC3	PC4	PC5
EV	-0.2500	0.4443	0.3779	0.1523	-0.2156
FI	0.4352	-0.1897	0.3659	0.1026	0.0834
DTH	0.2388	0.1165	0.6752	-0.2649	0.3957
PtHT	-0.2654	0.05568	0.2527	0.7799	0.1998
Tillers	-0.3394	0.1638	-0.1495	-0.3156	0.7217
CHLI	0.3956	0.3349	-0.2617	0.1699	0.2008
CHLII	0.3535	0.3714	-0.3287	0.2995	0.2499
CHLIII	0.1452	0.6344	0.06791	-0.2597	-0.3555
Yield	-0.4505	0.2657	-0.0002889	-0.04693	0.0063
			Loadings		
Eigen value	3.7886	1.5585	1.2160	0.8766	0.7018
Individual Percentage	42.0960	17.3170	13.5110	9.7401	7.7978
Cumulative variance	42.0960	59.4130	72.9240	82.6641	90.4619

The first 5 principal components, PC1 to PC5 obtained from original data accounted for 90.5% of total variation. Among all principal components PC1 contributed maximum (42.09%) to the total variation.

Characters with largest absolute value closer to unity within the first principal component influences the clustering more than those with lower absolute value closer to zero (Chahal and Gosal, 2002). Accordingly, the major contributing traits for diversity in first principal component were leaf firing and grain yield. Presence of positive and negative correlation trends between the components and the variables are interpreted by positive and negative loading. Similarly, for second principal component (PC2) CHL III and early vigor were major contributors for the diversity.

The major contributing character for the diversity in the third principal component 3 (PC3) was days heading, while plant height in principal component four (PC4) and tillers per meter and days to heading in principal component five (PC5).

Customary, one variable is selected from these identified groups depending on respective loadings. Hence, for the first group grain yield is the best choice, which had the largest loading

from PC1, CHL III for the PC2, days to heading for the third group and plant height and tillers/m for fourth and fifth group respectively.

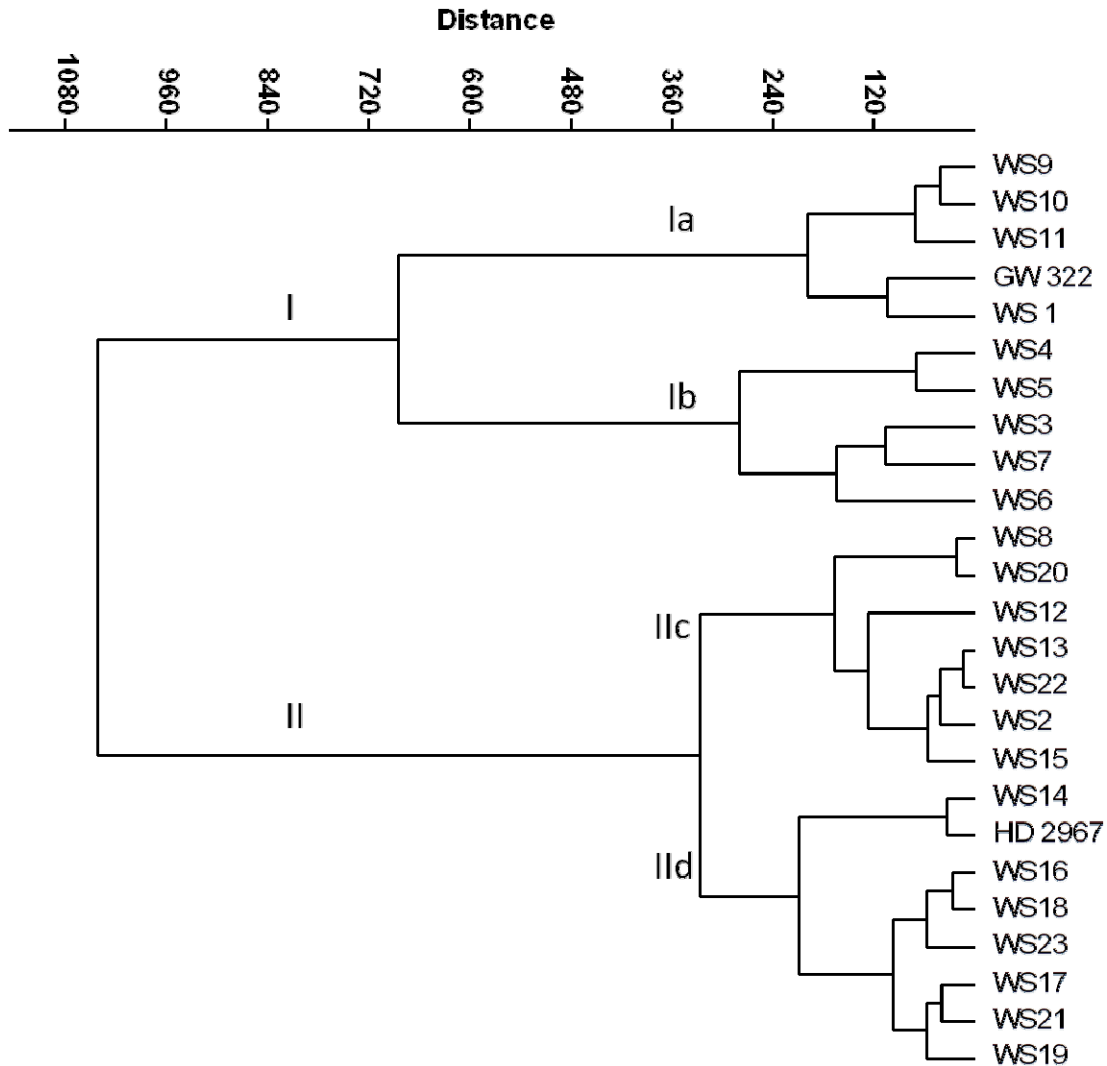
### Cluster Mean Analysis

The dendrogram was generated to examine the relationships among different genotypes based on Euclidean distances, calculated by the Wards method is presented in Figure 1, mean values of the traits in each cluster studied are presented in Table 3 and the feature of each cluster is described below.

Looking at the dendrogram, it could be seen that 2 major clusters were formed, Cluster I and cluster II. Cluster I have 2 sub-clusters which could be named as 'a' and 'b'. Cluster 'a' consisted of 5 genotypes *viz.*, WS9, WS10, WS 11, WS 1 and check variety GW322. The genotypes in this cluster had shown no leaf firing and were early in flowering with dwarf height, moderate yield and high CCI at maturity. Similarly cluster 'b' comprised of 5 winter x spring derivatives (WS3, WS4, WS 5, WS6 and WS7) derived from common cross (UP2425/Centruk//PHR1010) which were characterized by high leaf firing, delayed flowering, tall plant height, low tillering and low yield.

**Table 3.** Mean value of the traits in the 4 clusters.

Cluster		EV	FI	DTH	PtHT	Tillers	CHLI	CHLII	CHLIII	Yield
Cluster I a	Mean	3.80	0.00	95.40	90.49	115.90	24.91	23.75	3.91	2444.10
	Max	4.00	0.00	105.50	93.55	138.00	27.60	25.45	8.00	2562.50
	Min	3.00	0.00	90.50	85.40	93.50	22.55	22.15	1.95	2279.50
Cluster I b	Mean	3.80	1.00	106.70	90.55	85.20	27.14	23.80	3.25	1760.60
	Max	4.00	1.00	111.00	90.90	100.00	31.55	25.85	5.10	1980.50
	Min	3.50	1.00	103.00	90.10	78.00	24.60	21.80	1.90	1558.00
Cluster II c	Mean	4.21	0.00	99.79	92.86	128.07	21.15	18.44	2.73	2970.64
	Max	5.00	0.00	104.00	94.10	176.00	26.15	24.90	6.60	3110.50
	Min	3.00	0.00	96.00	91.75	102.50	14.20	11.65	1.60	2849.00
Cluster II d	Mean	4.56	0.00	100.00	93.79	129.69	22.46	19.54	3.09	3294.81
	Max	5.00	0.00	105.00	102.50	167.50	26.35	23.65	6.70	3459.50
	Min	3.50	0.00	95.50	90.10	105.00	17.55	14.55	1.55	3171.50

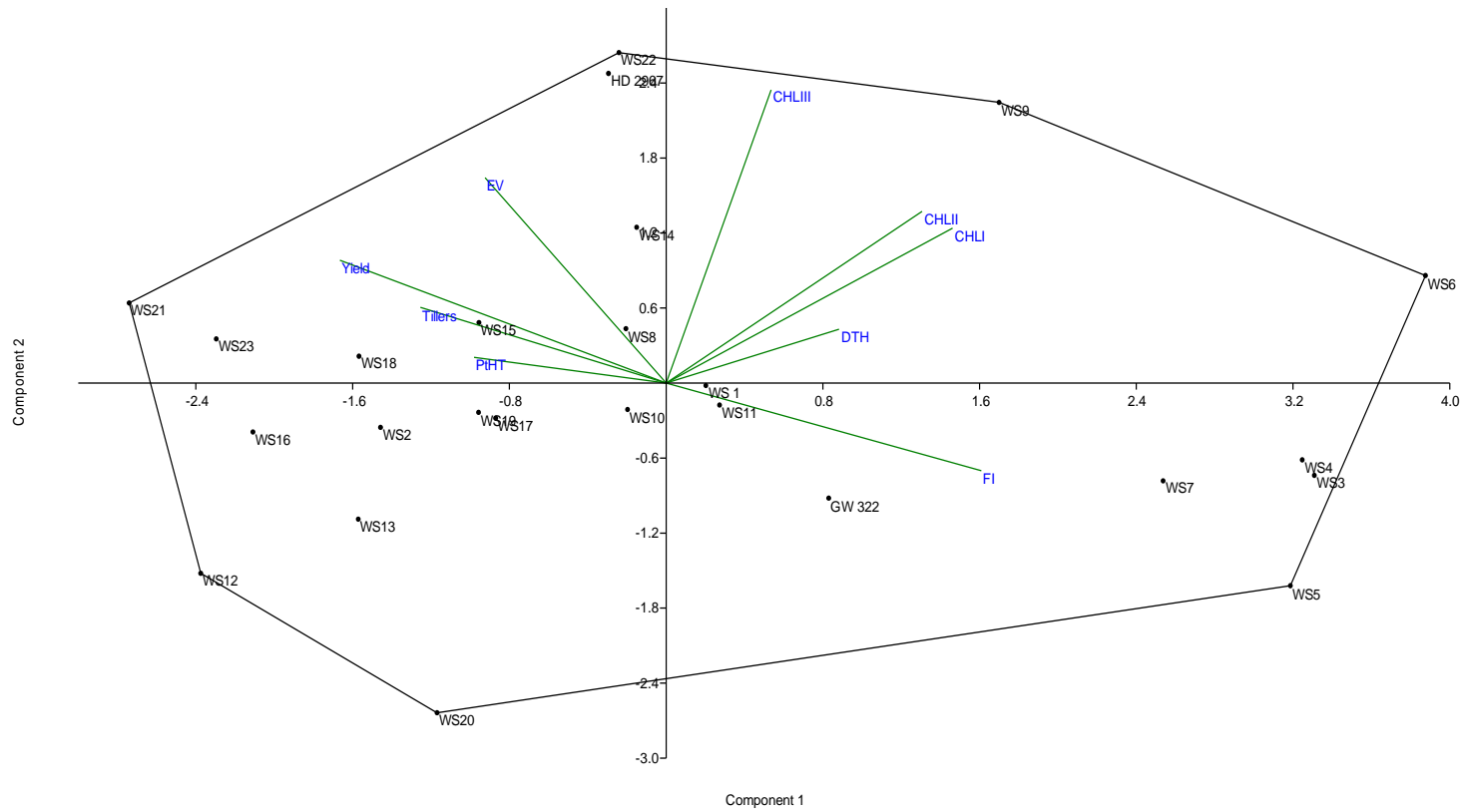


**Figure 1.** Dendrogram depicting genetic relationships among 25 wheat genotypes.

These genotypes possess high CHL I but high chlorophyll was not converted to high yield due to shorter time for grain filling caused by terminal heat. Both cluster represented 40% of total genotypes with an equal representation of 20% for each cluster.

Cluster II have 2 sub-clusters 'c' and 'd'. Cluster 'c' comprised of 7 genotypes (WS8, WS20, WS12, WS13, WS22, WS2 and WS 15) with characteristic feature of moderate early vigor, no leaf firing, moderate tillering and average yield. This cluster represented 28% of total genotypes.

Cluster 'd' consisted of 8 genotypes viz., WS 14, WS16, WS18, WS23, WS17, WS21 and WS19) and the recently released variety HD2967 with characteristic feature of high early vigor, no leaf firing, medium plant height, high tillering and high grain yield. This was the largest cluster with a representation of 32% of total genotypes. The cluster analysis showed that there was significant genetic variability among wheat genotypes tested that indicated the presence of



**Figure 2.** Genotype by trait (GT) biplot comprising of 25 wheat genotypes studied for 9 traits in 2012-13 cropping season.

excellent opportunity to bring about improvement through hybridizing genotypes from different clusters. The winter x spring derivatives from cluster II should be crossed with the genotypes of cluster I to reduce the flowering time. High yielding genotypes from cluster IV could be further tested for their combining ability. Thus the genotypes present in different clusters can be hybridized to assemble desirable traits with higher heterotic potential.

### Genotype by trait (GT) biplot

The biplot (Figure 2) displays the relationship of 25 wheat lines for 9 traits. The GT biplot of the mean performance of the wheat genotypes explained the 59.4 % of the total variation of the standardized data.

In GT biplot, a vector drawn from origin to each trait facilitates the visualization of interrelationships among traits. The vector length of the trait measures the magnitude of its effects on the yield (Yan and Tinker, 2005). The polygon view of GT biplot is best to visualize the interaction pattern between genotypes and traits, provided the biplot should explain a sufficient amount of the total variation. The correlation coefficient between any 2 traits is approximated by the cosine of the angle between their vectors (Yan and Rajcan, 2002). On this premise, 2 traits are positively correlated if the angle between their vectors is an acute angle ( $< 90^\circ$ ) while they are negatively correlated if their vectors are an obtuse angle ( $> 90^\circ$ ) (Yan and Kang, 2003).

Across the 25 tested wheat genotypes grain yield was positively associated with tillers/m and early vigor and negatively associated with leaf firing. Although the plant height was positively associated with grain yield but its magnitude was less. However, in regular breeding programs taller genotypes are not preferred. Days to heading were negatively associated with grain yield this might be due to the shortened grain filling duration. CCI measured at 3 stages were positively associated with each other indicating the loss of chlorophyll was similar in most of the genotypes tested. The vector trait yield made a 180 degree angle with leaf firing indicating traits to be opposite in genotype ranking. The distance between

genotype and the biplot origin is a unique measure of the genotype (i.e., how it differs from an “average” genotype), which is a hypothetical genotype that has an average level for all traits and is represented by the biplot origin (Yan and Fregeau-Reid, 2008). Therefore, genotypes WS 5, WS6, WS9, WS22, WS21, WS12 and WS 20 with long vectors are those that have extreme values for one or more traits. Such genotypes may or may not be superior, but they may be useful as parents for some useful traits

### CONCLUSION

The cluster analysis showed that there is significant genetic variability among tested wheat genotypes that indicates the presence of excellent opportunity to bring about improvement through hybridizing genotypes from different clusters. The winter spring derivatives need further improvement regarding days to heading. The GT biplot showed that the grain yield was positively associated with tillers/m and early vigor and negatively associated with leaf firing.

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