



Exploring Genetic Diversity in Wheat (*Triticum aestivum* L.) under Timely Sown and Terminal Heat Stress Conditions using Multivariate Approaches

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ABSTRACT

Background: Wheat, a vital food crop, is increasingly threatened by heat stress due to climate change. This significantly impacts yields and grain quality. India, a major wheat producer, urgently needs heat tolerant varieties and late sown wheat is particularly susceptible to terminal heat stress. This study aimed to identify diverse heat tolerant genotypes among evaluated genotypes that can be used in further breeding programs.

Methods: The study investigated genetic diversity in 29 wheat (*Triticum aestivum* L.) genotypes under timely and late sown environments at DRPCA, Bihar during *rabi* 2020-21 by employing 11 morpho-physiological traits in Randomized Block Design. Multivariate approaches such as principal component analysis, genotype by trait biplot and cluster analysis were employed to categorize and identify the genotypes with desirable traits for heat tolerance.

Result: Our study revealed a significant negative correlation between grain yield per plant and canopy temperature. Cluster analysis categorized genotypes into distinct groups under both timely and late sown conditions. The identified diverse lines from these clusters, exhibiting desirable traits, hold significant potential as parents for future breeding programs, emphasizing the crucial role of heat tolerance in wheat improvement.

Key words: Genotype by trait biplot, PCA, Terminal heat stress, Wheat.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is a major food crop for humans and animals and is the second most important cereal crop in the world after rice. India is the second largest producer of wheat in the world, after China (Zaveri and Lobell, 2019). However, wheat production in India is threatened by heat stress, which is becoming more frequent and severe due to climate change. Heat stress can affect wheat at all stages of growth, but it is most damaging at flowering and grain filling stages. Heat stress can reduce wheat yield up to 50% and can also lead to the development of heat-damaged grains, which are lower in quality and nutritional value. Elevated temperatures during the grain filling phase in wheat pose a significant challenge, leading to decrease in the quantity of grains per spike, grain weight and ultimately the harvest index. Consequently, this culminates in diminished grain yield (Bansal *et al.*, 2013). With each degree celsius increase in temperature above the cardinal temperature, there was considerable reduction of about 3% to 15% in wheat yield (Mondal *et al.*, 2013).

Late-sown wheat is highly susceptible to the detrimental effects of high temperature stress, resulting in decreased yield and compromised grain quality (Wardlaw and Wringley, 1994). Consequently, it becomes imperative to prioritize heat tolerance as a crucial trait in crop improvement programs. Therefore, exploring existing cultivars for their heat stress tolerance offers a valuable resource that can be readily utilized for late sown conditions.

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In order to enhance the breeding program and develop new and improved wheat genotypes with enhanced heat tolerance, it is necessary to actively search for cultivars that exhibit greater tolerance to high temperatures. Additionally, analyzing the diversity of cultivars based on morpho-physiological traits will aid in identifying diverse parental lines for crossbreeding programs, leading to the identification of superior offspring with improved terminal heat tolerance. In light of these considerations, an experiment was conducted to evaluate various wheat

cultivars for their performance under conditions of terminal heat stress *i.e.*, under late sown conditions. The objectives of the experiment were to identify potential donor cultivars with desirable heat tolerance and to assess the genetic diversity among the evaluated genotypes.

The estimation of genetic distance is a pertinent tool for the selection of parents in wheat hybridization programs. The greater the genetic distance between the parents, greater heterosis in the offspring can be observed (Anand and Murty, 1968). The selection of appropriate parents is crucial for their use in crossing programs to enhance the genetic recombination and thus increase potential yield (Islam, 2004). Currently, there exist several suitable methods such as cluster analysis, PCA and factor analysis for the identification of genetic diversity, parental selection and studying the interaction between the environment (Eivazi *et al.*, 2007).

The employment of principal component analysis (PCA) is a highly effective means of ascertaining which trait has made a positive contribution towards a crucial trait. As a result, these traits can then be utilized in crop breeding programmes (Bilal *et al.*, 2015). In addition to PCA, Genotype by Trait (GT) biplot represents an incredibly efficient biometrical technique for resolving data (Khan *et al.*, 2015). This technique graphically illustrates the usefulness of genotypes for production and facilitates the identification of traits that are suitable for indirect selection (Khodarahmpour *et al.*, 2011). The utilization of Hierarchical cluster analysis is a facile approach to evaluate the genetic heterogeneity and homogeneity within germplasm collections. This approach entails the computation of the distance and similarity between all types of genotypes, irrespective of their descriptors, which are then presented in the form of a dendrogram (Peeters and Martinelli, 1989).

MATERIALS AND METHODS

The present investigation consisted of 29 diverse wheat genotypes including one check (Table 1). The experiment was conducted at wheat research farm, DRPCA, Pusa, Bihar during *rabi* 2020. Randomized block design with three replications was used in both timely (15 November, 2020) and late sown conditions (15 December, 2020) with row to row spacing of 23 cm and 18cm for timely and late sowing respectively. The data was recorded on following morpho-physiological characters *viz.*, plant height (PH), tillers per plant (TPP), days to fifty per cent flowering (DFF), canopy temperature (CT), spike length (SL), grains per spike (GPS), chlorophyll content (CC), days to maturity (DM), 1000-grain weight (TGW), harvest index (HI), grain yield per plant (GYP), heat susceptibility index (HSI). The method of principal component analysis (Harman, 1976) was duly implemented in the extraction of the underlying components. The software application RStudio (2022), package 'FactoMineR' (Husson *et al.*, 2020) was utilized to

conduct the Principal Component Analysis and cluster analysis by using the Ward's method of hierarchical clustering technique (Ward, 1963).

RESULTS AND DISCUSSION

Principal component analysis

Principal component analysis (PCA) reflects the significance of the largest contributor to total variation at each axis of differentiation (Sharma, 1998). The eigenvalues are frequently used to decide which components should be retained. In most cases, the total of the eigenvalues equals the number of variables.

PCA under timely sown conditions

Only four principal components (PCs) with eigen values greater than one were found to account for the majority of the variability, accounting for about 76.37% of the variability among the attributes investigated in 29 diverse lines (Fig 1). Thus, the current study prioritized these four PCs for detailed explanation. Among these, PC1 contributed maximum variance 34.51, followed by PC2, PC3 and PC4 with contribution 17.58, 13.44 and 10.83 percent variance,

Table 1: List of 29 Wheat genotypes used in the present study.

Genotype no.	Genotype
G1	RAUW401
G2	RAUW402
G3	RAUW403
G4	RAUW404
G5	RAUW405
G6	RAUW406
G7	RAUW407
G8	RAUW408
G9	RAUW409
G10	RAUW410
G11	RAUW411
G12	RAUW412
G13	RAUW413
G14	RAUW414
G15	RAUW415
G16	RAUW416
G17	RAUW417
G18	RAUW418
G19	RAUW419
G20	RAUW420
G21	RAUW421
G22	RAUW422
G23	RAUW423
G24	RAUW424
G25	DBW16
G26	ANKUR
G27	HUW234
G28	HD2824
G29	RAJENDRA GHEHU 3 ©

respectively (Table 2). With a total of 76.37 percent, PC1, PC2, PC3 and PC4 accounted for the majority of the variability.

Characters with the highest absolute value closer to unity in the first principal component have a greater effect on clustering than those with the lowest absolute value closest to zero (Chahal and Gosal, 2002). Therefore, GYP (0.472) followed by GPS (0.390), harvest index (0.383), CC (0.382) and TPP (0.357) were major contributing traits towards diversity. The presence of positive and negative correlation trends among the components and variables are construed by positive and negative loading. Thousand grain weight (0.451) was major contributor towards the diversity amongst characters of PC2. Similarly, days to maturity (0.393) followed by thousand grain weight (0.348) were major contributors towards diversity in PC3. SL (0.838) reported maximum contribution in PC4 (Table 3). Depending on the respective loadings, one variable is chosen from the identified groups. Therefore, GYP, TGW, DM and SL reported greatest loadings in PC1, PC2, PC3 and PC4 respectively. Similarly, Ambati *et al.*, (2020) observed similar results with first four PCs accounting for 70.87% of cumulative variance in durum wheat germplasm.

PCA under late sown conditions/ terminal heat stress conditions

Under late sown conditions, only three principal components recorded eigen values of above one accounting for 71% of variance (Fig 2). Among these PC1 (48.53) reported maximum variance followed by PC2 (12.86) and PC3 (9.98) with cumulative variance of 71.39 per cent (Table 2). Similarly, Khan *et al.*, (2020) reported first nine components accounting for 68.23% of variation under heat stress conditions. Traits, GYP (0.388), CC (0.344), GPS (0.339), TPP (0.335) and HI (0.335) accounted major contribution towards diversity among PC1. Whereas DFF (0.64) followed by DM (0.604) reported maximum contribution towards diversity in PC2. In PC3, PH (0.784) and SL (0.340) were major contributors towards variance (Table 3).

Genotype by trait biplot

In the GT biplot, the interrelationships among traits can be visualized by drawing a vector from the origin to each trait. The magnitude of the trait's effects on the yield can be measured by the length of the vector associated with it (Yan and Tinker, 2005). The relationship value between

Table 2: Eigen value, percentage of variance and cumulative percentage of variance for all the principal components.

Principal components	Eigen value	variance %	Cumulative variance%
Timely			
1	3.79	34.51	34.51
2	1.93	17.58	52.10
3	1.47	13.44	65.54
4	1.19	10.83	76.37
5	0.74	6.76	83.14
6	0.61	5.62	88.76
7	0.43	3.96	92.73
8	0.36	3.36	96.09
9	0.18	1.70	97.80
10	0.15	1.38	99.18
11	0.08	0.81	100
Late			
1	5.82	48.53	48.53
2	1.54	12.86	61.40
3	1.19	9.98	71.39
4	0.98	8.24	79.63
5	0.61	5.13	84.76
6	0.49	4.11	88.87
7	0.41	3.42	92.30
8	0.30	2.51	94.81
9	0.25	2.09	96.90
10	0.16	1.34	98.25
11	0.15	1.27	99.52
12	0.05	0.47	100

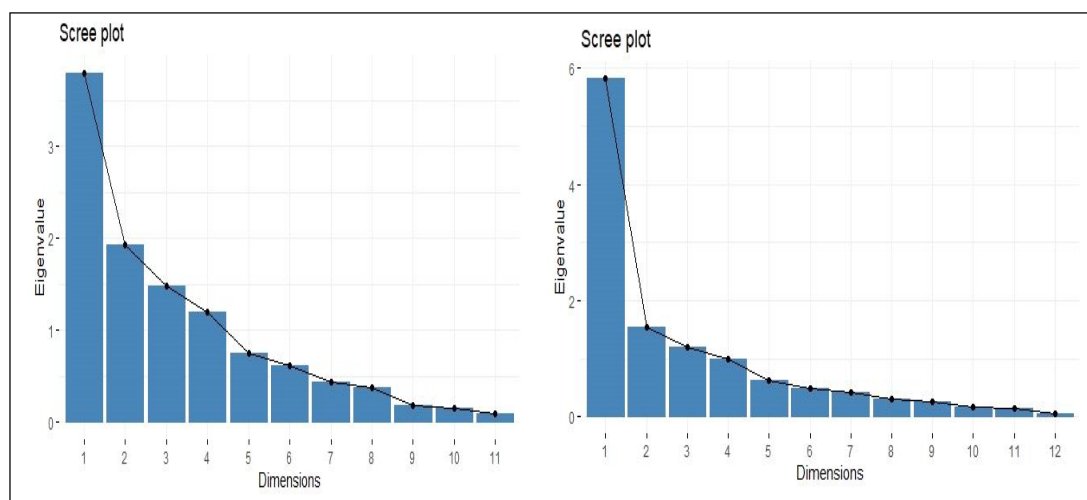


Fig 1: Scree plot depicting eigen values of 11 and 12 PCs timely sown (left) and late sown (right).

two characteristics can be estimated by the cosine of the angle formed by their respective vectors (Yan and Rajcan, 2002). Consequently, if the angle between two vectors is acute ($<90^\circ$), the two characteristics are positively correlated, while if their vectors form an obtuse angle ($>90^\circ$), they are negatively correlated (Yan and Kang, 2003). Amongst all the characters under study, GYP reported significant negative correlation with CT and significant positive correlation with characters like GPS, CC, TSW, HI and remaining traits. Ashik *et al.* (2023) ascertained the identical results with GYP. Similarly, CT was negatively correlated with most of the traits under evaluation as angles between them measuring more than 90° (Fig 2). These findings were in correspondence with Fouad *et al.* (2020).

The distance from the biplot origin to the genotype serves as a distinctive indicator of the genotype's characteristics, indicating its deviation from an "average" genotype. This hypothetical genotype is portrayed by the biplot origin and has an average level for all traits (Yan and Fregeau-Reid, 2008). Thus, under timely sown

circumstances genotypes such as G19, G11, G23, G5, G17, G18 and G29 (Rajendra Ghehu 3 ©) with elongated vectors exhibit extreme values in one or more traits (Fig 3). While these genotypes may or may not be superior, they can serve as potential parents for certain desirable traits. Whereas, under late sown conditions, G5, G19, G29 (Rajendra Ghehu 3 ©), G20 were potential genotypes that can be used as parents in crossing programs (Fig 3).

Cluster analysis

Using Ward's method, a hierarchical clustering approach was employed to group 29 wheat lines based on data from eleven quantitative traits. This resulted in the formation of nine clusters under timely sown conditions, whereas six clusters were formed under late sown conditions. The dissimilarity coefficients calculated using the morphophysiological traits of these genotypes varied between 1.22 to 8.83 and 1.99 to 9.13 under timely and late sown conditions respectively. The dissimilarity distances showed that the genotype G20 was most

Table 3: Principal component analysis for 11 yield and its attributing traits.

Trait	Timely				Late		
	PC1	PC2	PC3	PC4	PC1	PC2	PC3
PH	0.028	-0.006	-0.735	0.011	0.011	-0.258	0.784
TPP	0.357	0.106	0.272	-0.191	0.335	0.112	-0.029
DFF	0.015	-0.651	0.009	-0.188	-0.109	0.640	0.242
CT	-0.416	0.002	0.269	0.243	-0.363	-0.004	0.235
SL	0.050	-0.183	-0.135	0.838	0.232	-0.277	0.340
GPS	0.390	-0.090	-0.030	0.115	0.339	-0.155	0.171
CC	0.382	-0.009	-0.074	-0.183	0.344	0.114	0.002
DM	0.072	-0.559	0.393	0.046	0.064	0.604	0.269
TGW	0.150	0.451	0.348	0.165	0.281	-0.059	-0.054
HI	0.383	0.043	0.084	0.296	0.335	0.034	-0.205
GYP	0.472	-0.047	-0.063	0.027	0.388	0.080	0.044
HSI					-0.328	-0.140	-0.072

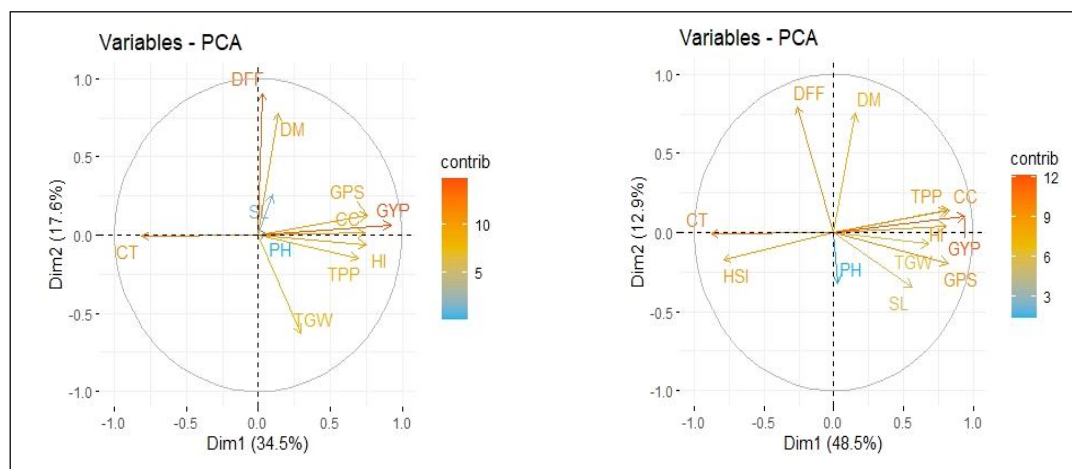


Fig 2: A two-dimensional biplot demonstrating the contribution of yield and yield attributing traits on principal component axes timely sown (left) and late sown (right).

dissimilar with G23 followed by G11, followed by genotype G15 with G23 and G11 under timely sown conditions (Table 4). Whereas in late sown conditions, the highest

dissimilarity distances were reported between genotypes G19 with G18 followed by G20, G15 and G10 (Table 4). In timely sown conditions, the pair of genotypes such as G14

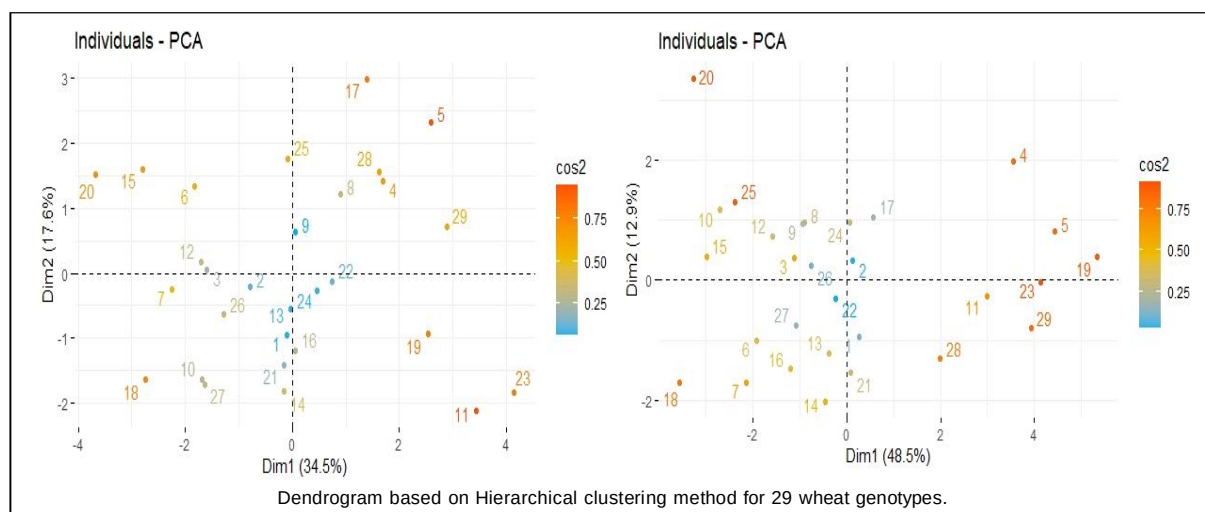


Fig 3: Biplot dispersion graph illustrating the relationship among 29 wheat genotypes timely sown (left) and late sown (right).

Table 4: d values based on yield and its attributing traits.

Timely			Late		
			Maximum		
G20	G23	8.83	G19	G18	9.13
G20	G11	8.18	G19	G20	9.1
G15	G23	7.91	G19	G15	9
G15	G11	7.59	G19	G10	8.87
			Minimum		
G14	G16	1.22	G2	G3	1.99
G17	G5	1.96	G2	G24	2
G4	G5	2.04	G3	G12	2.01

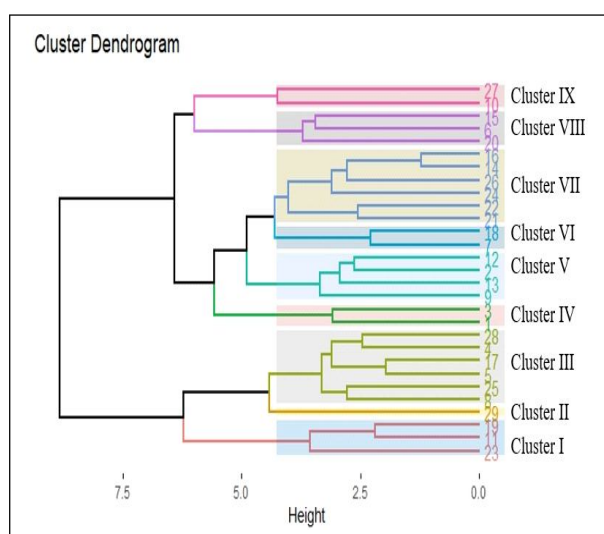


Fig 4a: Timely sown.

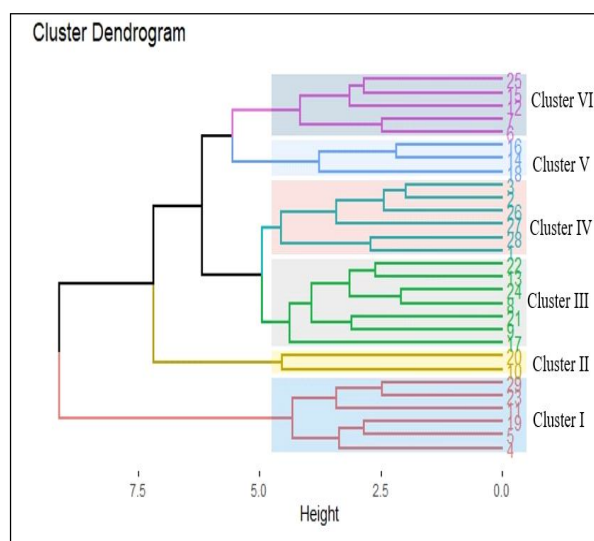


Fig 4b: Late sown.

and G16 followed by G17 and G5, G4 and G5 exhibited the smallest dissimilarity distances, indicating these genotypes are closely related likely due to shared parentage in their pedigree. Whereas, under late sown conditions, the pair of genotypes such as G2 and G3 followed by G2 and G24, G3 and G12 reported smallest dissimilarity distances.

In timely sown conditions, nine clusters were reported in the analysis, maximum number of genotypes (7) were reported cluster 3 and cluster 7 whereas cluster 2 comprised of only one genotype *i.e.*, G29 (Fig 4a). Cluster 1 comprised of three genotypes (G23, G11, G19) characterized by high tillers per plant, early days to fifty per cent flowering, low CT, high GPS, high CC and high GYP. Similar result was reported by Singh *et al.* (2019) in which a cluster showed considerable high values for TPP and GYP. Cluster 2 contained only one genotype *i.e.*, G29 (check Rajendra Ghehu 3) characterized by delayed DM, high TGW, high HI. Lowest PH was reported in cluster 9, while, cluster 4 exhibited a notably high SL.

Six clusters were reported in late sown conditions, cluster 3 contained maximum number of genotypes (7) and cluster 2 contained least number of genotypes (2) (Fig 4b). Cluster 1 comprised of genotypes (G4, G5, G19, G11, G23, G29) characterized with high TPP, low CT, high SL, high GPS, high CC, TGW, HI and low HSI. Cluster 2 characterized by dwarf PH, delayed DFF, DM. Similar findings were reported by Kandel *et al.* (2018), Singh *et al.* (2019).

CONCLUSION

The current investigation focused on assessing the genetic diversity of bread wheat lines and their associated yield-related traits using multivariate techniques. Through the application of Principal Component Analysis (PCA), key traits contributing to the variation within the lines were identified. In the case of timely sown conditions, GYP, GPS, HI, TGW, DM and SL emerged as the primary characteristics driving the observed variation. Conversely, under late sown conditions, GYP, CC, GPS and TPP were identified as the main contributing traits. The classification of genotypes into distinct clusters serves as a valuable tool for identifying diverse lines with desirable traits to be employed in future crop improvement programs. In this study, cluster analysis successfully categorized the 29 bread wheat genotypes into nine clusters for timely sown conditions and six clusters for late sown conditions. By crossing genotypes from different clusters possessing desired traits, there is an increased likelihood of harnessing high heterosis and subsequently obtaining improved performance in terms of various yield-related traits.

Author contribution

The research was conducted by Uttej Karla under the supervision of Satish Kumar Singh and Vinay Kumar Choudhary. The work was done and manuscript preparation

was done by Uttej Karla. Sonal Chavan assisted with statistical analysis. Vignesh S and Madan Lal assisted throughout the research and manuscript preparation. All authors reviewed and finalized the manuscript.

Conflict of interest

All authors declare that they have no conflicts of interest.

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