PRINCIPLE COMPONENT ANALYSIS (PCA) OF WHEAT GENOTYPES CONCERNING MORPHOLOGICAL RESPONSES TO HEAT STRESS

Abstract. This study was conducted at Dev Bhoomi Uttarakhand University in Dehradun, India, with three replications using the Randomized Block Design (RBD) pattern to assess morphological responses to stress conditions in eight wheat genotypes (PBW-550, PBW-343, PBW-292, RAJ-3765, HD-2967, HD-3086, and NABI MG {BLACK WHEAT}. Wheat genotypes were subjected to Principle Component Analysis (PCA) during the investigation. The 1^{st} principal component accounted for 47.2689% of the total variance (PC1). The second main component (PC2) contributed 24.6007% of the total variance. The 3^{rd} main component was responsible for 16.3402% of the overall variance (PC3). The fourth main component accounted for 8.3215 percent of the total variance (PC4). The ratio between the four major components to overall variance was 96.5311%. In the principal component, FLA had the largest positive component loading (0.294), followed by PH (0.038). There are six variables with the largest negative connection with this component: PL (-0.117), NPT (-0.319), DTH (-0.469), DTF (-0.477), DTM (-0.479), and GY (-0.346). The genotypes utilized in the study exhibit significantly different morphological responses. For breeders who additionally aspire to create variation, this is important, and it is a beneficial approach to use such genotypes as genitors in breeding studies.

Keywords: Heat stress, wheat, principal component Analysis (PCA), Scree plot, Biplot

INTRODUCTION

Wheat is a grain crop that is essential to the human diet as a source of calories and protein. Developing high-yielding cultivars that are tolerant to both abiotic and biotic stressors is essential to ensure food security. Owing to its high nutritional richness, Wheat contributes 21 percent of the total calorie intake and 20 percent of the protein consumed by the nearly 5.5 billion individuals living in approximately 100 nations that are less developed countries.(Braun et al., 2010). Carbohydrates account for around 70% of the wheat crop, followed by approx three and two percent mineral substances and fatty acids respectively two percent fiber, vitamin B complex, and riboflavin as well as minerals such as Fe, Zn, Se, and Mg (Sharma, 2004).

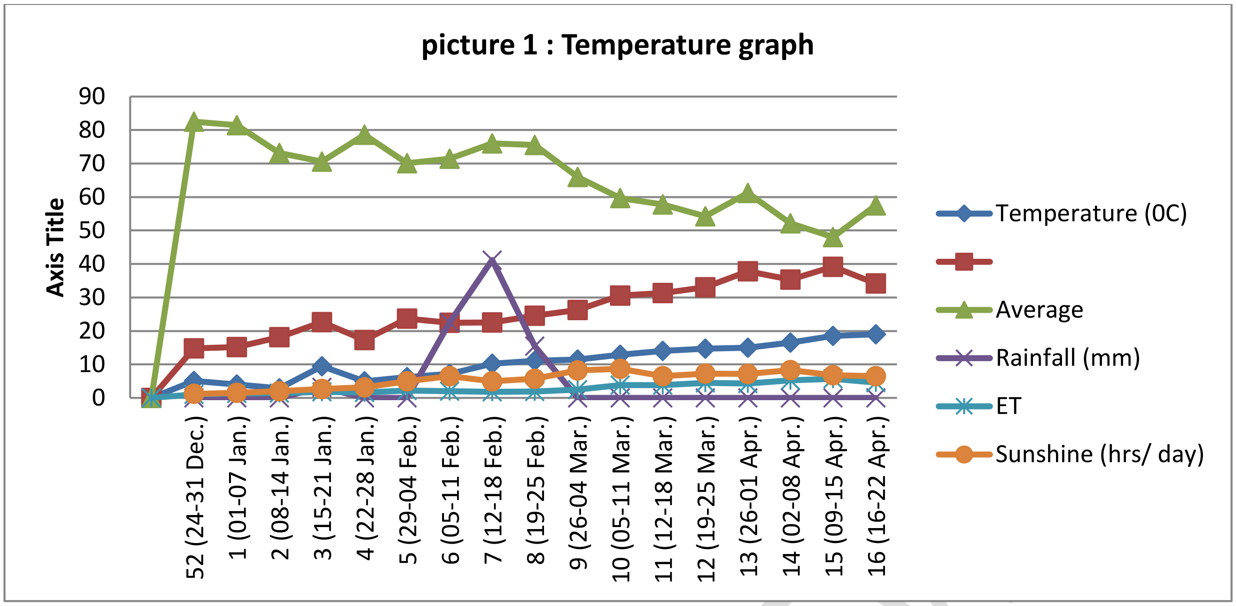
Grain yield is the most significant biometric characteristic of each crop and is influenced by genetics, environment, and the interaction of genetics and environment (Dia *et al.*, 2016). Crop output is limited by several biotic and abiotic variables, the most important of which are now the abiotic stresses—heat stress in particular is becoming more prevalent owing to climate change brought on by global warming (Chauhan et al., 2023; Joshi et al., 2024). According to a UNEP report from 2019, if greenhouse gas emissions continue to rise, by the end of the century, 3.5°C of expected temperature increase is on the upper end of the range. Wheat requires a temperature of 21±3°C for most of its crop stages. Heat stress has a large influence on production and the standard affecting around 0.036 billion hectares in the temperate zones and 7.0 million hectares in economically backward regions (Reynolds *et al.*, 2001).In cool-season cereal species, heat stress induces physiological changes via decreasing chlorophyll content, which results in leaf senescence. Excessive temperatures have a direct impact on the length and pace of grain filling (Kumar *et al.*, 2012; Lobell 2012; Gourdji *et al.*, 2013), which reduces the quantity of grains and size of the kernels (Ferris et al., 1998). Physiological screening methods for heat tolerance have included green fluorescent chlorophyll (Moffatt *et al.,* 1990) and fall in canopy temperature (Reynolds et al., 1998). The ability of a plant to flourish and provide a profit under high temperatures is known as heat tolerance. (Hall, 2001). Plant breeders have concentrated mostly on grain yield since it is a complicated quantitative characteristic that is influenced by several genes and has poor heritability because it depends so much on environmental factors. (Khairnar *et al.*, 2018). Because of this, breeders will be able to improve wheat's heat tolerance through indirect yield selection, which involves screening for connected qualities. Direct yield selection will thus be less successful. Principal component analysis are examples of a multivariate analytic technique. Evaluate diversity in genes and calculate the relative contributions of each visual and metabolic specific to the overall wheat yield (Phougat, 2022).

The creation of new varieties depends critically on the existence of genetic diversity and the application of that variability. To do this, the primary principal components and the primary attributes that have the largest positive or negative loadings in each component which determine how various genotypes cluster together are analyzed. Determining the critical components and characteristics that accounted for the majority of the variance and variety within breeding lines was the main objective of the current investigation.

MATERIALS AND METHODS

Site Description

In Rabi 2023–24 an experiment was conducted at the agricultural research farm of Dev Bhoomi Uttarakhand University, Dehradun, to establish the influence of thermal stress on the production of grains and the key causes of it. A total of eight types, PBW-550, PBW-343, PBW-292, RAJ-3765, HD-2967, HD-3086, and NABI MG (BLACK WHEAT), were sown on December 25, 2024, to maintain temperatures that are comparatively high during the reproductive period, particularly for grain filling.



The proposed study was conducted at Dev Bhoomi Uttarakhand University, Dehradun, Uttarakhand, India, situated at an altitude of about 640 meters (2,100 feet) above sea level, Dehradun has a subtropical environment with scorching summers and refreshing winters. Annual precipitation is around 2200 mm, with moderate to heavy rainfall falling during the monsoon season (usually July to September). Soils of many sorts, including clayey, sandy loam, and alluvial, are present in the area, and each has its own set of advantages and disadvantages when it comes to growing wheat. Laboratory space for biochemical analysis, chambers for controlled environment plant growth studies, and necessary infrastructure for field experiments are all provided by DBUU's advanced agricultural research facilities. Experimental plots, irrigation infrastructure, and data recording are all overseen by a group of agricultural experts and technicians at this location. Researchers and staff engaged in monitoring, data collecting, and other research operations find DBUU to be easily accessible by road and positioned near important transportation networks. Overall, the conducive climate, diversity of soil types, and state-of-the-art research facilities at DBUU, Dehradun make it an excellent site for wheat stress response and variety assessment studies.

List 1: **Different Wheat Genotypes and their characteristics**

S.No.	Name of Genotype	Area/Institute released & Year	Charcateristics
1.	**PBW-550**	Punjab Agriculture University (2017)	• A gene introgressed rust resistant version of the popular variety PBW 550 • Medium duration variety suitable for mid-late planting • Average plant height: 86 cm • Days to maturity: 145 • Resistant to yellow and brown rust • Average grain yield: 23.0 q/acre
2.	**PBW-343**	Punjab Agriculture University (2017)	• Unnat PBW 343 is a gene introgressed rust resistant version of mega-variety PBW 343 • First wheat variety developed through MABB (Marker Assisted Backcross Breeding) released and notified at the National Level for the North Western Plains Zone including Punjab • Average plant height: 100 cm • Days to maturity: 155 days • Average grain yield: 23.2 q/acre
3.	**PBW-292**		
4.	**RAJ-3765**	RAJASTHAN AGRICULTURAL RESEARCH INSTITUTE	• Raj 3765 has the tolerance to high-temperature • And rusts and are suitable for normal to very late sowing conditions
5.	**HD-2967**	ICAR-IARI, New Delhi (2014)	• Plant height: 98 cm (range: 72-112) • Maturity range: Seeding to flowering: 99 Days (range: 84-108) • Seed to Seed: 143 days (range: 127-160) • Average yield- 5.456 t/ha
6.	**DBW-187**		
7.	**HD-3086**		
8.	**NABI MG (BLACK WHEAT)**		

Experiment

The varieties were planted using a Randomized Block Design (RBD) with 3- replications, and the prescribed set of measures were carried out to guarantee a healthy harvest. The mean of high and low temperatures during the reproductive stage were 30.700 and 15.120 degrees Celsius, respectively.

Measurements

Seven morphological traits were monitored, including plant height (cm), flag leaf area (cm2), and the number of productive tillers. Days to seventy-five percent heading, days to seventy-five percent flowering, days to seventy-five percent maturity, and the amount of grain produced (per plant) were determined by selecting five plants at random from each replication line and computing the mean for different statistical features.

Principal Component Analysis, or PCA, is a statistical method that keeps crucial information while minimizing the number of variables to simplify complicated data sets. The conversion of the initial variables into a fresh set of independent variables known as principle components, aids in identifying patterns and correlations within data, and the primary variables resulting in the most total variability were determined using a scree plot. All data were examined using the OPSTAT application.

RESULT

Principal component analysis:-

We employed PCA to compress the size of the analyzed agricultural attributes dataset. The overall variation was determined using eight major component axes, Eigenvalues, Variability values (%), and Cumulative values (%). The first principal component was responsible for 47.2689% of the overall variance (PC 1). The second primary component(PC2) contributed 24.6007% of the total variance. The third main component (PC3) was responsible for 16.3402% of the overall variation. The 4th component accounted for 8.3215 percent of overall variation(PC4). The overall variance of the four major components was 96.5311%. The remaining components (PC5=1.8679%, PC6=1.5832, PC7=0.01755%, accounted for 3.4689% of the total variation. The PCA analysis yielded eight primary component axes, which contribute to all of the overall variance. The eight principal components accounted for 100% of the complete variance.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Eigenvalues	3.782	1.968	1.307	1.307	0.149	0.127	0.001
% of Variance	47.2689	24.6007	16.3402	8.3215	1.8679	1.5832	0.01755
Cumulative (%)	47.2689	71.8697	88.2099	96.5313	98.3992	99.9825	100

Table Eigenvalues, Variability, and Cumulative Values

Scree Plot (Graphical representation of Eigenvalues)

Figure **1** depicts a scree plot (Graphical visualization of Eigenvalues). Eigenvalues for PC1 were 3.782 and 1.968 (PC2), 1.307 (PC3), 1.307 (PC4), 0.149 (PC5) and 0.127 (PC6), 0.001 (PC7), and zero (PC8), respectively. If the Eigenvalues exceed one, It indicates that the assessed main component scores are reliable (Mohammadi 2003). On the other hand, Iezzoni (1991) found that PCs with Eigen values larger than 1 are more informative than the original variable. Thus PC1, PC2 , PC3, and PC4 have explained the major portion of genetic variation caused due to heat stress. Through this, we can therefore categorize the traits that are strongly impacted by heat stress and traits that are heavily impacted by heat stress.

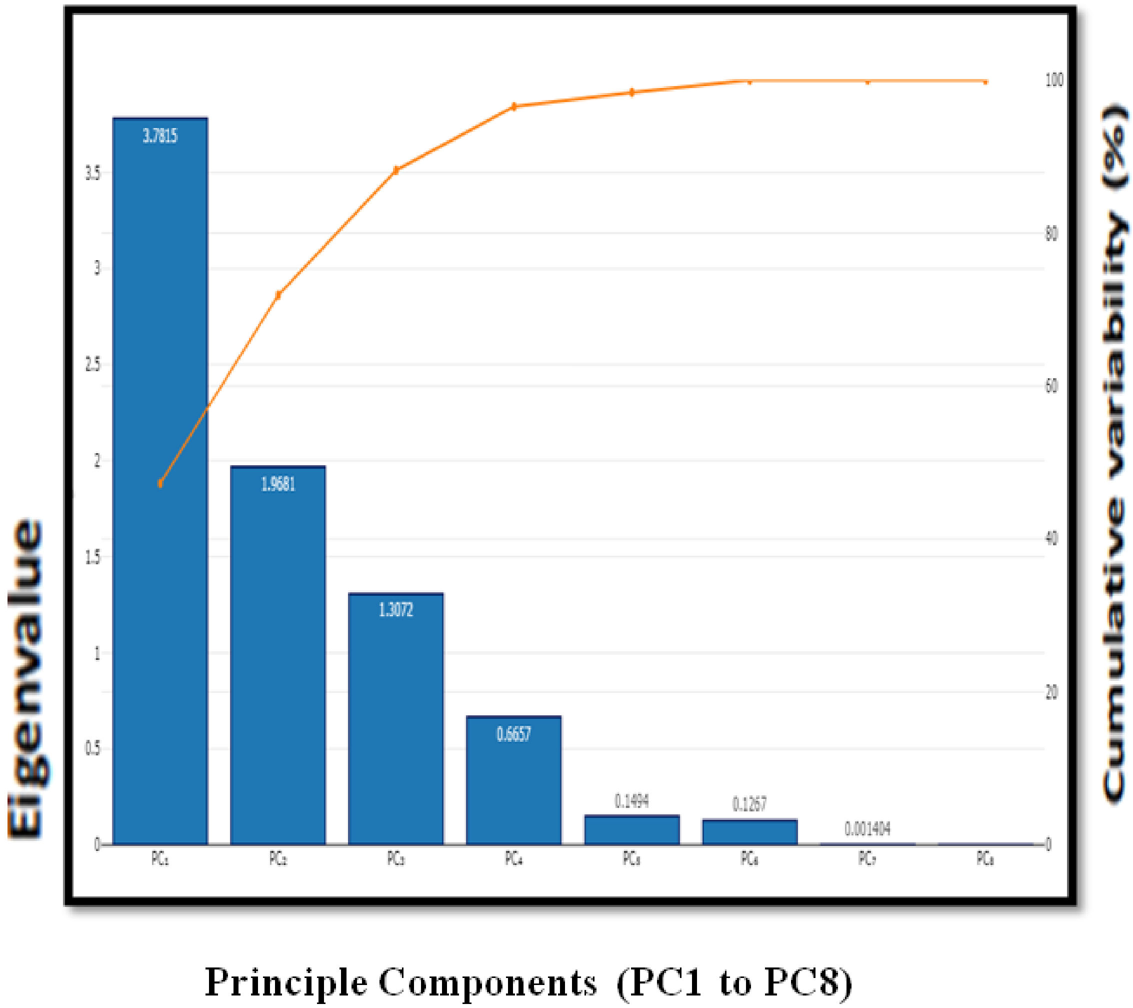


Figure-1. Graphical visualisation of Eigen values

Biplot

When viewing this biplot (Fig. 2), the narrow-angle features reveal a positive relationship. Right-angle characteristics are unrelated to each other. Wide-angle characteristics have unfavorable interactions with one another. The biplot approach allows for the identification of correlations between parameters as well as a detailed examination of a heterogeneous data set. (Yan,2002).

Biplot (axes PC1 and PC2: 71.87 %)

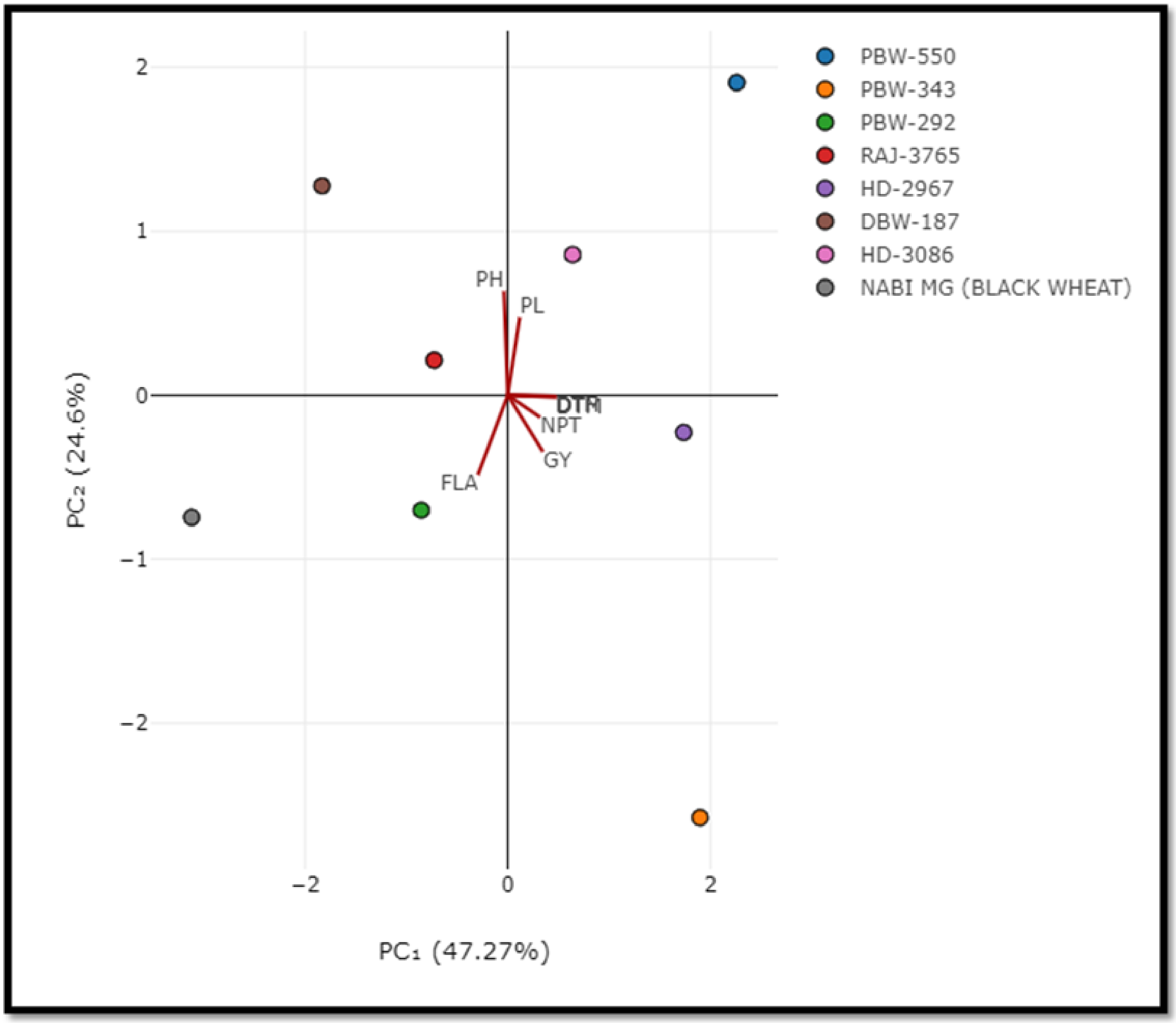


Figure 2. Biplot of the morphological responses to heat stress of 8-wheat varieties for the first two principal components.

Table: 2 Factor loading of morphological character about the major factor in wheat.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
PH	0.038	-0.634	0.348	0.023	0.327	0.478	0.337	-0.161
FLA	0.294	0.486	0.053	-0.500	0.505	-0.058	0.372	-0.168
PL	-0.117	-0.474	-0.580	-0.124	0.441	-0.463	-0.039	0.010
NPT	-0.319	0.135	-0.637	-0.102	-0.215	0.531	0.337	-0.149
DTH	-0.469	0.006	0.187	-0.426	0.073	0.097	-0.548	-0.498
DTF	-0.477	0.006	0.191	-0.367	0.085	0.063	0.088	0.763
DTM	-0.479	0.013	0.251	0.124	-0.207	-0.490	0.562	-0.304
GY	-0.346	0.345	0.000	0.626	0.585	0.131	-0.102	0.018

An eigenvector with reversed signs for all its components is still considered a valid solution!

PH: Plant height, FLA: Flag leaf area, PL: Peduncle length, NPT: No. of productive Tiller, DTH: Days to 75%Heading, DTF: Days to 75%flowering, DTM: Days to maturity, GY: Grain yield

DISCUSSION

The first main component had the largest positive component loading from FLA (0.294), followed by PH (0.038). Six variables are having PL (-0.117), NPT (-0.319), DTH (-0.469), DTF (-0.477), DTM (-0.479), and GY (-0.346) had the strongest negative connection. In the second primary component, FLA (0.486) explained the biggest positive contribution followed by GY (0.345) and NPT (0.135). Characters such as PH (-0.634) and PL (-0.474) showed the most negative relationship with this component. In 2ndcomponent, plant height and peduncle length were shown to contribute the most variability. In the third principal component PH (0.348), DTM (0.251), and

DTF (0.191) had the highest coefficients, respectively and the NPT (-0.637) and PL (-0.580) had highest negative coefficients. For the fourth principal component, GY (0.626) has highest coefficient and FLA (-0.500) has highest negative coefficient. For the fifth principal component, GY (0.585) is followed by FLA (0.505) having the highest coefficient and NPT (-0.215) having the highest negative coefficients. For the sixth principal component, NPT (0.585) is followed by PH (0.478) having the highest coefficient and DTM (-0.490) having the highest negative coefficient. For the seventh principal component, DTM (0.562) and DTH (-0.548) have the highest positive and negative values, correspondingly.

The Principal Component Analysis (PCA) conducted on our dataset revealed several key insights into the structure and relationships among the variables:

1. **First Principal Component (PC1)**:
    - **Positive Loadings**: The first principal component had the largest positive loading from Flag Leaf Area (FLA) (0.294), followed by Plant Height (PH) (0.038).
    - **Negative Loadings**: Six variables showed strong negative loadings: Peduncle Length (PL) (-0.117), Number of Productive Tillers (NPT) (-0.319), Days to Heading (DTH) (-0.469), Days to Flowering (DTF) (-0.477), Days to Maturity (DTM) (-0.479), and Grain Yield (GY) (-0.346).
    - **Interpretation**: PC1 captures a contrast between FLA and the other six variables, indicating a potential trade-off or inverse relationship between these groups of traits.
2. **Second Principal Component (PC2)**:
    - **Positive Loadings**: FLA (0.486) had the highest positive contribution, followed by GY (0.345) and NPT (0.135).
    - **Negative Loadings**: PH (-0.634) and PL (-0.474) showed the most negative relationships with PC2.
    - **Interpretation**: This component highlights the variability contributed primarily by plant height and peduncle length, with FLA and GY being the key positive contributors, suggesting distinct patterns of growth and productivity.
3. **Third Principal Component (PC3)**:
    - **Positive Loadings**: PH (0.348), DTM (0.251), and DTF (0.191) had the highest positive coefficients.
    - **Negative Loadings**: NPT (-0.637) and PL (-0.580) had the highest negative coefficients.

- **Interpretation**: PC3 differentiates between traits related to reproductive timing (DTM and DTF) and structural attributes (NPT and PL).

4. **Fourth Principal Component (PC4)**:
   - **Positive Loadings**: GY (0.626) had the highest positive coefficient.
   - **Negative Loadings**: FLA (-0.500) had the highest negative coefficient.
   - **Interpretation**: This component contrasts grain yield with flag leaf area, indicating a potential inverse relationship between these two important agronomic traits.
5. **Fifth Principal Component (PC5)**:
   - **Positive Loadings**: GY (0.585) and FLA (0.505) had the highest positive coefficients.
   - **Negative Loadings**: NPT (-0.215) had the highest negative coefficient.
   - **Interpretation**: PC5 emphasizes the positive association between grain yield and flag leaf area, contrasting with the number of productive tillers.
6. **Sixth Principal Component (PC6)**:
   - **Positive Loadings**: NPT (0.585) and PH (0.478) had the highest positive coefficients.
   - **Negative Loadings**: DTM (-0.490) had the highest negative coefficient.
   - **Interpretation**: This component shows a strong relationship between the number of productive tillers and plant height, against days to maturity.
7. **Seventh Principal Component (PC7)**:
   - **Positive Loadings**: DTM (0.562) had the highest positive coefficient.
   - **Negative Loadings**: DTH (-0.548) had the highest negative coefficient.
   - **Interpretation**: PC7 highlights the contrast between days to maturity and days to heading, which may reflect different aspects of plant developmental timing.

Correlation Coefficient Analysis

The correlation coefficient analysis (Pearson, 1900) revealed positive-significant associations between DTH and DTF, DTH and DTM, and DTF and DTM). This circumstance demonstrates that there is a close link between heading, blooming, and maturity.

Below mentioned the result of the correlation coefficient Analysis revealed that GY is negatively correlated with PH, FLA, and PL. As in heat stress conditions if plant height, flag leaf area, and peduncle length are more, due to more vegetative growth plants are unable to complete their need at later stages and decline in grain yield. Here our result also justifies the same that PH, FLA, and PL are negatively correlated with grain yield. On the other hand, GY is positively correlated with NPT, DTH, DTF, and DTM in heat stress conditions. According to this conclusion comes that if the variety takes more time for heading, flowering, and maturity it means that the variety is stable for heat stress conditions as most of the variety that are late sown takes more time for these above-mentioned traits, as they are late sown variety. In our research trial, we also selected 8 different genotypes and sown on the 25$^{th\ of}$ December 2023, to find the suitable variety that can easily tolerate heat stress and there is no effect on grain yield.

Table-3. Correlation matrix (Pearson (n)

	PH	FLA	PL	NPT	DTH	DTF	DTM	GY
PH	1							
FLA	-0.526	1						
PL	0.303	-0.546	1					
NPT	-0.483	-0.256	0.461	1				
DTH	0.013	-0.356	0.094	0.443	1			
DTF	0.013	-0.383	0.093	0.445	0.998	1		
DTM	-0.008	-0.557	0.014	0.338	0.867	0.891	1	
GY	-0.433	-0.220	-0.190	**0.456**	**0.448**	**0.484**	**0.661**	1

Values in bold are different from 0 with a significance level of alpha=0.05.

PH: Plant height, FLA: Flag leaf area, PL: Peduncle length, NPT: No. of productive Tiller, DTH: Days to 75%Heading, DTF: Days to 75%flowering, DTM: Days to maturity, GY: Grain yield

The candidate manuscript does not have a robust scientific discussion, I suggest the authors incorporate the suggested paragraphs, in this way, it would improve the scientific quality of the manuscript:

The findings are particularly relevant to agronomic studies in Latin America, where diverse environmental conditions and stressors (Rodriguez et al. 2013; Araya et al. 2020)—such as heat and drought (Cortez et al. 2016a; 2016b)—affect staple crops (Campos, 2023). Like the Indian wheat study, Latin American agriculture faces the challenge of sustaining productivity under adverse conditions (Olivares et al. 2022; Campos et al. 2023). PCA can be leveraged to evaluate genotype-environment interactions across the region's varied agroecological zones (Olivares et al. 2018; Calero et al. 2022; Viloria et al. 2023). For example, identifying traits like phenological stability or efficient resource use can guide the development of resilient cultivars for crops such as maize, beans, and rice, which are critical for food security in the region (Olivares and Franco, 2015; López et al. 2019; Lobo et al. 2023).

Moreover, the study underscores the interplay between genetics and environmental factors, with PCA delineating traits that are either positively or negatively correlated with stress resilience (Olivares et al. 2015; Rey et al. 2022). This is crucial for Latin America, where smallholder farmers often rely on traditional knowledge but lack access to advanced breeding technologies (Pitti et al. 2021; Montenegro et al. 2021a; Olivares et al. 2020). Integrating PCA-driven insights with local agricultural practices can foster region-specific solutions (Montenegro et al. 2021b). For instance, targeting traits like prolonged maturity stages (as identified for heat resilience) could align well with breeding strategies for crops in tropical climates, where prolonged growing seasons are beneficial (Hernandez and Olivares, 2019; Hernandez and Olivares, 2020).

This study exemplifies the potential of PCA in addressing environmental challenges in crop production. Its application in Latin America could enhance understanding of stress-tolerant traits in key crops, driving innovations in breeding programs (Hernandez et al. 2017; Hernandez et al. 2018a). Future research should focus on multi-location trials across varied climates in the region to validate the universality of PCA-derived trait associations (Hernandez et al. 2020; Hernandez et al. 2018b). By aligning statistical methodologies with agronomic realities, PCA not only bridges scientific knowledge gaps but also empowers the agricultural sector to adapt to global climate change challenges effectively.

CONCLUSION

In conclusion, this study underscores the importance of understanding the relationships among key agronomic traits under heat stress conditions. By identifying varieties that exhibit stable performance through prolonged phenological stages and optimal trait combinations, we can enhance grain yield resilience in the face of increasing heat stress challenges. Future research should focus on exploring the genetic basis of these traits to develop heat-tolerant crop varieties with improved yield potential.

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