



Assessment of terminal heat tolerance among wheat (*Triticum aestivum*) genotypes based on multivariate analysis and selection indices

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Received: 09 October 2023; Accepted: 08 July 2024

ABSTRACT

Terminal heat stress is of major concern for global wheat (*Triticum aestivum* L.) production as wheat prefers a cool climate. Using physiologically efficient parents in crossing programme and direct selection of elite genotypes would be useful for breeding climate-resilient wheat. A field experiment was conducted during winter (*rabi*) season of 2021–22 at Bihar Agricultural College, Sabour, Bhagalpur, Bihar to study the terminal heat tolerance among wheat genotypes based on multivariate analysis and selection indices. A set of 225 wheat genotypes was evaluated under normal sowing (Timely sown) and heat stress conditions (Late sown) during 2021–22 to perform multivariate analysis, viz. D² statistics, principal component analysis and selection indices, heat susceptible index (HSI) for grain filling duration, 1000-grain weight and yield. The clustering pattern as per Tocher's optimization method and magnitude of D² value revealed that wheat genotypes studied for 16 traits were grouped into seven clusters, where cluster V with 51 genotypes emerged as the largest cluster. Cluster VII and cluster III showed highest inter-cluster distance (6.958). HSI for grain filling duration (GFD), 1000 grain weight (TGW) and yield per plot (YPPT) ranged from 0.36 (genotype 30, GID: 7933202) to 2.51 (genotype 54, GID: 7933656), -0.84 (genotype 44: GID: 7933473) to 2.66 (genotype 36, GID: 7933333) and -0.49 (genotype 37 GID: 7933334) to 1.93 (genotype 49, GID: 7933509), respectively. Genotypes 88, 74 and 66 showed low HSI for GFD, TGW and YPPT, respectively. Six principal components (PC1 to PC6) accounted for 76.38% of the total variation and represented the core traits for further investigation. Identified promising lines and potential donors for yield components like genotype 24 (GID: 7933122), genotype 64 (GID: 7933762), genotype 71 (GID: 7933792), genotype 93 (GID: 7933947) and genotype 172 (GID: 7934632) could be utilized in the crossing programme to breed terminal heat-tolerant wheat.

Keywords: Canopy temperature, Chlorophyll content, Cluster analysis, Principal components, HSI, NDVI

Wheat (*Triticum aestivum* L.) is globally cultivated and one of the most important cereal for about two-billion people. It is sensitive to high ambient temperature, particularly during the reproductive stage posing a significant threat to sustainable wheat production (Blum *et al.* 2001, Reynolds and Borlaug 2006). Terminal heat stress is a condition in wheat where temperatures exceed 30°C after flowering, induces a variety of morphological, biochemical and physiological alterations in plants regulating growth and development including chlorophyll degradation, decreased fluidity in lipid membrane, disruption in cell organelle functions, reduced rate of photosynthesis, inhibition of protein synthesis, enhanced protein degradation and ultimately cell death (Wahid *et al.* 2007, Gonzalez-Navarro *et al.* 2015, Sharma *et al.* 2019). The development of

high-yielding wheat cultivars relies on understanding yield components, their genetic inheritance and the interaction of genotype and environmental factors (Baranwal *et al.* 2016). Genetically diverse lines could ensure breeding for elite varieties carrying variability for economic traits facilitating successful recombination breeding and selection. It can favour early adaptation due to diverse genetic background (Meena *et al.* 2014). To break the yield plateau, selection of transgressive segregants among derived segregating generations is crucial and it depicts level of genetic diversity in the parents (Reynolds *et al.* 2012). The heat susceptibility index indicates a penalty in grain yield and its components caused by unfavourable versus favourable environments (Fischer and Maurer 1978).

Different quantitative methods namely D² statistics (Rao 1952) and hierarchical euclidean cluster (Ward 1963) are commonly used to assess parental divergence for effective breeding. These genetic divergence approaches rely on similarity or dissimilarity criteria based on the aggregate effect of studied traits. Principal component analysis, a multivariate statistical method, condenses correlated

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variables or covariance into fewer principal components using linear transformations retaining the original meaning (Meena *et al.* 2014). This approach can determine key yield components and streamline future breeding programmes. Considering the above facts, a total of 225 wheat genotypes were evaluated to identify the terminal heat tolerant genotypes and assess the diversity among them using multivariate analysis.

MATERIALS AND METHODS

A field experiment was conducted during winter (*rabi*) season of 2021–22 at Bihar Agricultural College, Sabour, Bhagalpur (25°15'40"N and 87°2'42" E; 46 meter amsl), Bihar under normal (November 2022) and late sown conditions (December 2022). The experiment comprised of 225 wheat genotypes including 221 selected lines from Seeds of Discovery (SeeD) programme of the International Maize and Wheat Improvement Center (CIMMYT, Mexico) and four checks namely HD3249, HD2967, DBW14 and DBW187 (Supplementary Table 1). The SeeD programme primarily characterizes available wheat genetic diversity for further utilization in breeding programmes. The experiment was laid out in an alpha lattice design with two replications having 10 blocks and within each block 90 rows in normal sowing and heat stress (late sown) conditions were sown. Each genotype was planted with paired rows of 1.8 m. The standard agronomic package and practices were followed precisely under irrigated conditions.

Data were recorded from each plot for traits, viz. days to 50% flowering (DF), days to physiological maturity (DPM), days to anthesis (DA), grain filling duration (GFD), flag leaf area (FLA), plant height (PH), spike length (SL), peduncle length (PDL), 1000-grain weight (TGW) and yield/plot (YPPT). Physiological characteristics such as normalized difference vegetation index (NDVI), canopy temperature and chlorophyll content index were examined at

two growth stages: Zadoks growth scale ZS45-late booting and ZS75-mid milking. A portable GreenSeeker (Tribble Ag, Sunnyvale, California, USA) was used to record NDVI. The canopy temperature was measured with a handheld infrared thermometer (Fluke Infrared Corporation, WA, USA) as per standard procedure (Kumar 2022). A chlorophyll concentration meter (Apogee Instrument, MC-100, Logan USA) was used to measure the chlorophyll concentration index (CCI). Heat susceptibility index (HSI) was estimated for GFD, TGW and YPPT using recorded observations under heat stress (LS) and timely sown (TS) conditions as per Fischer and Maurer (1978) where HSI denotes $1 - YD/YP/D$; YD shows mean yield trait of genotype in a stress environment and YP indicates mean yield trait of the same genotype in a non-stress environment, D represents $1 - (\text{mean YD of all genotypes}/\text{mean YP of all genotypes})$.

Statistical analysis: The statistical analysis for genetic divergence, cluster mean analysis including dendrogram and principal component analysis were carried out using Factoextra, Nbclust and pca3dpackages, respectively in R (R Core Team 2020). The D^2 analysis using the recorded observations was performed as per Mahalanobis (1936) and Rao (1952).

RESULTS AND DISCUSSION

Genetic diversity analysis: Genetic diversity was assessed using Mahalanobis D^2 statistics resulting in the grouping of all genotypes into seven clusters, with cluster V comprising the largest cluster, consisting of 51 genotypes. The clusters I, II, IV, VII and VII have 36, 36, 21, 44 and 26 genotypes, respectively; cluster III has minimum genotypes (11) in it. (Supplementary Table 2 and Fig. 1). Supplementary Fig. 1 shows the per cent contribution of 16 traits for divergence among wheat genotypes with NDVI (ZS75) having the maximum contribution of 10.5% followed by peduncle length (9.8%) and plant height (9.6%). The

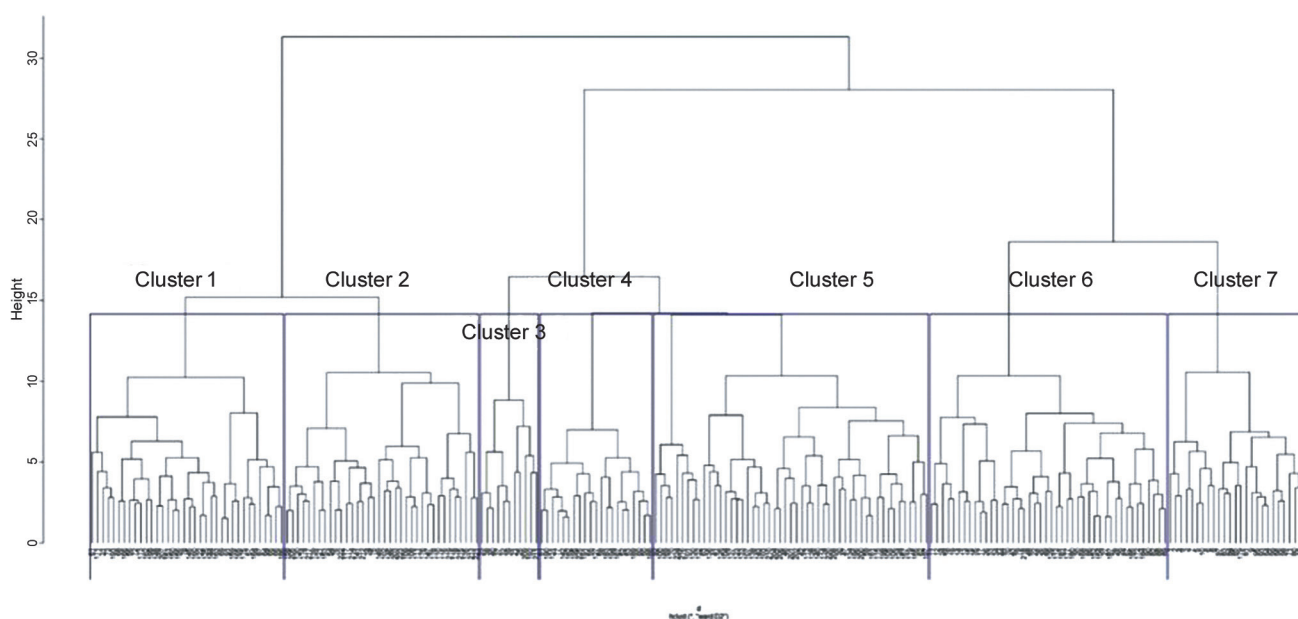


Fig. 1 Dendrogram (Cluster analysis tree chart) depicting genetic relationships among 225 wheat genotypes.

cluster means of each of the 16 traits under late-sown (heat stress) condition was calculated (Supplementary Table 3); cluster II was observed for the maximum cluster mean for grain yield/plot (283.33 g) followed by cluster III (198.67 g). Intra-cluster distance was maximum for cluster III (3.353) followed by cluster IV (3.311) and cluster I (3.152), while cluster VI (2.358) and cluster V (2.882) had slightly lower values of intra-cluster distance (Supplementary Table 4). Thus, genotypes included within the clusters III and IV showed a major magnitude of genetic diversity. The highest inter-cluster distance was noted for cluster VII and cluster III (6.958) which was followed by cluster V and III (6.499), cluster III and I (6.341). It revealed that genotypes exist within these clusters possess significant genetic diversity making them suitable candidates to generate favourable recombinants and promising lines. Minimum inter-cluster distance was observed between cluster VII and V (2.533). Solanki *et al.* (2022) classified 48 genotypes into seven clusters based on genetic diversity analysis and based on the percent contribution of different traits towards total genetic divergence, canopy temperature depression, total gluten and zinc content were contributed highest. Niyazi *et al.* (2023) also reported similar clustering pattern having five cluster as per Tocher's method considering D^2 value.

Genotypes 132 (GID 7934144) and 133 (GID 7934148) from cluster V for days to 50% flowering, genotype 98 (GID: 7933964) from cluster VII for flag leaf area, genotypes 31 (GID: 7933205) and 211 (GID: 7935056) from cluster I and III for peduncle length, and genotype 25 (GID: 7933135) from cluster III for both, 1000-grain weight and grain yield per plot are considered as genetically diverse and donor parents (Table 1). These genotypes can be employed in future breeding programmes for the desired traits. The relative contribution of yield per se towards genetic divergence was very high; Bellundagi (2013) and Sharma *et al.* (2018) also recorded similar observations. Yield traits namely peduncle length, plant height, flag leaf area, 1000-grain weight, days to heading, grain filling duration, spike length and chlorophyll content contributed towards genetic divergence (Supplementary Fig. 1). Thus, these traits aid in selecting diverse parents for breeding heat-tolerant high-yielding varieties adaptable to different agro-climatic zones.

Identification of donors using heat susceptibility index (HSI): Heat susceptibility index was calculated for GFD, TGW, yield and classified according to Baranwal *et al.* (2016) i.e. genotype bearing 0.0 to 0.75 HSI value can be accepted as terminal heat tolerant, 0.76 to 1.25 as moderate heat susceptible and genotypes with HSI >1.25 as heat susceptible. HSI for grain filling duration (GFD), 1000-grain weight (TGW) and yield per plot (YPPT) ranged from 0.36 (genotype 30, GID: 7933202) to 2.51 (genotype 54, GID:7933656), -0.84 (genotype 44, GID: 7933473) to 2.66 (genotype 36, GID: 7933333) and -0.49 (genotype 37, GID: 7933334) to 1.93 (genotype 49, GID: 7933509), respectively (Table 2). The selected genotypes on the basis of HSI of GFD, TGW and YPPT represented different clusters like genotypes 8 (GID: 7933029), 71 (GID: 7933792) and

Table 1 Superior diverse wheat genotypes identified for 16 phenological and physiological traits

Genotype	Cluster number	Desirable trait
132,133	V	DF
21,25	III	DPM
21	III	DA
1, 2, 3, 5, 8, 10, 11, 14, 15, 32, 37, 39, 62, 68, 97, 166, 171, 176, 197, 208, 211	VII, III, VI, I	GFD
98	VII	FLA
2, 3, 7, 120, 129	VII, VI, II	NDVI (ZS45)
1	VII	NDVI (ZS75)
18	VI	CT (ZS45)
119, 159	V, I	CT (ZS75)
100	V	CCI (ZS45)
8	VII	CCI (ZS75)
135	II	PH
63	V	SPL
31,211	I, III	PDL
25	III	TGW
25	III	YPPT

*DF, Days to 50% flowering; DA, Days to anthesis; DPM, Days to physiological maturity; GFD, Grain filling duration; NDVI, Normalized difference vegetation index; CT, Canopy temperature; CCI, Chlorophyll content index at ZS45 and ZS75; ZS, Zadoks Scale; ZS45, Late booting; ZS75, Medium milking; FLA, Flag leaf area (cm²); PH, Plant height (cm); SPL, Spike length (cm); PDL, Peduncle length (cm); TGW, 1000-grains weight (g); YPPT, Yield/plot.

93 (GID: 7933947) to cluster VII; genotypes 19 (GID: 7933096), 24 (GID: 7933122) and 172 (GID: 7934632) to cluster VI, genotypes 183 (GID:- 7934802) and 188 (GID: 7934847) to cluster IV, genotype 64 (GID:7933762) to cluster I and genotype 192 (GID: 7934855) to cluster III (Table 2 and Supplementary Table 2). Genotypes 88, 74 and 66 had low to moderate HSI for GFD, TGW and GY respectively. Based on per cent reduction in YPPT under heat stress, genotype 19 (GID: 7933096) exhibited lowest

Table 2 Categorization of 225 wheat genotypes based on three selection indices

Heat susceptibility index (HSI)	Number of genotypes		
	HSI GFD*	HSI TGW	HSI YPPT
Range	-0.36-2.51	-0.84-2.66	-0.49-1.93
Heat tolerant (0-0.75)	88	74	66
Moderate heat susceptible (0.76-1.25)	82	61	96
Heat susceptible (> 1.25)	55	90	63

*HSI GFD, Heat susceptibility index for grain filling duration; HSI TGW, Heat susceptibility index for 1000-grain weight; HSI YPPT, Heat susceptibility index for yield/plot.

reduction (-23.00%) along with HSI for YPPT and TGW are -0.49 and -0.60, respectively followed by genotype 224 (DBW14) with yield reduction and HSI for YPPT and TGW as 19.21%, 0.41 and -0.56, respectively (Table 3). HSI has been a robust indicator for identifying terminal heat tolerant genotype and classifying genotypes on various groups (Ubale Sonali *et al.* 2020, Kumar *et al.* 2024a, Kumar *et al.* 2024b).

Based on per cent reduction in TGW under heat stress, genotype 23 (GID: 7933111) exhibited lowest reduction (-20.00%) followed by 67 (GID: 7933773) (-19.2%) and 19 (GID: 7933096) (-14.29%) (Table 3). Among the selected genotypes, genotype 64 (GID: 7933762) showed the lowest percentage reduction in GFD i.e., 5.88% with HSI for GFD, TGW and YPPT are 0.32, 0.39 and 0.71 (Table 3). However, highest average yield per plot was recorded for genotype 192 (GID: 7934855) (310.0 g) followed by genotype 19 (GID: 7933096) (290.0 g). The wheat genotypes 8, 64, 71 and 93 exhibited higher NDVI at ZS75 0.42, 0.52, 0.44 and 0.46, respectively. The wheat genotypes 19, 24 and 188 exhibited lower CT at ZS75 16.8, 16.5 and 16.2, respectively (Table 3). A low CT value suggests favourable water absorption and root development potential, in alignment with prior research by Meena *et al.* (2014) and Baranwal *et al.* (2016) while a higher NDVI value indicates robust and dense vegetation health (Reynolds *et al.* 2012). Based on low HSI of TGW, GFD and GY and other selection indices, 10 genotypes namely 8, 19, 24, 64, 71, 93, 172, 183, 188 and 192 along with check DBW 14 were found tolerant to terminal heat stress; these donors could be used in breeding programme and mapping QTLs associated with terminal heat tolerance (Table 3).

Principal component analysis (PCA): Six principal components (PC1–PC6) were extracted from the original data accounting nearly 76.38% of the total variation (Supplementary Table 5). The maximum eigen value (3.99) was recorded for first PC which explained 24.92% variation. The remaining five PCs (PCs 2-6) explained 19.90%, 10.05%, 8.21%, 7.52% and 5.78% of variation, respectively. PCA biplot for the first two PCs depicted the distribution of all wheat genotypes considering observations recorded at late-sown condition (Fig. 2). It highlighted the importance of traits namely chlorophyll content index (CCI), NDVI, flowering-related parameters, spike length, plant height, TGW, canopy temperature, and flag leaf area in distributing genotypes across the biplot (Fig. 2). The first PC was predominantly related to phenological traits like days to 50% flowering, days to physiological maturity, day to anthesis and NDVI at ZS75 indicated that this component was more important towards the genetic diversity, whereas the second PC include variables related to yield like yield/plot and 1000-grain weight accounting for 19.90% variation. The third principal component was positively correlated with flag leaf area, CCI at ZS45 and CCI at ZS75. This factor accounted for 10.05% variation (Supplementary Table 5). The fourth principal component accounted for 8.21% of the variation and included components like plant height and peduncle length. The experimental results were well

Table 3 Average performance of promising wheat genotypes for important traits and selection indices

Geno- type	DF	DPM	DA	GFD	HSI GFD	Per cent R in GFD	FLA	NDVI (ZS45)	NDVI (ZS75)	CT (ZS45)	CT (ZS75)	CCI (ZS45)	CCI (ZS75)	PH	SPL	PDL	TGW	HSI TGW	Per cent R in TGW	YPPT	HSI YPPT	% R in YPPT
8	66	104	75	29	0.66	12.12	35.64	0.85	0.42	18.2	33.8	32.6	28.5	102	10	34	29	0.39	9.38	230	0.43	20.69
19	66	106	75	31	0.88	16.22	36.05	0.79	0.31	18.6	34.2	21.8	16.8	99	9	33	32	-0.60	-14.29	290	-0.49	-23.40
24	66	106	74	32	0.60	11.11	22.31	0.81	0.36	19.8	34.8	21.2	16.5	108	10	33	34	-0.26	-6.25	270	0.10	4.59
64	69	109	77	32	0.32	5.88	33.66	0.84	0.52	21.8	32.2	24.8	19.4	93	11	32	29	0.39	9.38	225	0.71	33.82
71	66	106	76	30	0.49	9.09	34.94	0.82	0.44	21.0	32.4	32.6	25.4	87	10	34	34	0.44	10.53	285	0.54	25.97
93	66	106	75	31	0.62	11.43	28.89	0.84	0.46	20.0	33.2	28.6	22.9	92	11	27	28	0.53	12.50	205	0.71	33.87
172	64	104	73	31	1.11	20.51	26.46	0.81	0.32	19.8	32.4	24.7	18.6	98	9	30	32	0.47	11.11	280	0.29	13.85
183	65	104	74	30	0.64	11.76	25.37	0.82	0.32	20.8	34.4	24.60	19.7	96	7	27	28	0.74	17.65	205	0.41	19.61
188	67	107	76	31	0.62	11.43	18.45	0.83	0.35	21.6	33.8	21.40	16.2	84	9	25	30	0.26	6.25	225	0.44	21.05
192	61	101	70	31	0.62	11.43	26.22	0.82	0.30	21.8	34.2	23.10	18.1	90	8	37	35	-0.39	-9.38	310	-0.22	-10.71
DBW14	60	101	69	32	1.48	27.27	36.99	0.84	0.26	20.2	31.8	29.8	22.4	75	9	28	34	-0.56	-13.33	245	-0.41	-19.51

*DF, Days to 50% flowering; DA, Days to anthesis; DPM, Days to physiological maturity; GFD, Grain filling duration; NDVI, Normalized difference vegetation index; CT, Canopy temperature; CCI, Chlorophyll content index at ZS45 and ZS75; ZS, Zadoks Scale; ZS45, Late booting; ZS75, Medium milking; FLA, Flag leaf area (cm²); PH, Plant height (cm); SPL, Spike length (cm); PDL, Peduncle length (cm); TGW, 1000-grains weight (g); YPPT, Yield/plot; HSI GFD, Heat susceptibility index for grain filling duration.

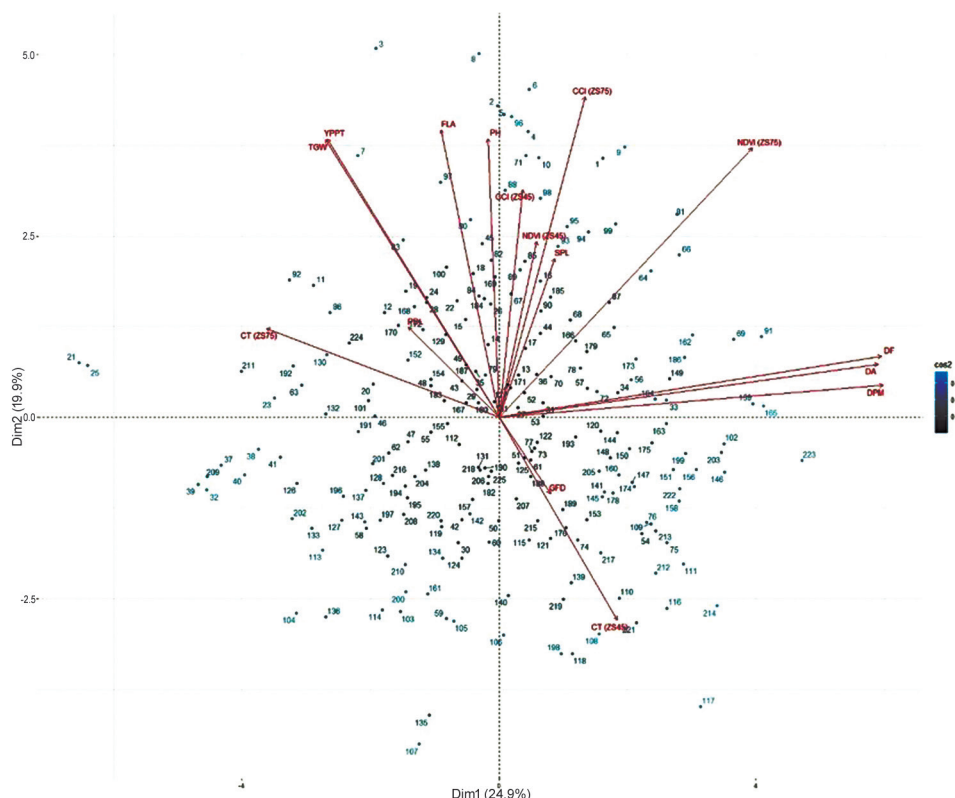


Fig. 2 PCA biplot: Grouping pattern of studied wheat genotypes.

supported by the findings of Morgunov (2020), Pandey *et al.* (2021) and Kumar *et al.* (2024b). The results on PC analysis indicated that these traits are important for selection and future breeding programme; it also represented genetic diversity contributed by these traits among the studied material.

Genetic diversity analysis among 225 wheat genotypes revealed seven diverse clusters. The highest intra-cluster distance was observed for cluster III followed by cluster IV and cluster I. Ten genotypes with lower HSI for selection indices namely 8, 19, 24, 64, 71, 93, 172, 183, 188 and 192 along with check DBW 14 were found tolerant to terminal heat stress. Hence they should be further study to validate their stability across the environments. PCA analysis preserved a significant portion of the original data's variance and indicated that days to 50% flowering, days to physiological maturity, yield/plot, 1000-grain weight and NDVI at ZS75 are important for trait manipulation and diversity analysis.

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