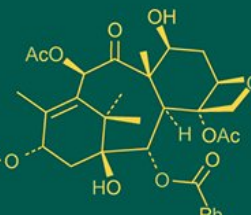
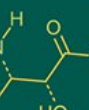
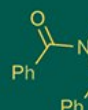


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Unraveling genetic variation and phenotypic plasticity for heat stress adaptation in diverse wheat germplasm

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Abstract

Heat stress is a major constraint to wheat productivity, making the identification of genetically stable traits essential for developing climate-resilient cultivars. This study evaluated genetic variation, heritability, and selection potential for agronomic, phenological, and physiological traits in diverse wheat germplasm under contrasting heat stress and non-stress environments. A panel of 230 unique bread wheat (*Triticum aestivum* L.) entries, comprising 225 advanced breeding lines and five check cultivars, was evaluated across Ludhiana, Jabalpur, and Pusa during two consecutive Rabi seasons (2021-22 and 2022-23) under Normal and Late-sown conditions using an alpha-lattice design with two replications. The five checks were repeated within incomplete blocks, resulting in 240 experimental plots per replication. Analysis of variance demonstrated substantial genetic variation for major phenological, agronomic, and physiological traits. Terminal heat stress accelerated crop maturity by up to 24 days and reduced grain yield by as much as 47.9% at Pusa, with a marked reduction in thousand-grain weight. Broad-sense heritability for grain yield declined under stress at Ludhiana ($H^2 = 0.03$) and Jabalpur ($H^2 = 0.05$), whereas Pusa retained moderate genetic control ($H^2 = 0.12$; GAM = 11.11%). Canopy temperature at anthesis was negatively associated with grain yield ($r = -0.28$) and thousand-grain weight ($r = -0.39$). Principal component analysis showed that the first two components accounted for 80.5% of total phenotypic variation. Multi-trait phenotypic plasticity identified G12072 as the most stable entry (Overall PI = 0.0929), followed by G12031, G12007, G12037, and G11066. Hierarchical clustering further supported distinct plasticity patterns among genotypes. These findings demonstrate substantial exploitable genetic variation and identify promising wheat germplasm with stable responses to terminal heat stress.

Keywords: Wheat, heat stress, genetic components, broad-sense heritability, genetic advance, correlation matrix, PCA, phenotypic plasticity

Introduction

Wheat (*Triticum aestivum* L.) is one of the world's most important cereal crops and contributes substantially to global food and nutritional security. Wheat is cultivated on more than 220 million hectares worldwide, with annual production exceeding 840 million tons, while India is the second-largest producer after China (USDA, 2025; FAOSTAT, 2024) [2]. Maintaining productivity under a warm climate is therefore a major priority for wheat improvement. High temperatures during reproductive and grain-filling stages speed up the development of crops, shorten the period of grain filling, lower the photosynthetic activity, and break the partitioning of assimilates, leading to reduced grain yield and grain weight (Asseng *et al.*, 2015; Zhao *et al.*, 2017) [3, 4]. Global predictions have shown that for every 1 °C increase above the optimum, wheat yields decrease by about 6%, highlighting the need for heat-resilient cultivars (Asseng *et al.*, 2015) [3]. The extent of damage caused by heat is genotype-dependent, allowing the possibility to exploit the genetic variation for adaptation. Grain yield is still the main breeding target, but its complex inheritance and sensitivity to environmental conditions may lower the efficiency of direct selection under stress. Phenological traits (booting, heading, flowering and maturity) influence the period of exposure to high temperature, while plant height and grain weight influence biomass and grain filling. These traits can serve as complementary selection criteria for heat adaptation (Qaseem *et al.*, 2019; Langridge & Reynolds, 2021) [5, 6]. Physiological traits can give more information on plant responses to heat stress. The Normalized Difference Vegetation

Index (NDVI) gives an indirect measure of canopy greenness and crop status, while canopy temperature (CT) measures plant cooling capacity and the balance between transpiration and atmospheric demand. NDVI and CT have been validated by field phenotyping approaches which are useful to assess wheat responses to environmental stress and for selection in breeding populations (Araus *et al.*, 2018) [7]. Quantitative genetic parameters are crucial to knowing how much of the observed phenotypic differences can be exploited by selection. Coefficients of variation for genotype and phenotype measure the extent of genetic and total phenotypic variation, respectively, while broad-sense heritability is a measure of the portion of phenotypic variation that is attributable to genetic differences. Heritability is an adjunct to genetic advance; it's the expected response to selection. These are beneficial in the conventional improvement especially if traits have high heritability with high genetic advance (Falconer & Mackay, 1996; Singh & Chaudhary, 1985) [8, 10].

Phenotypic plasticity offers a complementary framework for assessing genotype-specific responses to contrasting environments. Plasticity measures the extent of change in a trait across environments and can capture coordinated responses across agronomic, developmental, and physiological traits, rather than focusing on yield loss alone. Variation in phenotypic plasticity among wheat genotypes can reveal contrasting capacities to adjust agronomic and physiological traits across environments, providing an

additional criterion for assessing adaptation and stability (Elazab *et al.*, 2025) [11]. Recent wheat studies have demonstrated the significance of phenotypic plasticity in understanding the variation in yield components, physiological traits, and environmental adaptation (Saieed *et al.*, 2024; Elazab *et al.*, 2025) [9, 11]. Despite progress in wheat heat-stress research, relatively few studies have combined genetic variability, inheritance parameters, trait relationships, multivariate analysis, and multi-trait phenotypic plasticity within a diverse breeding panel. The present study therefore aimed to quantify genetic variability, heritability, and genetic advance for studies traits under Normal and Late-sown conditions; characterize relationships among key traits; identify stable heat-resilient wheat entries using an integrated multi-trait phenotypic performance approach.

2.1 Experimental material

The experimental material comprised 230 unique bread wheat entries, including 225 advanced breeding lines and five commercial checks. The experiment followed an alpha-lattice design with two replications; each divided into three incomplete blocks of 80 plots. Each block contained 75 breeding lines and the five checks, resulting in 225 unique breeding lines and 15 check plots (5 checks × 3 blocks) and 240 experimental units per replication. Details of the entries are provided in Supplementary Table S1.

Table 1: List of 230 bread wheat genotypes (accession codes) used in the present study, check cultivars are highlighted.

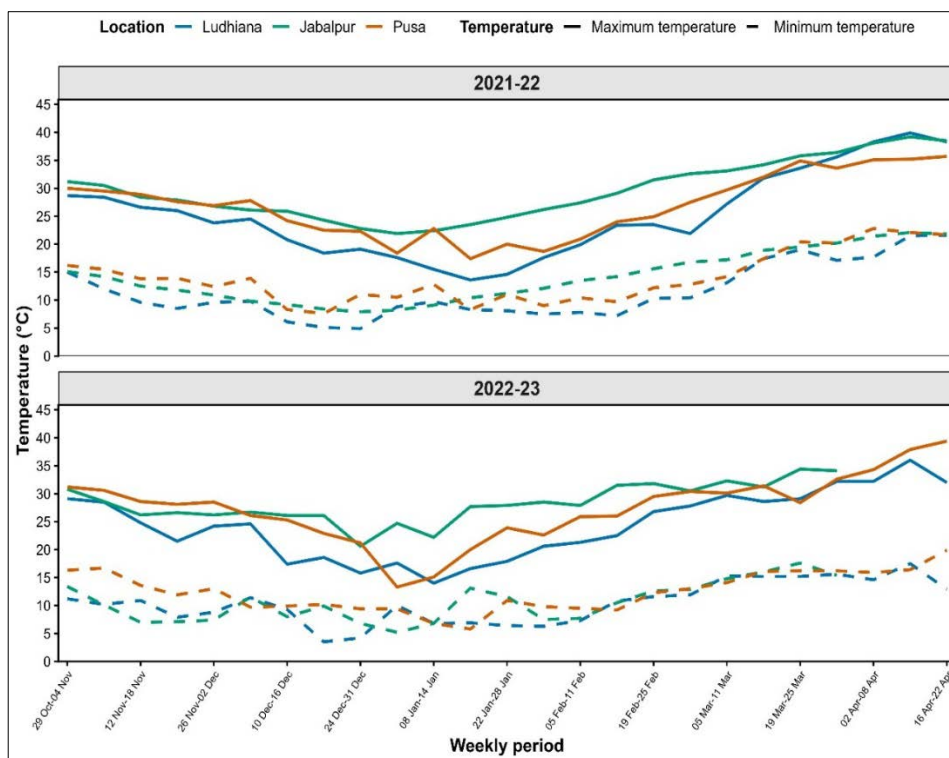
Entry	NBPGR Accession	Entry	NBPGR Accession	Entry	NBPGR Accession
10001	8	11001	466	12001	652
10002	RAJ3765 *	11002	RAJ3765 *	12002	RAJ3765 *
	(HD2402/VL639)		(HD2402/VL639)		(HD2402/VL639)
10003	9	11003	468	12003	653
10004	11	11004	479	12004	654
10005	16	11005	484	12005	682
10006	20	11006	485	12006	697
10007	21	11007	488	12007	702
10008	22	11008	491	12008	712
10009	26	11009	497	12009	713
10010	28	11010	499	12010	714
10011	DBW107 *	11011	DBW107 *	12011	DBW107 *
	(TUKURU/INQLAB)		(TUKURU/INQLAB)		(TUKURU/INQLAB)
10012	30	11012	501	12012	715
10013	31	11013	502	12013	748
10014	32	11014	503	12014	749
10015	33	11015	504	12015	752
10016	37	11016	506	12016	753
10017	40	11017	507	12017	754
10018	51	11018	513	12018	756
10019	52	11019	515	12019	763
10020	53	11020	517	12020	799
10021	54	11021	520	12021	812
10022	55	11022	523	12022	815
10023	56	11023	532	12023	818
10024	58	11024	533	12024	819
10025	59	11025	534	12025	821
10026	60	11026	535	12026	825
10027	61	11027	536	12027	844
10028	62	11028	538	12028	846
10029	63	11029	541	12029	868
10030	DBW71 *	11030	DBW71 *	12030	DBW71 *
	(PRINIA/UP2425)		(PRINIA/UP2425)		(PRINIA/UP2425)
10031	80	11031	5130	12031	869
10032	82	11032	545	12032	870

10033	83	11033	546	12033	873
10034	84	11034	547	12034	875
10035	99	11035	549	12035	880
10036	100	11036	550	12036	883
10037	102	11037	551	12037	884
10038	103	11038	552	12038	902
10039	106	11039	553	12039	906
10040	110	11040	556	12040	910
10041	111	11041	557	12041	930
10042	117	11042	560	12042	931
10043	160	11043	564	12043	934
10044	161	11044	565	12044	936
10045	162	11045	566	12045	937
10046	165	11046	572	12046	938
10047	197	11047	573	12047	956
10048	198	11048	575	12048	978
10049	199	11049	576	12049	979
10050	210	11050	578	12050	991
10051	HD2932 * (KAUZ/STAR/HD2643)	11051	HD2932 * (KAUZ/STAR/HD2643)	12051	HD2932 * (KAUZ/STAR/HD2643)
10052	214	11052	579	12052	992
10053	215	11053	581	12053	993
10054	216	11054	584	12054	998
10055	219	11055	585	12055	1022
10056	221	11056	588	12056	1023
10057	225	11057	590	12057	1026
10058	235	11058	591	12058	1027
10059	237	11059	592	12059	1031
10060	263	11060	595	12060	1040
10061	287	11061	596	12061	1047
10062	291	11062	597	12062	1062
10063	294	11063	598	12063	1358
10064	300	11064	600	12064	1113
10065	398	11065	601	12065	1117
10066	399	11066	602	12066	1119
10067	400	11067	603	12067	1159
10068	401	11068	604	12068	1166
10069	429	11069	606	12069	1171
10070	887	11070	615	12070	1178
10071	432	11071	616	12071	1180
10072	441	11072	625	12072	1183
10073	442	11073	632	12073	1184
10074	PBW771 * (BW9246/2*DBW71)	11074	PBW771 * (BW9246/2*DBW71)	12074	PBW771 * (BW9246/2*DBW71)
10075	443	11075	633	12075	1185
10076	445	11076	643	12076	1187
10077	446	11077	644	12077	1188
10078	447	11078	645	12078	1189
10079	448	11079	646	12079	1249
10080	453	11080	651	12080	1253
Check cultivars: RAJ3765, DBW107, DBW71, HD2932, PBW771.					
RAJ3765 *	https://www.researchsquare.com/article/rs-8710524/v1				
DBW107 *	https://www.aicrpwheatbarleyicar.in				
DBW71 *	https://www.researchgate.net/publication/279806820_Notification_of_a_wheat_variety_DBW_71				
HD2932 *	https://www.aicrpwheatbarleyicar.in/				
PBW771 *	https://www.researchsquare.com/article/rs-8710524/v1				

2.2 Experimental sites and environments

Field experiments were conducted during the 2021-22 and 2022-23 Rabi seasons at BISA Jabalpur (Central Zone), BISA Ludhiana (North-Western Plains Zone), and BISA Pusa (North-Eastern Plains Zone). At each location, two

contrasting sowing regimes were imposed: Normal sowing during the first fortnight of November and Late sowing during the second fortnight of December. Weekly maximum and minimum temperature profiles are presented in Supplementary Fig. S1.



Supplementary Fig 1: Seasonal trajectories of weekly maximum (solid) and minimum (dashed) temperatures across Ludhiana (blue), Jabalpur (green), and Pusa (orange) during two consecutive Rabi cycles (2021–22 and 2022–23). The increase in temperature after March is indicative of differential accumulation of terminal heat stress during the crop reproductive phase.

2.3 Experimental layout and trait assessment

Each genotype was grown in a standardized six-row plot measuring 2.80 m in length with 20 cm row spacing. Recommended regional agronomic practices and uniform crop protection measures were followed. Days to booting (DTB), days to heading (DTHD), days to flowering (DAYSFL), and days to maturity (DAYSMT) were recorded when 50% of plants in a plot reached the respective stage. Plant height (PH), grain yield (GRYLD), thousand-grain weight (TGW), NDVI at anthesis (NDVIANTH), and canopy temperature at anthesis (CTANTH) were also recorded.

2.4 Statistical analysis

Data were analyzed for individual environments and across environments using linear mixed models fitted by restricted maximum likelihood (REML). The combined model was specified as $Y_{ijk} = \mu + L_i + R_j(i) + B_m(ji) + G_k + (GL)_{ik} + \epsilon_{ijk}$, where Y_{ijk} is the observed value, μ is the overall mean, L_i is location, $R_j(i)$ is replication nested within location, $B_m(ji)$ is incomplete block nested within replication and location, G_k is genotype, $(GL)_{ik}$ is the genotype $\times$ location component, and ϵ_{ijk} is the residual. Location was considered fixed, while replication, incomplete block, genotype, genotype $\times$ location, and residual effects were treated as random. The model was used to partition variation appropriately for estimation of genetic parameters; interpretation focused on genotypic variation and selection potential. Broad-sense heritability across locations was estimated as $H^2 = \sigma^2_G / [\sigma^2_G + (\sigma^2_{GL}/e) + (\sigma^2_{e/re})]$, where σ^2_G , σ^2_{GL} , and $\sigma^2_{e/re}$ are the genotypic, genotype $\times$ location, and residual variance components, respectively, and e and r are the numbers of locations and replications. For individual locations, $H^2 = \sigma^2_G / [\sigma^2_G + (\sigma^2_{e/r})]$. Genotypic and phenotypic coefficients of variation

were calculated as $GCV = (\sqrt{\sigma^2_G} / \bar{X}) \times 100$ and $PCV = (\sqrt{\sigma^2_P} / \bar{X}) \times 100$, respectively. Genetic advance as per cent of the mean (GAM) was worked out at 5% selection intensity with $k = 2.06$ following standard quantitative-genetic procedures (Falconer & Mackay, 1996) [8]. The plasticity index (PI) was computed for each genotype and trait by formulating $PI = (XNS - XHS) / XNS$, where XHS represents the trait values under Late-sown heat-stress conditions and XNS represents the trait values under Normal-sown conditions (Saieed *et al.*, 2024) [9]. A smaller value of PI indicates lower environmental change and greater phenotypic stability. Plasticity was evaluated for GRYLD, TGW, DTB, DTHD, DAYSFL, DAYSMT, PH, NDVIANTH and CTANTH. The Overall PI for each genotype was calculated as $Overall\ PI = (1/n) \sum PI_i$, where n is the number of traits. Genotypes were ranked based on Overall PI, with lower values indicating better stability across multiple traits. We summarized multivariate variation by performing principal component analysis of standardized trait data. Associations among traits were assessed using Pearson correlation coefficients. Multi-trait plasticity indices were standardized and clustered with Ward hierarchical clustering based on Euclidean distance, D2 linkage. Analyses and plots were performed in R 4.5.2 using the packages lme4, lmerTest, FactoMineR, factoextra, corrplot, dplyr and related packages.

3. Results and Discussion

3.1 Anova

ANOVA showed significant genetic variation for all studies traits under both Normal and Late sown conditions (Tables 1 and 2). The genotypic effects were highly significant ($p < 0.001$) for DTB, DTHD, DAYSFL, DAYSMT, PH, GRYLD, and TGW indicating considerable genetic diversity for phenological and yield traits. These results

confirm the presence of exploitable genetic variation for traits associated with heat adaptation in the germplasm studied. The different thermal regimes resulted in evident phenotypic differences. Late sowing shortened crop development and maturity occurred up to 24 days earlier and reduced the grain yield as much as 47.9 per cent at Pusa.

Thousand Grain Weight reduce suggested that grain filling was very sensitive to terminal heat stress. Such developmental acceleration and reduced grain filling are consistent with established effects of high temperature during reproductive development (Lobell *et al.*, 2012; Qaseem *et al.*, 2019) [12, 5].

Table 2: Combined analysis of variance (ANOVA) for traits of wheat genotypes under Normal (Non-Stress) environments across three locations.

Source of Variation	df	DTB	DTHD	DAYSFL	DAYSMT	PH	GRYLD	TGW
Genotypes	224	248.2***	229.1***	218.4***	62.1***	2524.2***	3.35***	188.7***
Checks	4	84.5**	76.2**	71.3**	44.1*	812.5***	3.35**	94.2**
Treatments	229	251.3***	231.6***	220.5***	63.1***	2554.1***	3.35***	191.2***
Year	1	5.12*	4.88*	4.12*	2.05	18.4	136.29***	8.11*
Location	2	137552***	141972***	133767***	114845***	16777.9***	9.78***	6104.0***
Genotype × Location	458	1.31***	1.22***	1.28***	0.85***	14.21**	1.31***	24.62***
Year × Location	2	91.05***	88.42***	93.11***	104.50***	112.42	91.05***	18.91**
Checks vs Gen	1	948.44**	917.52**	899.31**	74.08	55582***	3.35*	1231.47***
Residuals	2181	0.38	0.44	0.52	0.72	14.25	0.79	4.22

Table 1. Combined ANOVA for Normal condition represents mean squares for all sources of variation for studies traits. significance levels are indicated by $p < 0.05$, $p < 0.01$, and $P < 0.001$, ns- non-significant. whereas DTB,

Days to booting; DTHD, Days to heading; DAYSFL, Days to flowering; DAYSMT, Days to maturity; PH, plant height; GRYLD, grain yield; TGW, thousand-grain weight.

Table 3: Combined analysis of variance (ANOVA) for traits of wheat genotypes under Terminal Heat Stress environments across three locations.

Source of Variation	df	DTB	DTHD	DAYSFL	DAYSMT	PH	GRYLD	TGW
Genotypes	224	1127.0***	1048.0***	954.0***	241.0***	5110.0***	2.38***	387.0***
Checks	4	185.2***	164.1***	152.4***	98.5**	1245.0***	2.38***	114.5**
Treatments (Total)	229	1142.1***	1061.2***	966.8***	243.2***	5521.8***	2.38***	395.7***
Year (Y)	1	3.11*	2.85*	2.99*	1.12	22.1	52.78***	11.22*
Location (L)	2	409969***	434514***	424106***	264436***	35073.0***	12.58***	11286.0***
Year × Location	2	225.07***	212.45***	218.90***	185.32***	142.11*	225.07***	42.12***
Checks vs Gen	1	5096.90***	4389.90***	4207.30***	841.75***	104594***	2.38*	2600.95***
Location × Entry	458	0.81***	0.76***	0.79***	0.51***	18.91***	0.82***	32.41***
Residuals	2181	0.22	0.28	0.31	0.42	11.02	0.5	3.11

Table 2. Combined ANOVA for Stress condition represents mean squares for all sources of variation for studies traits. significance levels are indicated by $p < 0.05$, $p < 0.01$, and $P < 0.001$, ns- non-significant. whereas DTB, Days to booting; DTHD, Days to heading; DAYSFL, Days to flowering; DAYSMT, Days to maturity; PH, plant height; GRYLD, grain yield; TGW, thousand-grain weight.

3.2 Heritability and genetic advance

Genetic parameters varied among traits and environments (Tables 3-5). Under Normal sowing, GRYLD showed comparatively higher GAM at Pusa (12.47%) and Ludhiana (8.56%) than at Jabalpur (2.13%). Under Late sowing,

GRYLD retained considerable genetic gain at Pusa (GAM = 11.11%), indicating exploitable genetic variation even under terminal heat stress. Broad-sense heritability for GRYLD was low under Late sowing at Ludhiana ($H^2 = 0.03$) and Jabalpur ($H^2 = 0.05$) but was moderate at Pusa ($H^2 = 0.12$). Phenological traits showed low to moderate genetic advance while DAYSMT recorded particularly low GAM under stress (0.11% at Pusa and 0.19% at Ludhiana). Heritability and genetic advance together can be useful in guiding selection for traits where genetic control and expected gain were greater, direct selection is likely to be more effective; traits with lower estimates may be better used as complementary indicators rather than sole selection targets.

Table 4: Genetic parameters for traits in diverse germplasm at Ludhiana.

Trait	Management	Mean	GCV (%)	PCV (%)	h ² (bs)	GAM (%)	Significance
DTB	Normal	98.59	3.49	7.88	0.2	3.18	**
	Stress	85.71	2.05	5.51	0.14	1.57	*
DTHD	Normal	106.06	3.11	6.79	0.21	2.93	**
	Stress	90.41	1.96	5.05	0.15	1.56	*
DAYSFL	Normal	111.72	2.55	5.88	0.19	2.28	**
	Stress	93.83	1.81	4.84	0.14	1.39	*
DAYSMT	Normal	144.76	1.08	2.66	0.16	0.9	*
	Stress	122.79	0.59	3.73	0.03	0.19	ns
PH	Normal	115.49	5.84	12.9	0.2	5.44	**
	Stress	110.42	6.31	13.43	0.22	6.12	**
GRYLD	Normal	4.77	10.69	27.5	0.15	8.56	**
	Stress	3.86	4.89	30.35	0.03	1.62	ns
TGW	Normal	36.35	3.91	15.78	0.06	2	*
	Stress	33	4.47	20.73	0.05	1.99	*

Table 3. GCV: Genotypic Coefficient of Variation; PCV: Phenotypic Coefficient of Variation; h² (bs): Broad-sense heritability; GAM: Genetic Advance as percent of Mean.

Significance levels: *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, ns = non-significant.

Table 5: Genetic parameters for traits in diverse germplasm at Jabalpur

Trait	Management	Mean	GCV (%)	PCV (%)	h ² (bs)	GAM (%)	Significance
DTB	Normal	67.34	6.65	14.85	0.2	6.14	**
	Stress	62.91	3.69	9.07	0.17	3.09	**
DTHD	Normal	72.88	6.05	13.77	0.19	5.48	**
	Stress	67.17	3.4	8.44	0.16	2.82	**
DAYSFL	Normal	77.86	5.57	12.87	0.19	4.97	**
	Stress	71.25	3.21	8.06	0.16	2.63	**
DAYSMT	Normal	119.34	2.34	6.3	0.14	1.79	*
	Stress	101.8	1.54	3.99	0.15	1.22	*
PH	Normal	105.58	5.79	14.85	0.15	4.65	**
	Stress	104.03	6.52	16.26	0.16	5.38	**
GRYLD	Normal	5.57	4.95	23.61	0.04	2.13	ns
	Stress	3.86	6	27.39	0.05	2.71	*
TGW	Normal	42.1	4.97	15.03	0.11	3.39	**
	Stress	36.73	5.78	16.81	0.12	4.09	**

Table 4. Estimation of GCV: Genotypic Coefficient of Variation; PCV: Phenotypic Coefficient of Variation; h² (bs): Broad-sense heritability; GAM: Genetic Advance as

percent of Mean. Significance levels: *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, ns = non-significant.

Table 6: Genetic parameters for traits in 240 diverse germplasm at Pusa

Trait	Management	Mean	GCV (%)	PCV (%)	h ² (bs)	GAM (%)	Significance
DTB	Normal	70.01	7.86	18.07	0.19	7.04	**
	Stress	67.99	3.4	8.46	0.16	2.81	**
DTHD	Normal	77.55	6.87	15.97	0.19	6.09	**
	Stress	72.57	2.97	7.42	0.16	2.45	**
DAYSFL	Normal	84.6	6.04	14.04	0.18	5.34	**
	Stress	76.58	2.67	6.57	0.17	2.24	**
DAYSMT	Normal	131.28	1.58	4.25	0.14	1.21	*
	Stress	106.94	0.36	2.45	0.02	0.11	ns
PH	Normal	110.76	6.74	19.01	0.13	4.92	*
	Stress	102.56	6.19	18.56	0.11	4.25	*
GRYLD	Normal	4.17	14.09	32.78	0.18	12.47	***
	Stress	2.17	15.8	46.28	0.12	11.11	***
TGW	Normal	40.35	6.28	19.66	0.1	4.13	*
	Stress	31.91	5.2	21.06	0.06	2.65	*

Table 5. Genotypic Coefficient of Variation; PCV: Phenotypic Coefficient of Variation; h² (bs): Broad-sense heritability; GAM: Genetic Advance as percent of Mean. Significance levels: *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, ns = non-significant.

3.3 Multivariate variation and associations among traits

GRYLD was positively associated with TGW ($r=0.55$) indicating that grain weight retention contributed to yield performance in the evaluated germplasm like recent reports of positive relationships between yield and grain weight under heat stress (Bhandari *et al.*, 2024) [13]. Similar studies

associated with canopy temperature with wheat performance under heat stress (Yu *et al.*, 2025) ^[16], CTANTH was negatively correlated with GRYLD ($r = -0.28$) and TGW ($r = -0.39$), indicating that cooler canopies were associated with better yield and grain-weight maintenance. The phenological traits were highly correlated with each other, indicating a synchronization of crop development, and NDVI and CT were complementary indicators of canopy condition and thermal regulation. (Fig. 2). In addition, the multivariate structure of the evaluated traits was

summarized using principal component analysis (PCA) (Fig. 3). PC1 and PC2 explained 56.9% and 23.6% of the total variation, respectively, and together accounted for 80.5% of GRYLD and TGW were the most important discriminators among genotypes, but phenological and physiological traits captured additional dimensions of variation. This is consistent with the present wheat research in which yield components along with phenological and physiological traits effectively phenotyped the genetic variation and identified the heat-adaptive genotypes (Khare *et al.*, 2024) ^[14].

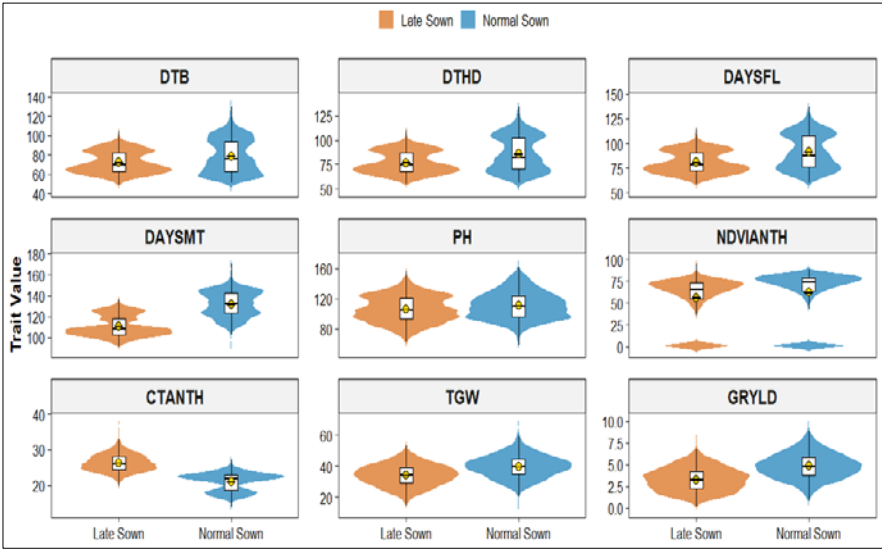


Fig 2: Normal Sown vs. Stress Sown: Phenological and Yield Variations Under Contrasting Thermal Environments. The violin plots illustrate the probability density and distribution of 240 wheat genotypes across three locations.

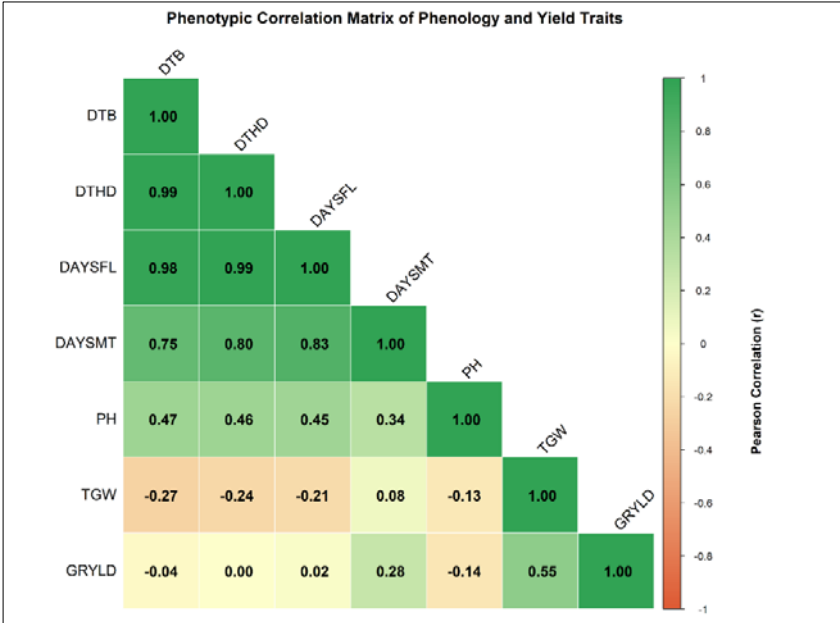


Fig 3: Phenotypic correlation matrix illustrating the relationships among phenological, physiological, and yield traits in 240 elite wheat genotypes under terminal heat stress. The color gradient indicates the strength of Pearson’s correlation coefficients (r).

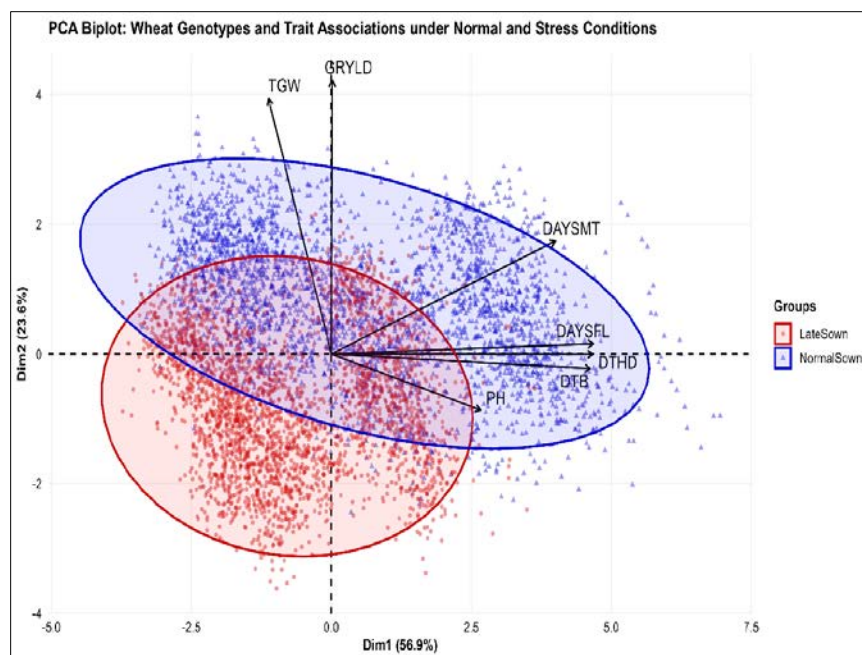


Fig 4: Combined PCA biplot displaying distinct phenotypic clustering of 240 elite wheat genotypes; the first two dimensions explained a robust 80.5% of the total phenotypic variance, while the 95% confidence ellipses statistically confirmed a highly significant spatial divergence between the optimal and heat-stressed environments.

3.4 Multi-Trait phenotypic plasticity

Phenotypic plasticity revealed substantial differences among traits under contrasting thermal conditions (Table 6). PI_PH had the highest variation (CV=84.97%) followed by PI_DTB (82.77%) and PI_DTHD (53.88%) indicating clear genotypic differences in developmental adjustment to late sown conditions. PI_DAYSMT (CV = 9.22%) and PI_CTANTH (10.94%) showed relatively narrow variation indicating more consistent responses across genotypes when compared. These trait-dependent differences in plasticity are in line with recent studies in wheat, where the magnitude and direction of phenotypic adjustment varies widely among traits and genotypes in different environments (Saieed *et al.*, 2024; Elazab *et al.*, 2025) [9, 11]. The Overall PI was an integrated measure of multi-trait stability and ranked genotypes based on their overall response for agronomic, phenological and physiological traits (Table 7; Fig. 4). The Overall PI was lowest for G12072 (0.093), followed by G12031 (0.099), G12007 (0.102), G12037 (0.103), G11066 (0.106) and G10040 (0.106). These entries were relatively less plastic across multiple traits, showing more phenotypic buffering across different thermal conditions. Similar genotype-dependent differences in plasticity and yield stability have recently been demonstrated in bread wheat tested in different environments including late-sown heat-stress conditions (Sangha *et al.*, 2025) [15]. Trait-specific responses further emphasized different components of adaptation. G12070 had the lowest plasticity for GRYLD (PI_Yield = 0.148) with relatively stable yield expression, while G12031 had low CTANTH plasticity (PI_CTANTH = 0.206), suggesting the canopy-temperature response across thermal regimes was relatively consistent. The stability of

TGW and NDVI among the selected entries further demonstrated the significance of preserving grain filling performance and canopy function under terminal heat stress. This is in line with recent evidence of plasticity of agronomic and physiological traits contributing to yield reliability and adaptation of wheat across stressful environments (Sangha *et al.*, 2025; Elazab *et al.*, 2025) [15, 11]. Hierarchical clustering of standardized multi-trait plasticity indices further separated the germplasm into distinct response groups (Fig. 4). PI_DTB, PI_DTHD and PI_DAYSFL showed similar clustering patterns, consistent with coordinated plastic responses of developmental traits. Conversely, PI_NDVIANTH and PI_CTANTH showed relatively separated patterns, with a complementary variability of canopy greenness and thermal responses. Also, it has been proved that canopy temperature is an auxiliary indicator for wheat yield and grain performance under the field condition (Yu *et al.*, 2025) [16]. Recent study for genetic analysis of wheat plasticity also reveals substantial genotype-dependent plasticity in flowering time and plant height further highlighting the genetic basis of developmental responses to environmental variation (Chen *et al.*, 2025) [17]. The clustering pattern gave wide support for the Overall PI ranking with G12072, G12031, G12007, G12037 and G10040 being among the most stable entries in the ranking across the clustering analysis. These results together demonstrate that multi-trait plasticity can add to conventional trait means by identifying germplasm that exhibits relatively stable expression across contrasting thermal regimes, offering promising candidates for follow-up validation in heat-resilience breeding.

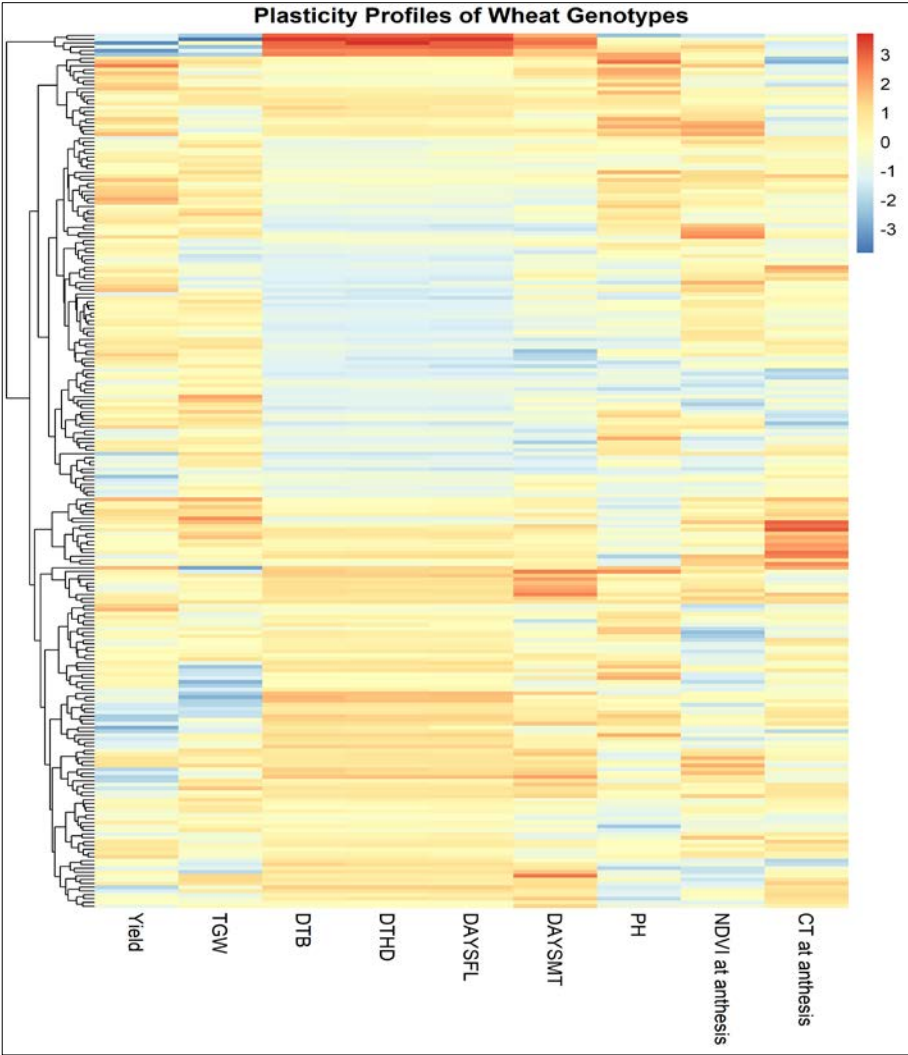


Fig 5: Hierarchically clustered heatmap showing multi-trait phenotypic plasticity profiles of wheat entries under contrasting normal- and late-sown conditions. The heatmap is based on standardized plasticity indices for yield, TGW, phenological traits, plant height, NDVI, CT at Anthesis stage with the row dendrogram representing hierarchical clustering using Euclidean distance and wards methods.

Table 6: Descriptive Statistics for Plasticity Index under Terminal Heat Stress

Trait	Mean	SD	Min	Max	CV
PI_Yield	0.313	0.069	0.08	0.496	22.056
PI_TGW	0.14	0.05	-0.052	0.26	35.893
PI_DTB	0.068	0.056	-0.017	0.268	82.773
PI_DTHD	0.091	0.049	0.012	0.273	53.882
PI_DAYSFL	0.109	0.041	0.037	0.256	37.278
PI_DAYSMT	0.161	0.015	0.125	0.203	9.222
PI_PH	0.04	0.034	-0.041	0.133	84.969
PI_NDVI	0.083	0.031	0.003	0.161	37.936
PI_CT	0.269	0.029	0.188	0.361	10.94

Supplementary Table 2. Summary statistics of trait-specific phenotypic plasticity indices in 230 wheat genotypes under contrasting thermal environments, whereas PI = Phenotypic Plasticity Index; PI_Yield = Grain yield; PI_TGW = Thousand-grain weight; PI_DTB = Days to booting;

PI_DTHD = Days to heading; PI_DAYSFL = Days to flowering; PI_DAYSMT = Days to maturity; PI_PH = Plant height; PI_NDVI = Normalized Difference Vegetation Index; PI_CT = Canopy temperature.

Table 7: Top 20 heat-resilient wheat genotypes identified using the Overall Phenotypic Plasticity Index (Overall PI).

Genotype	PI_Yield	PI_TGW	PI_DTB	PI_DTHD	PI_DAYSFL	PI_DAYSMT	PI_PH	PI_NDVIANTH	PI_CTANTH	Overall_PI
12072	0.217	0.118	-0.011	0.016	0.042	0.139	0.006	0.034	0.275	0.093
12031	0.252	0.135	0.000	0.033	0.059	0.152	0.008	0.048	0.206	0.099
12007	0.306	0.142	-0.014	0.024	0.050	0.140	0.022	0.030	0.217	0.102
12037	0.273	0.066	0.006	0.031	0.052	0.154	0.021	0.073	0.249	0.103
11066	0.258	0.089	0.021	0.057	0.071	0.140	-0.004	0.051	0.267	0.106
10040	0.252	0.165	-0.008	0.025	0.061	0.138	0.016	0.045	0.263	0.106
12048	0.295	0.135	0.009	0.042	0.075	0.140	-0.022	0.048	0.237	0.107
10056	0.231	0.171	0.003	0.018	0.056	0.150	-0.007	0.099	0.266	0.110
12044	0.328	0.165	-0.002	0.032	0.063	0.144	0.009	0.048	0.210	0.111
10042	0.223	0.128	0.033	0.058	0.082	0.168	0.007	0.046	0.257	0.111
11025	0.220	0.146	0.027	0.055	0.076	0.154	0.003	0.058	0.264	0.112
12070	0.148	0.124	0.045	0.063	0.086	0.160	0.024	0.077	0.286	0.113
10019	0.248	0.143	0.033	0.054	0.082	0.149	0.020	0.036	0.252	0.113
12027	0.213	0.112	0.025	0.055	0.080	0.157	0.031	0.085	0.263	0.113
12056	0.320	0.057	-0.008	0.030	0.054	0.155	0.040	0.107	0.269	0.113
10018	0.246	0.133	0.017	0.044	0.071	0.149	0.025	0.067	0.270	0.114
10006	0.353	0.136	0.001	0.027	0.052	0.143	-0.020	0.067	0.270	0.114
10007	0.319	0.153	-0.001	0.040	0.071	0.150	-0.003	0.044	0.258	0.114
11008	0.329	0.178	-0.005	0.020	0.037	0.134	-0.005	0.077	0.266	0.114

Table 7. Top 20 heat-resilient wheat genotypes identified using the Overall Phenotypic Plasticity Index. PI = Phenotypic Plasticity Index; Overall PI = mean of all trait-specific plasticity indices. Lower Overall PI values indicate greater phenotypic stability and heat resilience.

4. Conclusions

The study revealed substantial genetic variation for key agronomic, phenological, and physiological traits in diverse bread wheat germplasm evaluated under contrasting thermal regimes. Terminal heat stress accelerated crop development and substantially reduced grain yield, with pronounced effects on thousand-grain weight. Variation in heritability and genetic advance indicated differential potential for selection among traits, while trait associations and PCA demonstrated the complementary contributions of productivity, phenology, and canopy-related traits to heat-response characterization. Multi-trait phenotypic plasticity further differentiated genotypes according to their stability across thermal conditions and identified G12072, G12031, G12007, G12037, and G10040 as the most promising stable entries. These genotypes provide valuable breeding material for further multi-environment validation and incorporation into wheat improvement programs aimed at enhancing yield stability and adaptation to terminal heat stress.

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