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Trait-based insights of grain yield and major adaptive traits in wheat under terminal heat stress conditions

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Abstract

Terminal heat stress is a significant global limitation to wheat (*Triticum aestivum* L.) crop worldwide, especially during post-reproductive stages. In this study, 200 recombinant inbred lines (RILs) developed from WH711 × WH1021 were assessed under timely (stress-free) and late-sown (terminal heat stress) environments during the 2018–19 *Rabi* season. All traits exhibited significant genetic variability across environments, confirming the presence of adaptive diversity. GYP and AGBYP were markedly reduced under stress, with reductions of > 42% across RILs. Traits such as days to heading, anthesis, and maturity were shortened, leading to a reduced grain filling duration. Correlation and path coefficient analyses revealed that AGBYP and HI were the strongest positive contributors to GYP under both environments, while traits such as number of productive tillers, thousand-kernel weight, number of grains per spike, and seed density contributed indirectly. Regression and stepwise analyses identified AGBYP, HI, SD, and NPTM as the most predictive traits, explaining over 99% of yield variation. Principal component analysis (PCA) captured >70% of total trait variations, with biomass and partitioning efficiency emerging as key drivers of yield stability. Multivariate clustering grouped RILs into six clusters, with cluster V (containing WH1021 and RILs 15, 20 and 100) classified as heat-resilient high yielding. Overall, our results highlighted biomass production, efficient assimilate partitioning, and reproductive resilience as critical adaptive traits for breeding heat-resilient wheat. These findings provide trait-based selection indices and elite germplasm resources for future breeding programs targeting warming climates.

Keywords: Wheat, RILs, correlation, regression, PCA, cluster, terminal heat stress

Introduction

Wheat (*Triticum aestivum* L.) ranks among the most vital cereal crops globally, cultivated on over 220 million hectares and playing a key role in ensuring global food security (Redhu *et al.*, 2025) ^[39]. Global wheat production for the 2024/25 marketing year was projected around 800 million metric tons, reflecting a modest year-on-year rise (USDA 2024) ^[48]. With average global yields now approaching 3.54 t ha⁻¹, wheat remains central to cereal production (USDA 2024) ^[48]. Given its broad cultivation under both irrigated and rainfed systems, wheat ranks among the top cereals in many regions, frequently second to rice in irrigated systems and first under rainfed conditions (Qaseem *et al.*, 2019) ^[36].

However, these global statistics mask regional vulnerability to climate change. Wheat yield and stability are increasingly threatened by rising temperatures, erratic rainfall, and more frequent heat extremes, especially in arid and semi-arid zones. Among various abiotic stresses, terminal heat stress, especially occurring during post-reproductive phase, is particularly damaging and represents a major constraint on wheat productivity (Redhu *et al.*, 2025) ^[39]. Estimates suggest that in irrigated wheat-growing areas, up to ~ 40% may face episodic heat stress annually, contributing to global economic losses currently estimated in the multi-billion dollar range (Reynolds *et al.*, 2007) ^[40].

Even a single heat event can cause significant losses if it occurs at critical growth stages (Asseng *et al.*, 2011) ^[3]. Both anthesis and grain filling are two highly important stages as compared to prior developmental stages because there are few chances for recovery as compared to prior

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growth stages (Khadka *et al.*, 2020) [25]. A rise of 1 °C in mean temperature during the reproductive period is projected to reduce wheat yields by around 6% globally, mostly via shortened grain filling (by 4.1 to 6.4%), reduced seed set, and compromised source–sink dynamics (Devi *et al.*, 2024; Hassan *et al.*, 2021) [10, 18]. Heat stress has been shown to accelerate spikelet initiation and sporogenesis, ultimately resulting in spikelet sterility (Porter and Gawith 1999) [35]. Exposure to elevated temperatures (>20 °C) during the heading and anthesis stages promotes rapid spike enlargement but concomitantly decreases spikelet number (Semenov 2009) [42]. At the stage of floral initiation, heat stress exerts detrimental effects on microspore formation and pollen cell differentiation, frequently leading to complete male sterility (Freeha Anjum *et al.*, 2008; Kaur and Behl 2010) [16, 24]. In wheat, even a short-term (3-day) heat episode during flowering can cause structural abnormalities in florets (Hedhly *et al.*, 2009). Furthermore, heat stress at 31/20 °C (day/night) has been reported to reduce grain size by disrupting endosperm cell structure and impairing the development of the aleurone layer (Dias *et al.*, 2008) [12]. The combined effect is a contraction of yield potential and grain quality under heat stress episodes.

Thus, developing wheat genotypes with enhanced tolerance to terminal heat stress is of high priority. One of the most reliable approach involves screening and selection of potential germplasm in diverse environments that can provide reliable data on grain yield performance under both environments *i.e.* stress-free and heat-stressed conditions (Langridge and Reynolds 2021) [27]. However, achieving heat tolerance solely through yield-based selection is highly challenging due to its complex heritability, which has limited the attained genetic gain (Redhu *et al.*, 2024) [38]. A more effective strategy is to dissect component traits that confer adaptation under high temperature stress. Traits such as productive tiller number, biomass accrual, harvest index, kernel weight, and seed density are frequently implicated as yield determinants under stress (Dabi *et al.*, 2016; Kumbhar and Larik 1983) [9, 26]. To unravel the complex interplay among yield traits under stress, multivariate statistical methods (e.g. correlation, path analysis, multiple regression, and principal component analysis) provide powerful tools. These methods allow differentiation of direct and indirect effects, dimension reduction, and trait prioritization for breeding (Allard 1960; Bramel *et al.*, 1984) [2, 7]. In particular, biplot analyses based on vector angles (via cosine laws) help visualize associations and trade-offs among traits (Msundi *et al.*, 2021) [32].

In this study, 200 recombinant inbred lines (RILs) developed from WH711 × WH1021 were assessed under timely and late-sown environments. This study endeavours to discern an ideal model of wheat crop, capable of producing high-yielding genotypes under terminal heat stress. Our objectives were to (i) evaluate the impact of terminal heat stress on grain yield and its component traits, (ii) explore trait interrelationships under stress via multivariate approaches, (iii) identify key adaptive traits for breeding heat-tolerant genotypes, and (iv) selection of heat-tolerant genotypes.

2. Material and Methods

2.1 Experimental site and climate

The field trials were carried out in the 2018–19 *Rabi* season at CCSHAU farms, Hisar (29°09' N, 75°43' E; elevation 215.2 m above mean sea level). The soil is characterized as coarse sandy loam, slightly alkaline (pH ~7.6) with low organic matter content. The region experiences high solar radiation and

episodic heat stress during late spring; full supplemental irrigation was provided as needed, ensuring non-water limitation. Meteorological data (maximum, minimum temperatures, rainfall, and sunshine hours) were recorded from a nearby weather station and summarized in Table S1 and Fig. 1.

2.2 Experimental Design and Sowing Treatments

To evaluate genotype performance under contrasting thermal regimes, two sowing dates, *i.e.*, timely sowing (stress-free / yield potential environment) and late sowing (terminal heat stress environment) were used. Each genotype (line) was planted in an Alpha lattice design (ALD) with 5 incomplete blocks and 2 replications. Each plot comprised of 2 rows, each 2.5 m long spaced at 0.20 m (row-to-row), with a seeding rate of 100 kg ha⁻¹. Border rows were used to minimize edge effects. All agronomic practices, including fertilization, irrigation scheduling, weed management, and pest/disease control followed the recommended package for the Northwestern Plain Zone (NWPZ) to minimize non-genetic variation.

2.3 Plant materials

A population of 200 RILs developed from WH 711 × WH 1021 via the single-seed descent method was used (Table S2). WH 1021 is known for heat tolerance and high yield under late sowing, while WH 711 is agronomically superior under optimal, non-stress conditions but more heat susceptible. All entries (RILs and parents) were grown under both sowing conditions.

2.4 Measured traits and data collection

Phenological, morphological, and yield traits were systematically recorded for each recombinant inbred line (RIL). Phenological traits included days to anthesis (DA), days to heading (DH), and days to physiological maturity (DPM). For each RIL, heading was recorded when at least 50% of plot spikes had emerged from the booting stage. The grain filling duration (GFD) was calculated as the interval between DPM and DA. Morphological and yield component traits measured were plant height (PH), number of productive tillers per meter (NPTM), number of spikelets per spike (NSS), spike length (SL), number of grains per spike (NGS), thousand-kernel weight (TKW), 100-seed volume (HSV), and seed density (SD). Yield traits were assessed as grain yield per plot (GYP, g) and above-ground biological yield per plot (AGBYP, g). The harvest index (HI) was estimated as the proportion of grain yield to the total above-ground biomass (CM 1962). All phenological, morphological, and yield traits were measured randomly selected five plants from each entry, following standard agronomic protocols to ensure accuracy and comparability.

2.5 Statistical analysis

Mean values for each RIL under the two sowing environments were computed prior to analysis. A series of univariate and multivariate statistical approaches were applied. Pearson's correlation coefficients were estimated to assess pairwise trait associations (Al-Jibouri *et al.*, 1958) [1], and correlation matrices were visualized following Wei and Simko (Taiyun Wei 2021) [47]. Path coefficient analysis was conducted to partition the direct and indirect contribution of various traits to yield (Dewey and Lu 1959) [11]. Multiple linear regression was conducted to model yield as a function of component traits (Snedecor and Cochran 1967) [45], while stepwise regression was used to select the most predictive subset of traits (Draper and Smith 1966) [13]. To explore multivariate patterns, principal component analysis (PCA) was employed to investigate multivariate patterns by

reducing data dimensionality and examine covariance structures among traits (Kaiser 1958) ^[23]. In addition, cluster analysis was performed using Ward's method to group genotypes based on trait similarity. All statistical analyses were conducted using OPSTAT (Sheron *et al.*, 1998) ^[43], IBM SPSS Statistics v.27, and R software (packages: stats, factoextra, corplot) (Kassambara and Mundt 2016) ^[23].

3. Results

All the RILs and their parental lines were evaluated under two different environmental conditions, including a yield potential environment conducive to the expression of stress-free and heat heat-stressed environment where the environment had heat stress with an increase in temperature. Maximum, minimum temperature, and bright sunshine hours (in days) were presented in Table S1 and Fig. 1. An increasing trend of temperature was observed throughout the season, indicating the occurrence of heat stress during post-reproductive phase, especially in late-sown trials. Additionally, the maximum number of sunshine hours was recorded in April (7.8 hours), which showed an increase in heat units, shortened the crop cycles, and consequently had adverse effects on grain yield.

3.1 Impact of terminal heat stress on grain yield and its component traits

Analysis of variance (ANOVA) for stress-free (a) and heat-stressed (b) environments was shown in Table 1. The genotypes mean square (MS) was highly significant for all traits under both environments; however, most traits exhibited lower MS values under the heat-stressed environment, with exception of PH, ET, and NSS. On the other side, the mean square of variance due to blocks was found non-significant for all the traits under both environments except DA and GFD under heat-stressed environments. Table 2 presented the phenotypic variations and distributions of phenological and yield-related traits among RILs for. GYP and AGBYP were the most heat-sensitive traits among all the traits studied. The reduction score values of 37% and 30%, 21% and 23%, 34% and 27% were observed for WH 711 and WH 1021, respectively. GYP was recorded as 1050g and 660g for WH 711, 905g and 710g for WH 1021 under stress-free and heat-stressed environments, respectively. Likewise, GYP and AGBYP in RILs varied from 540-1220g and 217-900g; 1450-3500g and 900-2450g; 30.27-46.00% and 22.15-42.38% under stress-free and heat-stressed environments, respectively. On the contrary, HI for heat-tolerant parent (WH 1021) had a positive response under heat stress conditions with a negative (-2.11) reduction value.

Among phenological traits, both parents and population showed an average reduction of 5, 7, and 18 days in terms of DH, DA, and DPM stages under a heat stress environment. Consequently, the highest reduction percent was recorded for grain filling duration with 27.50%, 21.95%, and 24.26% in WH 711, WH 1021, and RILs population, respectively. For spike-related traits, 12-20% of the reduction was recorded for WH711 and RILs, whereas 0.89% to 10% reduction was observed for the tolerant parent WH1021. NSS showed the lowest reduction among them. Substantial phenotypic variation was observed among RILs across both environments, with greater reductions (22.22%) in productive tillers comparatively (Table 2).

3.2 Trait associations and yield contributing patterns

The phenotypic correlation revealed that GYP exhibited a positively significant association with spike related traits including SL (0.191** and 0.332**), NSS(0.256** and 0.468**)

and NGS(0.286** and 0.349**), yield component traits including AGBYP (0.802** and 0.808**), HI (0.395** and 0.732**) and other agronomic traits such as GFD (0.228** and 0.351**), NPTM (0.434** and 0.642**), TKW (0.230** and 0.286**) and SD (0.395** and 0.431**) under stress-free and heat-stressed environments, respectively (Fig. 2 and Table S3). The AGBYP also exhibited a significant and positive relationship with NPTM (0.311** and 0.534**), SL (0.280** and 0.238**), NSS (0.232** and 0.307**), NGS (0.299** and 0.269**), and SD (0.208** and 0.332**) under both conditions. Furthermore, HSV showed a significantly positive association with thousand kernel weight (0.594** and 0.601**). On the other hand, other remaining traits, i.e., DH, DA, and DPM, showed negative correlation with GYP (-0.414**, -0.513**, -0.368**, -0.486**, and -0.388, -0.210** respectively) except GFD (0.288** and 0.351**). TKW also showed a negative association with DH (-0.519** and -0.376**) and DA (-0.431** and 0.389**).

Path coefficient analysis revealed that the AGBYP contributed maximum (0.8814 and 0.8592) to GYP, followed by HI (0.5415 and 0.6257), TKW (0.2411 and 0.3413), HSV (0.1693), and NPTM (0.1512 and 0.1691) under both environments (Tables 3 and 4). Although direct effects on GYP via DA (0.3858) and HSV (0.1693) were positive, the overall correlation was negative among them in the yield potential environment. Likewise, the direct effects on GYP via GFD (-0.9228), SL (-0.0492), NSS (-0.0213), and SD (-0.4276) were negative under heat heat-stressed environment, but they had a positive correlation with GYP.

The traits, viz. NPTM (0.2097), SL (0.2800), NSS (0.2040), NGS (0.2925), TKW (0.1460) and SD (0.2267) contributed towards GYP through AGBYP under yield potential environment, whereas NPTM (0.8148), SL (0.6661), NSS (0.9546), NGS (0.9060), AGBYP (0.4220), HI (0.3033), TKW (0.5391), HSV (0.1833) SD (0.6137) contributed towards GYP via DA under heat stressed environment. Under stress-free conditions, GFD (0.1390) and SD (0.1805) contributed towards GYP via HI, whereas NPTM (0.3922, 0.2871), SL (0.2688, 0.2315), NSS (0.2901, 0.3117), NGS (0.2716, 0.2229), and TKW (0.1408, 0.2687) contributed towards GYP via AGBYP and HI. DH (0.2360), DA (0.5692), AGBYP (0.3159), and TKW (0.1708) also contributed positively towards GYP through GFD under heat heat-stressed environment.

2.3 Predictive modeling of grain yield

A simple regression model formula explained more than 99% (R^2) of the total variations of GYP for both environment, and the remaining less than 1% is probably due to the residual effects. The regression test showed that SD (104.51** and 63.735**), HI (22.46** and 17.113**), and AGBYP (0.349** and 0.309**) contributed significantly to GYP under both conditions, while HSV (37.39*) and NPTM (0.084*) were significant for both environments (Table 5). Multiple regression further predictive following equations for GYP:

Model for stress-free environment

GYP: - 823.7 - 0.205DH - 1.803DA + 1.154DPM - 1.534GFD - 0.063PH + 0.014NPTM + 0.714SL + 0.076NSSP + 0.217NGPS + 0.349AGBYPP + 22.461HI - 3.245TKW + 37.397HSV + 104.514SD

Model for heat heat-stressed environment

GYP: -623.76 + 0.455DH + 0.226DA - 0.654DPM + 0.970GFD - 0.059PH + 0.084NPTM + 0.387SL - 0.543NSSP +

$$0.029\text{NGPS} + 0.309\text{AGBYPP} + 17.113\text{HI} - 1.543\text{TKW} + 20.496\text{HSV} + 63.735\text{SD}$$

Stepwise regression analysis revealed that AGBYP (62.74%), HI (36.59%), NGS (0.02%), SD (0.02%) and DA (0.2%) played a significant role in influencing GYP under yield potential environment whereas, four variables, including AGBYP (62.40%), harvest HI (36.80%), SD (0.08%), NPTM (0.02%) were included into the model in form of most important variables under heat stressed environment (Table 6). According to the results, more than 99% of the total variation in GYP was ascribed to these aforementioned variables under both conditions. The rest of the variables were removed from the models due to their low relative contributions. Moreover, the regression analysis predicted following equations for GYP:

Model for stress-free environment

$$\text{GYP: } -803.384 + 0.3514\text{AGBYPP} + 22.7522\text{HI} + 0.2672\text{NGS} + 16.1568\text{SD} - 0.3364\text{DA}$$

Model for heat stress environment

$$\text{GYP: } -563.434 + 0.3100\text{AGBYPP} + 17.0128\text{HI} + 24.0709\text{SD} + 0.0800\text{NPTM}$$

3.4 Principal component analysis of yield-related traits

GYP and its associated traits were mapped on principal component (PC) axes to delineate the proportion of variance explained by each trait (Fig. 3 a and b). Out of the 15 axes of differentiation, only 5 PCs were found with >1 eigen values and cumulatively contributed for more than 70% of the total variations of GYP under both conditions (Supplementary Table S4). The first PC was moderately correlated with GYP (0.37 and 0.40), GFD (0.35 and 0.28), AGBYP (0.24 and 0.31) and HI (0.22 and 0.28) under both conditions, while TKW (0.35) and NPTM (0.26) had significant contribution only under stress-free and heat stressed environments, respectively (Supplementary Table S5). The PC-2 was correlated with SD (0.39 and 0.36) and NPTM (0.31 and 0.26) under both conditions, whereas, GYP (0.32), AGBYP (0.29) and DA (0.22) showed contribution towards this component under stress-free environment. Rest of three PCs cumulatively accounted for more than 25% of total variation with the highest contributions from DPM, GFD, NSS, HI, HSV and SD in different components under both conditions. Acute angles were also observed among GYP, AGBYP, NPTM and SD which depicted positive association among traits and their positive contribution towards both components under both conditions.

3.5 Multivariate clustering of RILs based on adaptive trait profiles

On the basis on the relative magnitude of distance, parents and two hundred RILs were grouped into six clusters under both environments (Fig. 4a and b). The intracluster distance ranged from 709.22 (IV) to 1311.39 (II) and 587.11 (VI) to 900.81 (IV) under stress-free and heat-stressed environments and indicating less diversity within cluster, whereas the inter-cluster distance varied from 862.71 (between cluster I and IV) to 1935.37 (between cluster V and VI) and 765.36 (between cluster I and III) to 1412.78 (between cluster II and V) under stress-free and heat-stressed environments, respectively (Table S6 and S7). Cluster III comprised of largest number of RILs (75) followed by cluster IV (33 entries) and cluster II (31 RILs) under a stress-free environment, whereas cluster IV was recorded with the maximum number of RILs (66), followed by cluster VI (44 RILs) and cluster III (39 RILs) under a heat-stressed

environment, respectively. Cluster V was comprised of 21 RILs and WH711 (agronomically superior parent) under a stress-free environment, and 9 RILs (15, 20, 33, 34, 59, 74, 100, 106, 117) with WH1021 (heat-tolerant parent) under a heat-stressed environment. This cluster can be categorized as heat-resilient high-yielding, supported by higher mean values for NPTM (138.45 and 131.85), NGS (59.45 and 49.43), GYP (1042.52 g and 760.05 g), AGBYP (2790.91 g and 2005 g), and SD (1.51 g/cc and 1.50 g /cc), respectively (Table S8 and S9).

4. Discussion

4.1 Genetic variability and environmental effects

The evaluation of a large RIL population (WH711 × WH1021) under contrasting thermal regimes provided valuable insights into wheat yield performance and adaptive trait expression under terminal heat stress. The highly significant genotypic variance for all studied traits indicated the presence of ample genetic variability, which is essential for identifying promising heat-resilient lines. Heat stress clearly harms wheat crop growth, development, total biomass, and potential grain yield (Zhang *et al.*, 2018) [49]. Yield reductions under terminal heat stress can largely be attributed to pollen sterility, impaired assimilate partitioning to developing spikes, and shortened grain filling duration (Ji *et al.*, 2010; Sinclair and Jamieson 2006; Sobhaninan *et al.*, 2019) [21, 44, 46]. The extent of trait reduction is influenced by both severity and duration of heat stress (Barnabás *et al.*, 2008) [5]. The meteorological profile confirmed exposure to elevated temperature, extended sunshine hours, and low precipitation during the post-anthesis period, which accentuated early senescence and led to yield losses (Fig. 1). In our study, substantial reductions were recorded for GYP, AGBYP, and HI, with the magnitude of loss varying between the parents and the RILs. The heat-susceptible parent (WH711) exhibited greater reductions compared with WH1021, reinforcing the utility of tolerant parents in broadening the genetic base of heat-resilient wheat (Table 2). The RILs showed a wide spectrum of responses, validating their suitability for dissecting adaptive traits under stress. Terminal heat advanced phenological development (reduced DH, DA, DPM, and GFD) and compressed the grain-filling window, which in turn reduced grain number and weight (Table 2). These results corroborate mechanistic studies that attribute yield loss under reproductive heat to pollen sterility, impaired endosperm development, and shortened assimilate accumulation (Farooq *et al.*, 2014; Zhang *et al.*, 2018) [15, 49].

4.2 Trait associations: correlation and path analyses

Correlation analysis demonstrated that the AGBYP consistently exhibited the strongest positive association with grain yield in both environments (Table S3). This is expected, as biomass accumulation underpins assimilate availability for grain filling (Gaju *et al.*, 2009) [17]. Other yield contributing traits, such as NPTM, HI, SL, and NGS, also correlated positively with yield (Fig. 2). These results were corroborated with earlier studies (Leilah and Al-Khateeb 2005) [28]. However, phenological traits such as DH, DA, and DPM generally showed negative associations with yield, likely reflecting an escape mechanism, whereby early-maturing genotypes partially avoid heat injury during grain filling. A negative association between DH and grain yield per meter has been reported earlier (Mansouri *et al.*, 2018) [29]. GFD also had a significant positive correlation with GYP. Heat-tolerant wheat genotypes had been reported with 38% longer GFD as compared to heat-susceptible genotypes (Ihsan *et al.*, 2016) [20]. Moreover, the grain filling rate is more

imperative than the grain filling period (Borrill *et al.*, 2015) [6]. The stressed plants may also have a higher grain filling rate, which, when combined with a shorter grain filling period, causes improper assimilate translocation and, as a result, lower yield production (Ihsan *et al.*, 2016) [20]. Interestingly, plant height showed contrasting associations across environments (negative under optimal, positive under heat), implying selection must consider the G×E context (Moghaddam 1998) [31]. Under stress, slightly taller plants may sustain higher biomass and lower plant area index to minimize moisture demand and reduce moisture loss through transpiration (Khadka *et al.*, 2020) [25]. The differential response of component traits with respect to grain yield could be attributed to environmental factors affecting plant growth (Asseng *et al.*, 2002) [3]. Path analysis further highlighted the direct importance of AGBYP and HI on grain yield, corroborating earlier reports that emphasized biological yield and partitioning efficiency as critical determinants of productivity under stress (Leilah and Al-Khateeb 2005) [28]. Traits such as NPTM, NGS, TKW, and SD also exerted appreciable direct or indirect contributions, making them reliable selection indices. The positive relation between PH and GYP under a heat-stressed environment indicated its direct significant role in the determination of biological yield. Although the direct effects of GFD, SL, NSS, and SD on GYP were observed to be negative under stressed conditions but overall, they had a positive correlation with GYP, suggesting that these traits were strongly affected by associated traits. The residual effect was very low, indicating that the traits under study are sufficient for explaining the variability in grain yield.

4.3 Predictive models and key determinants of yield

Regression and stepwise analyses revealed that AGBYP, HI, and SD were the most consistent predictors of grain yield under both environments, while NPTM, HSV, and TKW showed environment-specific effects. These predictors cumulatively explained over 99% of yield variation, underscoring their robustness for breeding applications (Tables 5 and 6). The overall results reflected the importance of AGBYP, HI, SD, and NPTM four variables under heat stress. These results reinforce the value of combining biomass accrual and partitioning efficiency with kernel traits in selection indices for heat resilience. Previous studies also indicated that the biological yield per unit, HI, thousand grain weight, and number of spikelets/m² are major contributors to variations in grain yield under a heat-stressed environment (Leilah and Al-Khateeb 2005; Msundi *et al.*, 2021; Saed-Moucheshi *et al.*, 2013; Sobhaninan *et al.*, 2019) [28, 32, 41, 46].

4.4 PCA insights, trait integration, and RILs selection

PCA reduced dimensionality to five major components (>70% variance) (Table S4 and S5) and highlighted coherent trait groupings: biomass and HI components aligned closely with GYP, while other components captured phenology and kernel attributes. Traits, DPM, GFD, NSS, GYP, HI, TKW, and SD under a stress-free environment and PH, SL, GYP, HSV, and SD under heat heat-stressed environment are the most important variables observed in different principal components, indicating a wide scope of improvement for heat tolerance. Acute vector angles among GYP, AGBYP, NPTM, HI, and NSS. NGS and SD again confirmed concerted positive effects on yield (Fig. 3). Previous studies also listed grain filling duration, thousand kernel weight, grain yield, biological yield and harvest index as the most important traits for yield contribution (Leilah and Al-Khateeb 2005; Munjal 2017; Muñoz-Sanz *et al.*, 2020) [28, 33, 34]. Multivariate clustering grouped both parental genotypes into different clusters under both environments, which evidently showed a significant source of genetic diversity and recombination phenomena in the segregating population. A total of six clusters were made with a lower intracluster distance and a higher intercluster distance, showing the potential opportunity of hybridization among diverse clusters to produce promising segregants for heat tolerance (Table S6). Nine RILs were grouped into cluster V along with WH1021, clearly exhibiting superior heat resilience and higher mean values for yield-related traits (GYP, AGBYP, NPTM, NGS, and SD) (Table S7- S9). Out of which, RIL 15, 20, and 100 were classified in the same cluster with an agronomically superior parent (WH 711) under a stress-free environment and a heat-tolerant parent (WH 1021) under a heat-stressed environment (Fig. 4). These results were matched with previous studies (Elahi *et al.*, 2020; Mecha *et al.*, 2017; Rajshree 2018) [14, 30, 37].

4.5 Breeding implications and selection strategy

Collectively, results indicate that selection for (i) robust biomass production (AGBYP), (ii) efficient partitioning to grain (HI), and (iii) compact, heavy kernels (SD, TKW) will most effectively increase yield under terminal heat (Table S10). Number of productive tillers (NPTM) also emerges as an important secondary target under heat stress. We recommend a trait-based selection index combining AGBYP, HI and SD (with environment-specific weightings) rather than sole reliance on plot yield. The identified heat-resilient cluster and specific RILs (e.g., RILs 15, 20, 100) provide immediate breeding material for crossing and further validation in multi-location trials.

Table 1: Analysis of variance of GYP and its component traits of wheat RILs under stress-free (a) and heat-stressed (b) environments

Traits	Df	DH	DA	DM	GFD	PH	ET	SPL	NSS	NGS	GYP	BY	HI	TGW	V	SD
Rep	1	9.61	17.22	3.42	5.29	0.49	29.16	0.9	2.682.74	11.78	976.56	62001	5.88	5.91	0.03	0.001
Trt	199	59.52**	56.11**	9.08**	48.52**	60.51**	840.01**	2.19**	4.16**	99.45**	33514.85**	233386.81**	23.16**	22.80**	0.25**	0.03**
Blocks	8	2.18	6.94	1.38	6.53	14.2	8.05	0.43	1.4	19.07	3620.06	62419.12	4.57	1.89	0.04	0.01
Error	191	8.98	9.92	2.23	10.72	12.6	99.22	0.44	1.36	21.03	1998.07	29658.88	5.18	2.95	0.03	0.004

Traits	Df	DH	DA	DM	GFD	PH	ET	SPL	NSS	NGS	GYP	BY	HI	TGW	V	SD
Rep	1	6.76	6.25	0.56	3.06	37	13.69	0.06	2.83	7.93	1176.49	3025	1.42	4.48	0.002	0.003
Trt	199	14.02**	16.02**	4.74**	18.04**	92.95**	1061.85**	1.58**	4.96**	62.93**	22810.12**	109739.20**	23.64**	17.76**	0.25**	0.03**
Blocks	8	5.03	6.23*	1.27	11.30**	20.23	11.88	0.49	1.13	16.02	4200.5	9581.36	12.68	4.87	0.08	0.01
Error	191	3.37	3.11	1.35	4.16	23.79	59.64	0.43	1.2	14.29	3023.98	12868.19	5.93	2.54	0.04	0.01

Abbreviations: DH = Days to heading, DA = Days to anthesis, DPM = Days to physiological maturity, GFD = Grain filling duration, PH = Plant height, NPTM = Number of productive tillers per meter, SL = Spike length, NSS = Number of spikelets/spike, NGS = Number of grains/spike, GYP = Grain yield/plot, AGBYP = Above ground biological yield/plot, HI = Harvest index, TKW = Thousand kernel weight, HSV = Hundred seed volume, SD = Seed density

Table 2: Mean and range values of GYP and its component traits of wheat RILs and parents under different environments

Traits	Under stress-free environment				Under heat-stressed environment			
	WH711	WH1021	RILs Average± SE(m)	RILs Range	WH711	WH1021	RILs Average± SE(m)	RILs Range
DH	95.50	91	97.74±0.55	86-110	90	86	90.29±0.28	81-99
DA	102.50	99	104.27±0.52	91-116	95	92	96.24±0.29	85-105
DPM	142.50	140	141.28±0.18	136-147	124	122	124.27±0.14	120-130
GFD	40	41	37.01±0.81	24-43	29	32	28.03±0.56	18-35
PH	92.00	104.50	103.49±1.82	86-117	81.93	103.07	92.77±2.32	71.67-107.67
NPTM	167.50	160	127.28±1.82	76-204	136	150	99.00±2.32	62-160
SL	11.11	12.11	11.49±0.31	8.4-15	9	10.87	9.75±0.29	7-13
NSS	21.33	20.18	21.13±0.31	15-23	18	20	18.42±0.36	13-21
NGS	65	57	57.57±0.93	40-83	52	53.50	46.02±0.83	27-69
GYP	1050	905	855.05±4.43	540-1220	660	710	563.99±4.50	217-900
AGBYP	2350	2200	2369.75±7.02	1450-3500	1630	1690	1731.00±5.59	900-2450
HI	44.68	41.14	36.25±0.57	30.27-46.00	40.49	42.01	32.47±0.64	22.15 - 42.38
TKW	44.32	42.01	40.87±0.53	27.52-46.00	40.10	41.67	38.48±0.49	25.20 - 42.00
HSV	3.40	3.36	3.19±0.20	2.20-4.30	3.25	3.35	3.12±0.20	1.60 - 4.20
SD	1.27	1.25	1.29±0.11	1.00-1.93	1.23	1.24	1.25±0.11	0.92-1.84

Abbreviations: DH = Days to heading, DA = Days to anthesis, DPM = Days to physiological maturity, GFD = Grain filling duration, PH = Plant height, NPTM = Number of productive tillers per meter, SL = Spike length, NSS = Number of spikelets/spike, NGS = Number of grains/spike, GYP = Grain yield/plot, AGBYP = Above ground biological yield/plot, HI = Harvest index, TKW = Thousand kernel weight, HSV = Hundred seed volume, SD = Seed density

Table 3: Path coefficient analysis under a stress-free environment. Direct effects are shown bold and diagonally, whereas indirect effects are off-diagonal.

Variables	Effects via														r with GY
	DH	DA	DPM	GFD	PH	NPTM	SPL	NS/S	NG/S	AGBY	HI	TKW	HSV	SD	
DH	-0.0129	0.3917	-0.0665	-0.2871	-0.0031	0.0002	-0.002	-0.0001	-0.0032	-0.2666	-0.1713	0.0799	-0.0509	-0.022	-0.414
DA	-0.01	0.3858	-0.0686	-0.2782	-0.0035	0.0002	-0.0009	-0.0001	-0.0027	-0.1926	-0.2026	0.068	-0.0433	-0.0194	-0.368
DPM	-0.0053	0.2388	-0.1609	-0.0162	-0.0022	0.0002	0.0003	-0.0001	-0.0027	-0.2421	-0.199	0.0453	-0.012	-0.0317	-0.3875
GFD	0.0037	-0.3903	0.0073	0.358	0.0029	-0.0001	0.0011	0.0001	0.0019	0.1096	0.139	-0.0558	0.0427	0.008	0.228
PH	-0.0034	0.1117	-0.0298	-0.0417	-0.0121	0.0002	0.0044	0.0001	0.0016	-0.0354	-0.1853	0.014	0.0089	-0.0262	-0.193
NPTM	0.0016	-0.0995	0.0198	0.0311	0.0024	0.1512	-0.0005	0.0002	-0.0003	0.2097	0.1092	0.0137	-0.0468	0.0422	0.434
SPL	0.002	-0.0263	-0.0039	0.0142	-0.0041	0.0001	0.0128	0.0001	0.0033	0.28	-0.0831	-0.0111	0.0057	0.0013	0.191
NS/S	0.0022	-0.0491	0.0225	0.029	-0.0009	-0.0003	0.0022	0.0009	0.002	0.204	0.042	-0.0257	-0.0132	0.0404	0.256
NG/S	0.003	-0.0752	0.031	0.0447	-0.0014	0	0.0031	0.0001	0.0139	0.2925	-0.0267	0.0161	-0.0069	-0.0083	0.286
AGBY	0.0039	-0.0843	0.0442	0.0164	0.0005	-0.0003	0.0041	0.0002	0.0046	0.8814	-0.0711	-0.0234	-0.0135	0.0393	0.802
HI	0.0041	-0.1444	0.0592	0.0443	0.0041	-0.0003	-0.002	0.0001	-0.0007	-0.1157	0.5415	-0.0273	-0.0188	0.0509	0.395
TKW	0.0073	-0.2646	0.0516	0.0116	0.0012	0.0001	0.001	0.0002	-0.0016	0.146	0.0047	0.2411	0.0046	0.0269	0.23
HSV	0.0039	-0.0986	0.0138	0.0903	-0.0006	0.0003	0.0004	-0.0001	-0.0006	-0.0703	-0.0602	-0.0872	0.1693	-0.1003	-0.14
SD	0.0019	-0.0791	0.028	0.0187	0.0021	-0.0003	0.0001	0.0002	-0.0008	0.2267	0.1805	-0.0248	-0.1111	0.1528	0.395

Residual Effect = 0.00006

Abbreviations: DH = Days to heading, DA = Days to anthesis, DPM = Days to physiological maturity, GFD = Grain filling duration, PH = Plant height, NPTM = Number of productive tillers per meter, SL = Spike length, NSS = Number of spikelets/spike, NGS = Number of grains/spike, AGBYP = Above ground biological yield/plot, HI = Harvest index, TKW = Thousand kernel weight, HSV = Hundred seed volume, SD = Seed density, r with GYP = Genotypic correlation with grain yield/plot

Table 4: Path coefficient analysis under a heat-stressed environment. Direct effects are shown bold and diagonally, whereas indirect effects are off-diagonal.

Variables	Effects via														r with GY
	DH	DA	DPM	GFD	PH	NPTM	SPL	NS/S	NG/S	AGBY	HI	TKW	HSV	SD	
DH	0.0097	-0.717	0.0736	0.7735	0.0111	-0.0209	0.0115	0.0067	0.0054	-0.4094	-0.2785	-0.1682	0.0338	0.1561	-0.513
DA	0.3797	-0.408	0.057	0.44	0.0101	-0.0182	0.0116	0.0067	0.0052	-0.3832	-0.612	-0.1706	0.0278	0.1684	-0.486
DPM	0.6099	-0.6254	0.2894	-0.2851	0.0177	-0.0154	0.0087	0.0093	0.0045	-0.1108	-0.1537	0.0107	-0.0706	0.1007	-0.21
GFD	0.236	0.5692	0.0894	-0.9228	-0.0009	0.0099	-0.0069	-0.0048	-0.0033	0.3159	0.0733	0.1708	-0.0623	-0.1129	0.351
PH	-0.2929	0.3045	-0.0566	-0.009	0.0907	0.0356	-0.0175	-0.0063	-0.0023	0.2357	0.1081	0.0462	-0.0396	-0.0084	0.388
NPTM	-0.7243	0.8148	-0.0645	-0.1327	-0.0218	0.1691	-0.0087	-0.0037	-0.0021	0.3922	0.2871	0.0174	0.1264	-0.2095	0.64
SPL	-0.5572	0.6661	-0.0509	-0.1296	-0.0323	0.0123	-0.0492	-0.0095	-0.0024	0.2688	0.2315	0.1137	-0.0824	-0.0486	0.33
NS/S	-0.753	0.9546	-0.0444	-0.2092	-0.0267	0.012	-0.0219	-0.0213	-0.0056	0.2901	0.3117	0.1019	0.0178	-0.1545	0.451
NG/S	-0.7661	0.906	-0.06	-0.1849	-0.0124	0.0085	-0.0072	-0.0071	0.0167	0.2716	0.2229	0.0492	0.0627	-0.1498	0.35
AGBY	-0.1695	0.422	-0.0376	-0.3393	-0.046	0.0396	-0.0154	-0.0072	-0.0053	0.8592	0.1834	0.0559	0.1027	-0.2123	0.83
HI	-0.0644	0.3033	-0.0711	-0.2556	-0.0157	0.0317	-0.0182	-0.0106	-0.006	0.2518	0.6257	0.1466	-0.0142	-0.1712	0.732
TKW	-0.1782	0.5391	0.0091	-0.4619	-0.0123	0.0035	-0.0164	-0.0064	-0.0024	0.1408	0.2687	0.3413	-0.3293	-0.0048	0.291
HSV	-0.155	0.1833	0.0392	-0.1101	-0.0069	-0.0167	-0.0078	0.0007	0.002	-0.1691	0.0171	0.2154	-0.5217	0.398	-0.132
SD	-0.4594	0.6137	-0.0553	-0.1975	-0.0015	0.0275	-0.0045	-0.0062	-0.0047	0.3457	0.203	0.0031	0.3936	-0.4276	0.43

Residual Effect = 0.01184

Abbreviations: DH = Days to heading, DA = Days to anthesis, DPM = Days to physiological maturity, GFD = Grain filling duration, PH = Plant height, NPTM = Number of productive tillers per meter, SL = Spike length, NSS = Number of spikelets/spike, NGS = Number of grains/spike, AGBYP = Above ground biological yield/plot, HI = Harvest index, TKW = Thousand kernel weight, HSV = Hundred seed volume, SD = Seed density, r with GYP = Genotypic correlation with grain yield/plot

Table 5: Multiple regression analysis of different morphological components under stress-free and heat-stressed environments

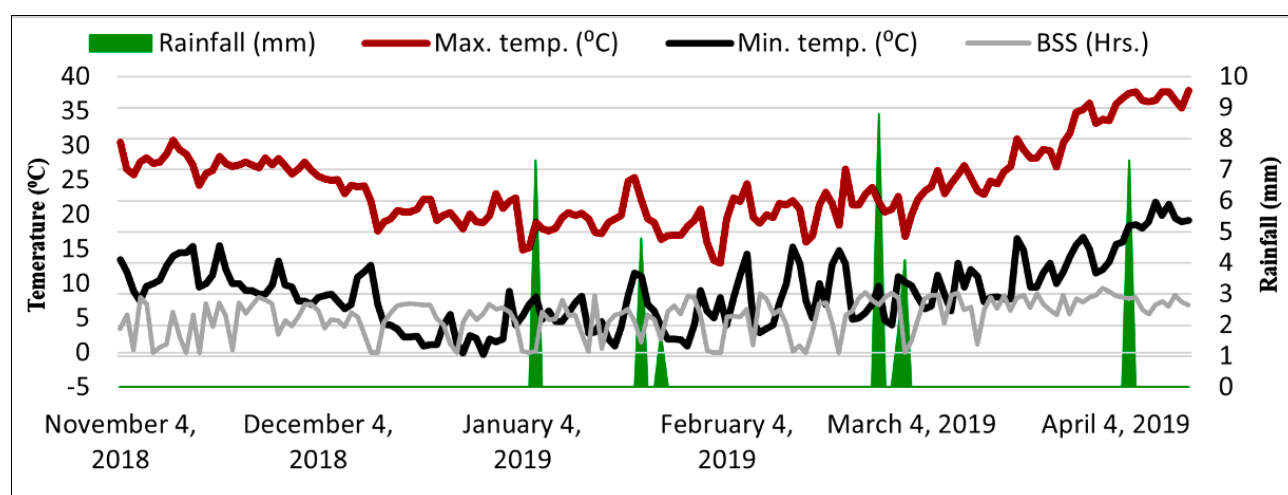
Traits	Stress-free environment	Heat stressed environment
Constant	-823.70±76.13**	-623.76±73.39**
DH	-0.21±0.29	0.46±1.08
DA	-1.80±7.39	0.23±7.39
DPM	1.15±7.39	-0.65±7.35
GFD	-1.53±7.39	0.97±7.34
PH	-0.06±0.15	-0.06±0.11
NPTM	0.01±0.04	0.08±0.04*
SL	0.71±0.78	0.39±0.83
NSS	0.08±0.54	-0.54±0.50
NGS	0.22±0.11	0.03±0.13
AGBYP	0.35±0.00**	0.31±0.00**
HI	22.46±0.28**	17.11±0.25**
TKW	-3.25±1.32*	-1.54±0.91
HSV	37.40±15.95*	20.50±11.11
SD	104.51±37.81**	63.74±25.59**

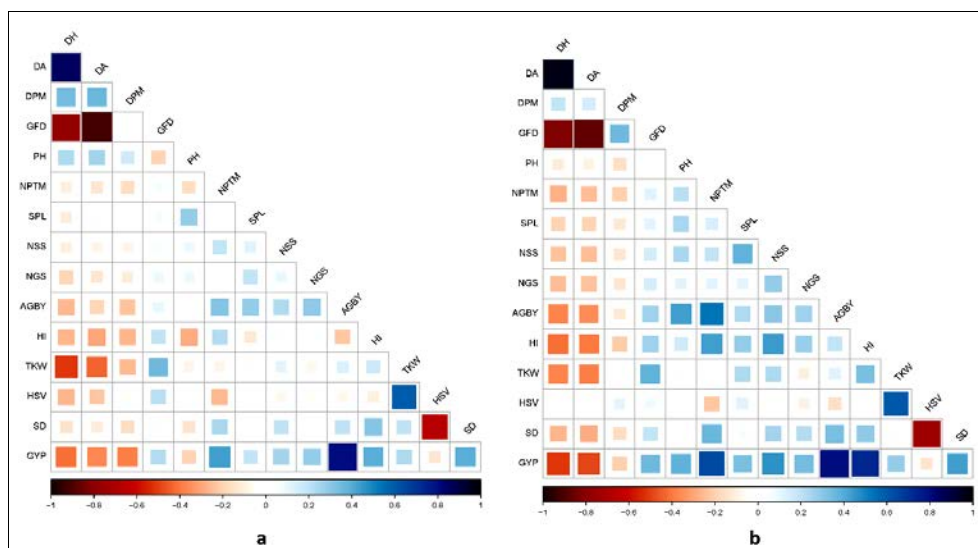
Abbreviations: DH = Days to heading, DA = Days to anthesis, DPM = Days to physiological maturity, GFD = Grain filling duration, PH = Plant height, NPTM = Number of productive tillers per meter, SL = Spike length, NSS = Number of spikelets/spike, NGS = Number of grains/spike, GYP = Grain yield/plot, AGBYP = Above ground biological yield/plot, HI = Harvest index, TKW = Thousand kernel weight, HSV = Hundred seed volume, SD = Seed density, *Significant at 5 per cent and ** at 1 per cent

Table 6: Stepwise regression model explaining variation in grain yield under contrasting temperature environments

Stress-free environment							
Step	Variables entered	Partial R^2	Model R^2	P value ER	Parameter estimate	Standard error	P value M
1	AGBYP	0.6274	0.6274	0.0000	0.3514	0.0025	0.0000
2	HI	0.3659	0.9933	0.0000	22.7522	0.2437	0.0000
3	NGS	0.0002	0.9934	0.0156	0.2672	0.1095	0.0241
4	SD	0.0002	0.9936	0.0138	16.1568	6.5028	0.0204
5	DA	0.0002	0.9937	0.0255	-0.3364	0.1495	0.0255
Heat stressed environment							
1	AGBYP	0.6240	0.6240	0.0000	0.3100	0.0033	0.0000
2	HI	0.3680	0.9920	0.0000	17.0128	0.1941	0.0000
3	SD	0.0008	0.9928	0.0000	24.0709	5.5848	0.0000
4	NPTM	0.0002	0.9930	0.0241	0.0800	0.0352	0.0241

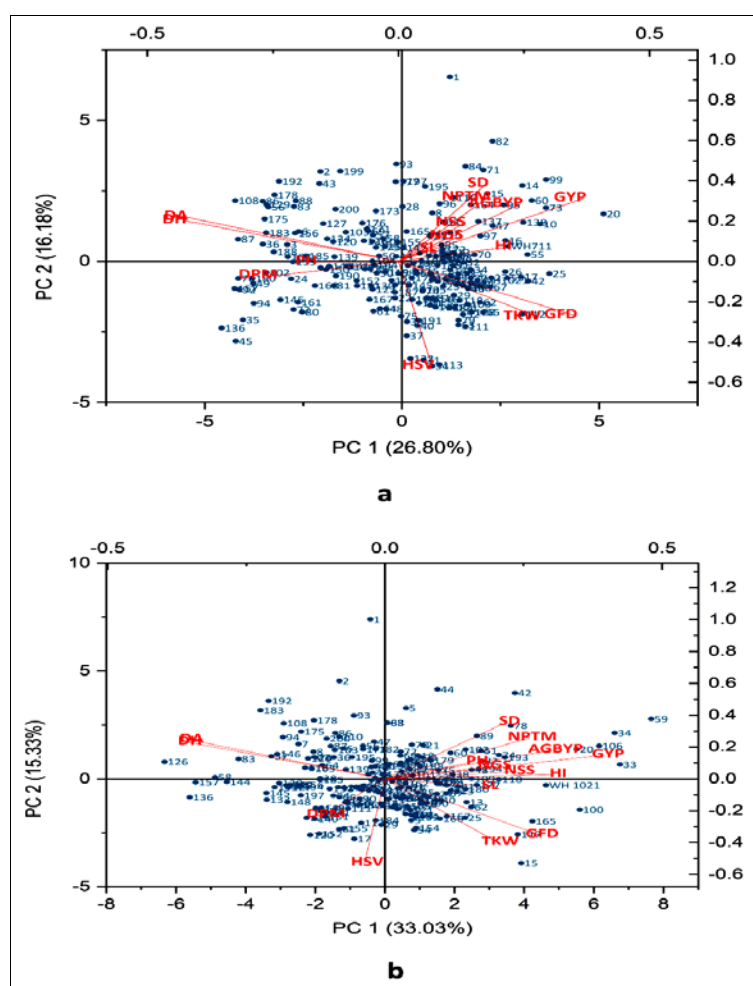
Abbreviations: DA = Days to anthesis, NPTM = Number of productive tillers per meter, NGS = Number of grains/spike, AGBYP = Above ground biological yield/plot, HI = Harvest index, SD = Seed density, R^2 : coefficient of determination, P value ER: P value for entered or removed variables, P value M: P value for final model.

**Fig 1:** Weekly weather parameters recorded during the 2018–2019 Rabi season at CCS Haryana Agricultural University, Hisar. Data includes maximum and minimum temperatures (°C), total weekly rainfall (mm), and weekly bright sunshine hours.



Abbreviations: DH = Days to heading, DA = Days to anthesis, DPM = Days to physiological maturity, GFD = Grain filling duration, PH = Plant height, NPTM = Number of productive tillers per meter, SL = Spike length, NSS = Number of spikelets/spike, NGS = Number of grains/spike, GYP = Grain yield/plot, AGBY = Above ground biological yield/plot, HI = Harvest index, TKW = Thousand kernel weight, HSV = Hundred seed volume, SD = Seed density

Fig 2: Pearson's correlation coefficients among grain yield per plot (GYP) and yield component traits in 200 wheat RILs under (a) stress-free (timely-sown) and (b) heat-stressed (late-sown) environments during 2018–19. Positive correlations are shown in blue, negative correlations in red, with intensity reflecting correlation strength.



Abbreviations: DH = Days to heading, DA = Days to anthesis, DPM = Days to physiological maturity, GFD = Grain filling duration, PH = Plant height, NPTM = Number of productive tillers per meter, SL = Spike length, NSS = Number of spikelets/spike, NGS = Number of grains/spike, GYP = Grain yield/plot, AGBY = Above ground biological yield/plot, HI = Harvest index, TKW = Thousand kernel weight, HSV = Hundred seed volume, SD = Seed density

Fig 3: "Which-won-where" biplot of 200 wheat RILs showing the distribution of genotypes and yield-related traits along the first two principal components (PC1 and PC2) under (a) stress-free (timely-sown) and (b) heat-stressed (late-sown) environments. Vectors represent the contribution and direction of individual trait.

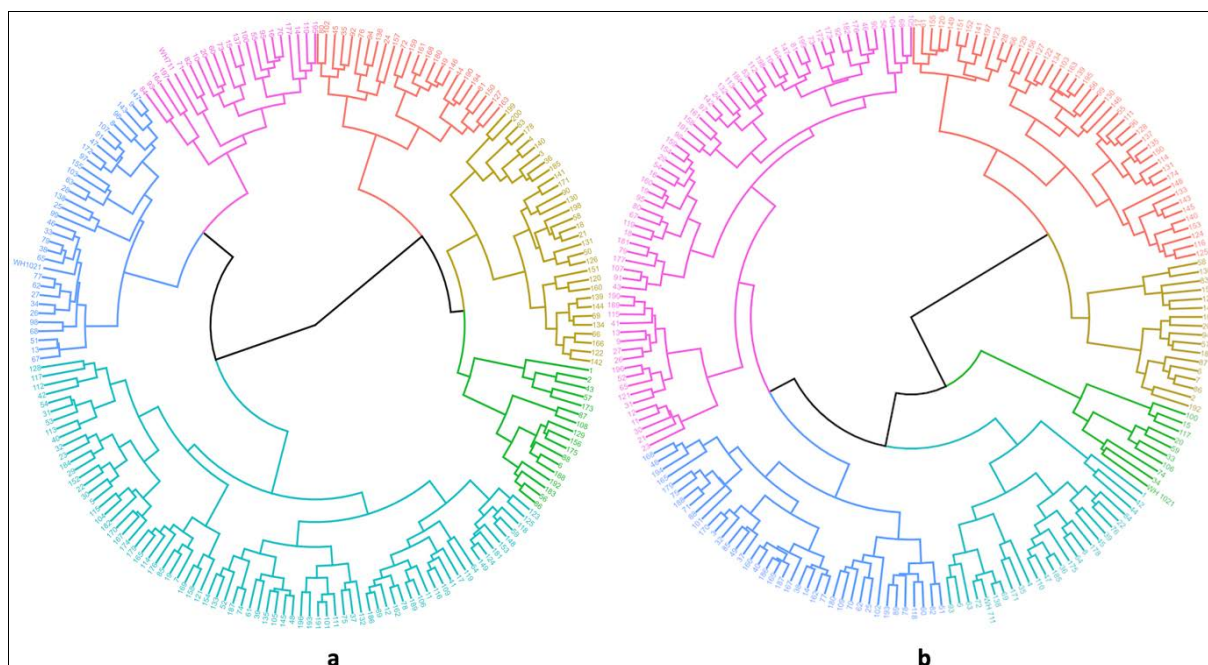


Fig. 4: Hierarchical cluster dendrogram of 200 wheat RILs and parental lines based on grain yield and associated adaptive traits under (a) stress-free and (b) heat-stressed environments. Clustering was performed using Ward's method, grouping genotypes with similar phenotypic responses and highlighting RILs with high yield potential and heat resilience.

Table S1: Weather parameter during crop season 2018-2019

Month	Min Temperature (°C)	Max Temperature (°C)	Bright Sunshine Hours	Rainfall (mm)
November 2018	11.7	27.9	4.2	0.0
December 2018	4.9	21.9	4.9	0.0
January 2019	5.2	19.2	4.6	13.8
February 2019	8.0	20.4	4.6	8.8
March 2019	10.5	26.5	6.8	6.0
April 2019	18.4	36.7	7.8	15.5

Table S2: List of parents used in the present study

S. No:	Genotype	Developing center	Pedigree
1	WH 711	CCSHAU, Hisar	ALD'S'HUAC/HD2285/3/HFW-17
2	WH 1021	CCSHAU, Hisar	NY0T95(GW 296)/SONAK
3	200 RILs	CCSHAU, Hisar	WH711/WH1021

Table S3: Phenotypic correlation coefficient among grain yield and component traits under yield potential (above diagonal) and heat stressed (below diagonal) environments

Traits	DH	DA	DPM	GFD	PH	NPTM	SPL	NSS	NGS	AGBY	HI	TKW	HSV	SD	GYP
DH	1	0.859**	0.347**	-0.774**	0.237**	-0.109	-0.114	-0.109	-0.186**	-0.257**	-0.264**	-0.519**	-0.262**	-0.144*	-0.414**
DA	0.970**	1	0.369**	-0.916**	0.251**	-0.137	-0.057	-0.09	-0.133	-0.189**	-0.293**	-0.431**	-0.223**	-0.115	-0.368**
DPM	0.193**	0.156*	1	0.036	0.163*	-0.169*	0.027	-0.081	-0.113	-0.233**	-0.267**	-0.256**	-0.056	-0.163*	-0.388**
GFD	-0.820**	-0.866**	0.352**	1	-0.199**	0.074	0.073	0.062	0.094	0.102	0.200**	0.353**	0.216**	0.053	0.228**
PH	-0.116	-0.091	-0.157*	0.012	1	-0.161*	0.283**	0.09	0.093	-0.014	-0.290**	-0.09	0.044	-0.147*	-0.193**
NPTM	-0.273**	-0.246**	-0.204**	0.13	0.214**	1	-0.011	0.196**	0.02	0.311**	0.229**	-0.089	-0.254**	0.249**	0.434**
SPL	-0.197**	-0.191**	-0.127	0.118	0.249**	0.148*	1	0.129	0.196**	0.280**	-0.125	0.059	0.027	0.002	0.191**
NSS	-0.234**	-0.246**	-0.138	0.160*	0.241**	0.196**	0.388**	1	0.108	0.232**	0.058	0.113	-0.077	0.203**	0.256**
NGS	-0.242**	-0.236**	-0.136	0.157*	0.12	0.122	0.118	0.284**	1	0.299**	-0.011	-0.08	-0.055	-0.007	0.286**
AGBY	-0.380**	-0.357**	-0.121	0.267**	0.431**	0.534**	0.238**	0.307**	0.269**	1	-0.226**	0.131	-0.071	0.208**	0.802**
HI	-0.413**	-0.391**	-0.212**	0.268**	0.168*	0.431**	0.284**	0.440**	0.272**	0.199**	1	0.175*	-0.11	0.310**	0.395**
TKW	-0.376**	-0.389**	0.021	0.383**	0.056	0.043	0.235**	0.237**	-0.105	0.127	0.334**	1	0.594**	0.202**	0.230**
HSV	-0.038	-0.027	0.111	0.081	0.035	-0.229**	0.13	-0.046	-0.098	-0.158*	-0.007	0.601**	1	-0.656**	-0.140*
SD	-0.262**	-0.285**	-0.165*	0.192**	0.019	0.366**	0.063	0.259**	0.229**	0.332**	0.297**	0.057	-0.743**	1	0.395**
GYP	-0.513**	-0.486**	-0.210**	0.351**	0.388**	0.640**	0.332**	0.468**	0.349**	0.808**	0.732**	0.286**	-0.131	0.431**	1

*Significant at 5 per cent and ** at 1 per cent

Table S4: Principal component, eigen value, percentage of total variation and cumulative percentage of morphological traits in the RILs of the cross WH711/WH1021 under stress-free and heat stressed environments

Principal Component	Stress-free environment			Heat stressed environment		
	Eigen Values	% Variance	Cumulative %	Eigen Values	% Variance	Cumulative %
1	4.02	26.80	26.80	4.95	33.03	33.03
2	2.43	16.18	42.98	2.30	15.33	48.36
3	1.84	12.26	55.24	1.72	11.46	59.82
4	1.19	7.92	63.16	1.12	7.47	67.29
5	1.08	7.20	70.36	1.00	6.65	73.95
6	0.92	6.15	76.51	0.91	6.09	80.03
7	0.86	5.74	82.25	0.84	5.61	85.64
8	0.78	5.19	87.44	0.62	4.16	89.80
9	0.68	4.55	91.99	0.60	4.01	93.81
10	0.58	3.87	95.86	0.53	3.56	97.37
11	0.45	2.99	98.95	0.34	2.29	99.66
12	0.16	1.08	99.93	0.03	0.22	99.89
13	0.01	0.05	99.98	0.01	0.09	99.97
14	0.00	0.02	100.00	0.00	0.03	100.00
15	0.00	0.00	100.00	0.00	0.00	100.00

Table S5: Factors loading of all studied characters in the RILs of the cross WH711/WH1021 under stress-free and heat stressed environments

Traits	Yield potential environment					Heat stressed environment				
	PC-1	PC-2	PC-3	PC-4	PC-5	PC-1	PC-2	PC-3	PC-4	PC-5
DH	-0.43	0.20	0.02	-0.16	0.02	-0.35	0.24	0.27	-0.13	-0.25
DA	-0.42	0.22	-0.05	-0.25	0.07	-0.35	0.25	0.30	-0.12	-0.20
DPM	-0.25	-0.07	-0.06	0.52	0.09	-0.10	-0.22	-0.17	-0.65	-0.52
GFD	0.35	-0.27	0.03	0.49	-0.04	0.28	-0.36	-0.37	-0.22	-0.08
PH	-0.17	0.00	-0.41	0.11	0.30	0.16	0.12	0.36	-0.37	0.24
NPTM	0.18	0.31	0.11	-0.04	-0.17	0.26	0.26	0.08	-0.16	0.21
SL	0.07	0.07	-0.48	0.16	0.17	0.18	-0.04	0.32	0.01	-0.27
NSS	0.12	0.19	-0.17	0.05	0.47	0.24	0.05	0.21	0.15	-0.46
NGS	0.13	0.13	-0.32	0.12	-0.44	0.20	0.08	0.05	0.18	-0.27
GYP	0.37	0.32	-0.11	-0.19	-0.10	0.40	0.16	0.14	-0.14	0.02
AGBYYP	0.24	0.29	-0.40	-0.16	-0.14	0.31	0.19	0.06	-0.42	0.19
HI	0.22	0.07	0.42	-0.08	0.05	0.31	0.04	0.18	0.23	-0.16
TKW	0.28	-0.27	-0.04	-0.33	0.45	0.21	-0.39	0.20	0.12	-0.13
HSV	0.07	-0.51	-0.21	-0.39	-0.01	-0.03	-0.52	0.42	-0.01	0.14
SD	0.17	0.39	0.23	0.15	0.43	0.22	0.36	-0.33	0.11	-0.26

Table S6: Inter cluster average distance among six clusters of RILs of the cross WH711/WH1021 under stress-free (above diagonal) and heat-stressed (below diagonal) environments

Clusters	I	II	III	IV	V	VI
I		1483.09	1327.41	862.71	1661.9	1286.5
II	1223.46		1459.69	1324.8	1489.79	1756.31
III	765.36	1364.61		1167.51	1638.45	1597.85
IV	782.54	1302.23	927.98		1503.91	1147.55
V	813.78	1412.78	704.04	972.42		1935.37
VI	1021.49	787.75	1163.90	1112.20	1210.99	

Table S7: Clustering of RILs and parents based on Euclidean distance under stress-free and heat-stressed environments

Clusters	Environment	Intra cluster Distance	Number of entries	Cluster members
I	Stress-free	1016.11	17	1, 2, 6, 43, 56, 57, 86, 87, 88, 108, 129, 156, 173, 175, 183, 188, 192
	Heat-stressed	625.10	26	1, 4, 5, 8, 22, 35, 36, 39, 42, 44, 45, 47, 63, 64, 69, 72, 76, 84, 93, 110, 138, 171, 175, 178, 185, WH 711
II	Stress-free	1311.39	31	3, 4, 18, 21, 36, 50, 58, 66, 69, 83, 90, 120, 122, 126, 130, 131, 134, 139, 140, 141, 142, 144, 151, 160, 166, 171, 178, 185, 198, 199, 200
	Heat-stressed	807.96	17	2, 6, 7, 57, 58, 83, 86, 87, 94, 108, 126, 136, 144, 157, 183, 192, 200
III	Stress-free	1300.28	75	5, 7, 11, 12, 17, 19, 22, 23, 29, 30, 31, 32, 37, 39, 40, 41, 42, 48, 52, 53, 54, 59, 61, 64, 74, 75, 78, 85, 89, 101, 104, 105, 106, 109, 111, 112, 113, 114, 115, 116, 117, 118, 119, 121, 123, 124, 125, 128, 132, 133, 135, 145, 148, 149, 152, 153, 154, 158, 162, 165, 167, 169, 170, 174, 176, 179, 181, 182, 184, 186, 187, 189, 191, 193, 196
	Heat-stressed	664.55	39	3, 14, 25, 32, 37, 38, 40, 48, 49, 51, 60, 62, 70, 71, 75, 77, 78, 82, 85, 88, 89, 101, 102, 109, 118, 162, 165, 166, 167, 168, 169, 170, 179, 180, 186, 187, 188, 193, 194
IV	Stress-free	709.22	33	8, 9, 13, 25, 26, 27, 28, 33, 34, 38, 46, 47, 51, 62, 63, 65, 67, 68, 77, 79, 91, 96, 97, 98, 99, 103, 107, 143, 147, 155, 172, 138, WH1021

	Heat-stressed	900.81	66	9, 10, 11, 12, 13, 16, 18, 19, 21, 23, 24, 26, 27, 29, 30, 31, 41, 43, 46, 50, 52, 53, 54, 65, 67, 68, 73, 79, 80, 81, 90, 91, 92, 95, 97, 98, 104, 105, 107, 112, 113, 115, 119, 121, 132, 142, 147, 154, 158, 159, 160, 161, 164, 172, 173, 176, 177, 181, 182, 184, 189, 190, 191, 196, 198, 199
V	Stress-free	1279.36	22	10, 14, 15, 16, 20, 55, 60, 70, 71, 73, 82, 84, 93, 95, 100, 110, 137, 164, 177, 195, 197, WH711
	Heat-stressed	709.42	10	15, 20, 33, 34, 59, 74, 100, 106, 117, WH 1021
VI	Stress-free	922.06	24	24, 35, 44, 45, 49, 72, 76, 80, 81, 92, 94, 102, 127, 136, 146, 150, 157, 159, 161, 163, 168, 180, 190, 194
	Heat-stressed	587.11	44	17, 28, 55, 56, 61, 66, 96, 99, 103, 111, 114, 116, 120, 122, 123, 124, 125, 127, 128, 129, 130, 131, 133, 134, 135, 137, 139, 140, 141, 143, 145, 146, 148, 149, 150, 151, 152, 153, 155, 156, 163, 174, 195, 197

Table S8: Mean value of different clusters for all studied traits in the RILs of the cross WH711/WH1021 under stress-free environment

Traits Clusters	DH	DA	DPM	GFD	PH	NPTM	SL	NSS	NGS	GYP	AGBYP	HI	TKW	HSV	SD
I	105.62	110.88	143.47	32.59	103.06	129.29	10.81	21.68	57.96	786.00	2229.41	35.49	34.63	2.62	1.33
II	100.53	107.48	140.84	33.35	106.55	121.76	11.86	21.64	57.38	826.42	2433.06	34.10	39.47	3.14	1.26
III	94.69	101.62	141.57	39.95	103.88	125.33	11.80	21.15	58.34	859.27	2438.67	35.40	42.43	3.42	1.25
IV	94.85	100.88	139.74	38.86	101.01	142.20	11.10	21.14	58.34	920.08	2266.67	40.65	41.68	3.22	1.29
V	94.91	101.70	140.05	38.34	101.56	138.45	11.57	21.61	59.45	1042.52	2790.91	37.63	42.11	2.80	1.51
VI	104.27	110.40	142.44	32.04	103.40	111.38	10.99	19.50	52.65	676.73	1919.79	35.45	40.20	3.26	1.24

Table S9: Mean value of different clusters for all studied traits in the RILs of the cross WH711/WH1021 under heat-stressed environment

Traits Clusters	DH	DA	DPM	GFD	PH	NPTM	SL	NSS	NGS	GYP	AGBYP	HI	TKW	HSV	SD
I	92.35	98.56	123.31	24.75	99.76	109.85	9.71	18.59	46.5	581.79	1785.58	32.70	37.03	2.99	1.27
II	93.65	100.03	124.71	24.68	88.87	89.24	9.10	16.52	43.95	451.5	1558.82	28.88	33.06	2.79	1.21
III	89.04	94.97	124.05	29.08	96.28	120.12	10.01	19.12	45.53	670.5	1937.05	34.73	39.72	3.22	1.24
IV	89.82	95.72	124.42	28.70	92.61	90.97	9.98	18.98	48.25	572.39	1731.82	33.2	39.78	3.26	1.23
V	83.85	89.15	123.95	34.80	93.84	131.85	10.00	19.12	49.43	760.05	2005.00	38.01	39.6	2.67	1.50
VI	90.98	96.84	124.64	27.80	87.02	84.2	9.41	17.45	43.14	450.85	1495.45	30.21	38.25	3.15	1.22

Table S10: Wheat traits identified as crucial in grain yield with each of the used statistical techniques

Wheat Traits	Correlation	Path analysis	Multiple regression	Stepwise regression	PCA	Cluster analysis
DH						
DA						
DPM		√				
GFD	√	√				
PH	√				√	
NPTM	√	√	√	√	√	√
SL	√					
NSS	√					
NGS	√					√
AGBYP	√	√	√	√	√	√
HI	√	√	√	√	√	
TKW	√	√			√	
HSV					√	
SD	√		√	√	√	√

5. Conclusion

This study revealed that terminal heat stress significantly reduces wheat yield through shortened grain filling, decreased biomass, and limited assimilate partitioning. Nonetheless, considerable genetic variability among RILs enabled the identification of adaptive traits associated with stress resilience. Multivariate analyses emphasized biological yield, harvest index, and reproductive traits such as thousand-kernel weight, grains per spike, and seed density as key determinants of yield across environments. Predictive modelling further confirmed the role of biomass production and efficient partitioning in yield stability. These findings underscore that breeding strategies should prioritize trait-based selection indices beyond yield alone, focusing on biomass accumulation, partitioning efficiency, and reproductive resilience to mitigate the effects of

rising temperatures. Several RILs including 15, 20 and 100 clustered with heat-tolerant parent WH1021, offering valuable germplasm for breeding climate-resilient wheat cultivars. While these findings are based on replicated field trials and comprehensive trait measurements, limitations include evaluation at a single site/season and reliance on late sowing as the sole heat-stress treatment. Future studies should validate promising RILs across multiple environments and years, integrate physiological/biochemical markers and high-throughput phenomics, and employ genomic approaches (QTL mapping, GWAS, genomic selection) to accelerate the identification and deployment of heat-adaptive alleles. Such steps will enhance the translational value of these trait-based insights for climate-resilient wheat improvement.

Data Availability Statement

The data supporting the findings have been provided in supplementary information.

Conflict of Interest

There authors no conflict of interest.

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Author Contributions

All authors contributed equally to the study and approved the final manuscript.

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