



# Assessment of heat tolerance indices and multivariate approaches for screening chickpea (*Cicer arietinum* L.) genotypes under field stress conditions

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## Abstract

Heat stress significantly affects chickpea growth, development, and productivity, making the identification of tolerant genotypes a key breeding priority. In the present study, thirty desi chickpea genotypes were evaluated over two years (2021–2022 and 2022–2023) in two different environments: timely sowing (non-stress) and late sowing (heat stress). A randomised complete block design (RCBD) with three replications was used to set up the experiments. Twelve parameters of heat tolerance Indices (TOL, STI, SSPI, YI, YSI, RSI, MP, GMP, HM, MRP, PYR, and HSI) and grain yield were analysed through ANOVA, correlation analysis, principal component analysis (PCA), biplot visualization, and hierarchical clustering. There was notable genotypic variance across all indices, with grain yield under stress (Ys) showing positive associations with STI, MP, YSI, GMP, HM, and YI, but negative associations with TOL, SSPI, RSI, and PYR. Strong interrelationships were recorded among MP, GMP, and YI, confirming their robustness as selection tools. PCA explained 99.28% of the total variance with two principal components, of which PC1 (66.64%) was strongly associated with Ys, YI, YSI, HM, and MRP, thereby identifying BRCHT-1, BRCHT-13, BRCHT-29, and IPC 11–20 as highly tolerant and stable genotypes. In contrast, BRCHT-20 and BRCHT-28 exhibited high values for TOL, SSPI, and PYR, reflecting susceptibility. Biplot analysis confirmed positive associations of Yp and Ys with MP, GMP, HM, and STI, while negative associations were evident with susceptibility indices. Hierarchical clustering grouped genotypes into five distinct clusters, with Cluster 2 showing superior tolerance (high Ys, STI, GMP, HM, and YSI), while Clusters 1 and 5 contained the most susceptible lines. Overall, this integrated analysis demonstrates that MP, GMP, HM, STI, and YSI are the most reliable indices for screening heat-tolerant chickpea genotypes, with BRCHT-1, BRCHT-13, BRCHT-18, BRCHT-29, IPC 11–20, and Sabour Chana-02 emerging as the most resilient candidates for future breeding programs.

**Keywords** Heat tolerance · Chickpea · PCA biplot analysis · Stress tolerance indices · Yield

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## Introduction

Chickpea (*Cicer arietinum* L.) has been recognised the most significant pulse crop owing to its dual role in agricultural sustainability and human nutrition. It ranks as the 2nd most widely consumable legumes worldwide, following common beans (Jha et al., 2015). It provides an affordable and sustainable source of proteins, carbohydrates, and essential micronutrients, making it a vital component of food and nutritional security, particularly in regions where vegetarian diets dominate. Beyond its dietary importance, chickpea contributes to soil fertility through biological nitrogen fixation, thereby supporting sustainable cropping systems.

Globally, chickpeas are cultivated on 14.84 million hectares, yielding yearly production of around 11.5 million tonnes, with an average of 1016.3 kg/ha (FAOSTAT, 2023). India is the largest producer, accounting for nearly two-thirds of the global area and output, with 11.08 million tonnes harvested from 10.94 million hectares in 2020 (FAOSTAT, 2023). Despite this dominance, India continues to face a demand–supply gap, as chickpea alone contributes nearly half of the nation’s total pulse production (13.12 Mt in 2021–22). At the state level, Bihar contributes only ~2.6% to national output, cultivating chickpea on just 0.057 million hectares. However, the presence of vast rice fallows covering over one million hectares provides immense opportunities for horizontal expansion of chickpea cultivation by introducing high-yielding, stress-tolerant, and high-yielding varieties.

Despite its nutritional and economic significance, chickpea production remains constrained by abiotic stresses, with heat stress emerging as the most critical under ongoing climatic change (Choudhary et al., 2024; Kumar et al., 2023). Projections suggest that global chickpea yields may decline by approximately 53 kg/ha by the end of the 21st century with a temperature rise of 1–4 °C (Driedonks et al., 2016). Elevated temperatures, particularly during delicate reproductive stages like flowering and pod filling, over 35 °C, accelerate crop maturity and disrupt fertilization, ultimately leading to substantial yield losses (Devasirvatham et al., 2012; Jha et al., 2017). Late-sown chickpeas, which often face terminal heat stress, are especially vulnerable, highlighting the urgent need for genetic solutions to this challenge.

Developing heat-resilient chickpea cultivars is critical to sustaining production under changing climatic conditions, enabling expansion into non-traditional growing areas, and providing for the dietary requirements of the world’s constantly expanding population. To evaluate crop performance under stress, several selection indices have been proposed, ranging from simple measures such as mean productivity (MP) and tolerance index (TOL) (Rosielle & Hamblin, 1981) to more comprehensive indices like the heat susceptibility index (HSI) (Fischer & Maurer, 1978) and stress tolerance index (STI) (Fernandez, 1992). Breeders may identify genotypes that are stable and heat-tolerant by using these stress tolerance parameters, which combined yield performance under stress and non-stress situations. Their use is particularly valuable for environments like rice fallows, where late sowing exposes chickpea to high terminal temperatures (Table S3).

The increasing incidence of heat stress in chickpea-growing regions, it is hypothesized that tolerance-based selection indices can effectively identify heat-resilient genotypes capable of maintaining yield stability across environments.

The present investigation’s objective was to assess the performance of diverse chickpea genotypes under contrasting environments i.e., normal and heat-stressed environments; apply tolerance-based selection indices to identify stable and heat-resilient genotypes. Identifying and selecting chickpea genotypes that exhibit superior heat-tolerance indices enables breeders to deliver cultivars that can reliably thrive in the brief, high-temperature residual moisture window that occurs after the rice season. This directly helps in bringing rice fallow lands under chickpea cultivation, thereby expanding area, stabilizing yields, and improving profitability in Northern Eastern Plains Zone (NEPZ).

## Materials and methods

### Experimental material and environment

Systematic evaluation for heat tolerance in chickpea was initiated in 2013–14 with the screening of ~300 genotypes. This long-term effort culminated in the release of Sabour Chana-2 in 2020 a high-yielding, heat-tolerant variety specifically recommended for late-sown conditions (second fortnight of December). through multi-year testing under both normal and late-sown environments, the initial pool of 300 genotypes was progressively narrowed to 55 promising entries, based on yield performance and yield-contributing traits under late sowing. From this subset, 30 diverse genotypes were selected for the present study, ensuring representation of a wide genetic base (Figure S1; Table S1). The experimental material was sourced from major institutions: IIPR (Kanpur), JNKVV (Jabalpur), IARI (New Delhi), DRPCA (Dholi), GBPUAT (Pantnagar), and BAU (Sabour). Among these, PG-186 and Sabour Chana-2 were included as heat-tolerant checks to provide robust benchmarks for comparison.

The experiment was conducted at the area specified to pulse Sect. (25°15'40" N and 87°2'42" E longitude), Bihar agricultural university, Sabour, Bhagalpur, which is situated 46 m above mean sea level and comes under Agro-climatic zone No. IIIA of the state of Bihar. The fields had a sandy loam soil that was productive, well-drained, and had a pH range of 7–7.5. The trials were conducted consecutively for two cropping years. Each year, two trials were grown; one under timely sown condition (November, 15 & 17), and the second under late sown condition (December, 18 & 20) conditions during *Rabi* cropping season 2021–2022 and 2022–23.

The study was conducted using a Randomised Complete Block Design (RCBD) with three replications to reduce experimental error and improve the reliability of the results. Every genotype was grown on a 1.2 m<sup>2</sup> plot with two rows,

each measuring 4 m in length, for each replication. To raise a healthy crop, best agronomic inter-cultural practices were followed on a regular basis.

## Environmental evaluation

Meteorological data of the two-year crop-growing period (November to April) were collected from the BAU, Sabour, Bhagalpur, Meteorological Department (Figure S2; Table S2). During the anthesis stage, the highest temperature recorded for timely sown genotypes in 2021–2022 was 27.8 °C, while for late sown genotypes, the temperature reached 32.8 °C. During stress conditions, the reproductive phase experienced a temperature increase of nearly 5 °C, which hastened flowering and maturity while significantly reducing grain yield. However, in the year 2022–2023 experiment laid under timely sown genotypes experienced a maximum temperature of 28.2 °C, relative humidity of 93%, 8.0 h of bright sunshine, and zero (mm) rainfall during the anthesis stage. In contrast, stressed genotypes experienced (late-sown) a maximum temperature of 33.5 °C, relative humidity of 89%, 7.3 h of sunshine, and zero (mm) rainfall during the reproductive stage.

Figure S2 and Table S2a & b present the weekly minimum and maximum temperatures (°C), relative humidity (morning and evening), bright sunshine hours, and rainfall (mm) recorded at the experimental site during the chickpea growing season. Notably, temperatures exceeding 30 °C in March coincided with the anthesis and pod-filling stages of late-sown genotypes, adversely affecting their performance.

After crop maturity, each genotype was harvested plot-wise, and grain yield (kg/ha) was recorded. Stress indices were estimated using both the mean yield of all genotypes under normal and late sowing ( $X_p$ ,  $X_s$ ) and the genotype-specific yields recorded under normal ( $Y_p$ ) and late sowing ( $Y_s$ ) conditions. Based on these parameters, the heat tolerance indices were determined using the formulas as in Table S3.

Stress tolerance indices were calculated in Microsoft Excel, while all advanced statistical analyses were conducted in R. Analysis of Variance (ANOVA) quantified genotypic differences across environments, and correlation analysis measured relationships among yield traits and stress indices. Multicollinearity analysis was performed to assess whether the heat tolerance indices could be reliably used together in regression-based models and multivariate analyses without causing redundancy or instability in parameter estimation. Principal Component Analysis (PCA) was used to reduce dimensionality and identify major patterns in genotype performance, with PCA biplots visualised in R to illustrate associations among genotypes and indices. Hierarchical Cluster Analysis further grouped genotypes

based on similarity in their stress responses, enabling the identification of tolerant and susceptible clusters (details of the R packages are provided in Supplementary Table S12). Together, these analytical tools provided a comprehensive and robust assessment of genotype behaviour under heat stress.

## Results

In this study, grain yield and stress indices of 30 chickpea genotypes were evaluated under two contrasting environments—timely sown (non-stress) and late sown (heat stress). Under late sowing, a yield reduction was observed due to exposure to higher temperatures ( $\approx 5$  °C above timely sown conditions) during critical stages such as anthesis and pod filling. To identify heat-tolerant genotypes, twelve stress indices were employed: heat susceptibility index (HSI), relative stress index (RSI), tolerance index (TOL), mean productivity (MP), yield stability index (YSI), harmonic mean (HM), stress tolerance index (STI), geometric mean (GMP), yield index (YI), mean relative performance (MRP), stress susceptibility percentage index (SSPI), and percent yield reduction (PYR). The primary objective of this investigation was to identify and characterize heat-tolerant chickpea genotypes based on their yield performance and stress indices. The use of ANOVA, PCA, correlation coefficients, biplots, and cluster analysis suggested a comprehensive and multidimensional approach to analysing the data.

### Combined analysis of variance of grain yield and associated stress indices

Results of the combined ANOVA indicated that heat stress imposed by late sowing had a significant impact on the growth, developmental traits, and grain yield of chickpea genotypes. To minimize seasonal variability, data were averaged across two years, thereby providing a more reliable and consistent assessment of genotype performance. The yield under timely and heat stress scenarios, together with all heat tolerance parameters indicates statistically significant variance ( $P < 0.001$ ) across chickpea genotypes. This is a key finding that highlights the importance of genotype selection based on their response to both normal and stress environments (Table 1). Significant variances in stress behaviours seen in conditions of various chickpea-growing seasons serve as evidence of the association between genotype and environment. Furthermore, a significant genotype  $\times$  environment interaction was observed, indicating that chickpea genotypes exhibited distinct responses to the two sowing conditions in terms of grain yield and associated traits.

**Table 1** Pooled ANOVA for yield (Yp, Ys) and heat tolerance indices across chickpea genotypes under timely and late sown environments

Category	Trait / index	Source of variation	DF	Mean sum of square (MSS)	Sig-nificance	
Yield	Yp (Timely)	Replication	2	1027.64	ns	
		Genotype	29	195787.8	**	
		Residual	58	16758.52	–	
	Ys (Late)	Replication	2	6418.36	ns	
		Genotype	29	245 123.70	**	
		Residual	58	10 183.60	–	
Stress susceptibility	HSI	Replication	2	7.4	ns	
		Genotype	29	1.27	**	
		Residual	58	0.0004	–	
	PYR	Replication	2	6.94	ns	
		Genotype	29	580.63	**	
		Residual	58	10.52	–	
	SSPI	Replication	2	3.14	ns	
		Genotype	29	158.9	**	
		Residual	58	3.88	–	
Tolerance / stability	TOL	Replication	2	4047.3	ns	
		Genotype	29	222 841.40	**	
		Residual	58	5425.6	–	
	YSI	Replication	2	0.0006	ns	
		Genotype	29	0.581	**	
		Residual	58	0.001	–	
	RSI	Replication	2	0.1821	ns	
		Genotype	29	265 761.00	**	
		Residual	58	0.004	–	
	Productivity	MP	Replication	2	2711.2	ns
			Genotype	29	164 745.40	**
			Residual	58	12 114.70	–
GMP		Replication	2	3178.6	ns	
		Genotype	29	171 563.60	**	
		Residual	58	11 816.50	–	
HM		Replication	2	3674.7	ns	
		Genotype	29	180 210.20	**	
		Residual	58	11 560.80	–	
MRP		Replication	2	0.0001	ns	
		Genotype	29	0.094	**	
		Residual	58	0.0017	–	
Comprehensive	STI	Replication	2	0.0026	ns	
		Genotype	29	0.143	**	
		Residual	58	0.0124	–	
	YI	Replication	2	0.003	ns	
		Genotype	29	1130	**	
		Residual	58	0.004	–	

\*\* = significant at  $p < 0.01$ 

ns = non-significant

Full distributions for Yp, Ys, and all indices are provided in Supplementary Figures S4 and S5, as recommended by the reviewer

The study's findings showed that, for all of the examined stress indices, the combined analysis of variance suggested substantial genotype-by-genotype changes (Lamba et al., 2023). Hamza et al. (2023) revealed that Results from the combined analysis of variance (ANOVA) showed that genotypes differed highly significantly for all measured indices, suggesting substantial genetic variation in their adaptive responses to heat stress.

### Selection of indices

*Assessment of heat tolerance stress indices:* Grain yield was assessed under both timely-sown (normal) and late-sown (heat-stressed) environments using twelve stress indices, specifically TOL, STI, SSPI, YI, YSI, RSI, MP, GMP, HM, MRP, PYR, and HSI. These indices provided a multi-dimensional perspective on genotype performance, capturing susceptibility, tolerance, stability, and overall productivity under contrasting environments (Table S4).

*Heat-susceptible genotypes:* Among the evaluated genotypes, BRCHT-20 emerged as the most heat-susceptible. Although it produced high yield under timely sowing, its yield declined sharply under late-sown conditions. This susceptibility was further confirmed by its highest values of TOL, SSPI, RSI, and PYR, which are indicators of yield instability and sensitivity to stress. Hence, BRCHT-20 appears better suited for environments where timely sowing can be ensured, rather than heat-prone conditions.

*Heat-tolerant genotypes:* BRCHT-1 showed consistent performance under both environments, with low TOL, SSPI, and YI values, reflecting minimal yield reduction and strong heat tolerance. This agrees with earlier findings linking low TOL and SSI with superior stress resilience in chickpea (Yucel & Mart, 2014; Sabaghnia & Janmohammadi, 2014). BRCHT-10 also performed well, showing high STI, MP, GMP, and HM values, whereas IPC-2000-14 and BRCHT-29 displayed low index values, identifying them as heat-sensitive.

### Key indices for reliable selection

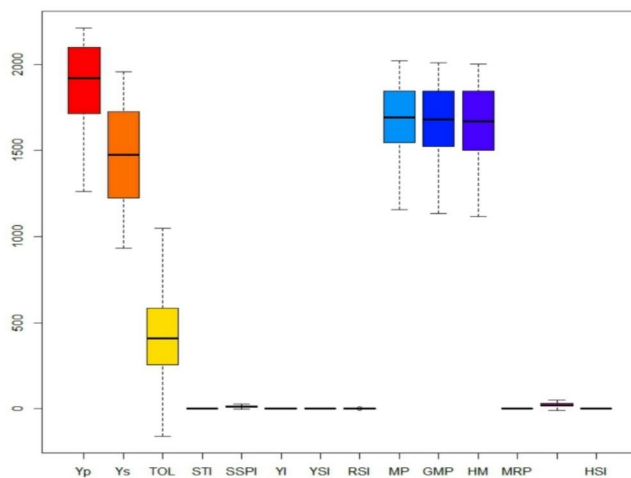
The results underscore the value of productivity-based indices such as MP, GMP, and HM, which showed strong positive associations with yield in both environments. These indices are widely recognized as reliable selection tools in crops like wheat and rice (Anwar et al., 2011) and are equally effective for chickpea. YI further distinguished tolerant lines, while susceptibility indices such as SSI/HSI classify genotypes with values  $> 1$  as susceptible and  $< 0.5$  as highly tolerant (Fischer & Maurer, 1978; Porch, 2006).

## Classification using heat susceptibility index (HSI)

Fischer and Maurer's (1978) Heat Susceptibility Index (HSI) was used to classify genotypes into four categories: highly heat-tolerant ( $HSI < 0.50$ ), heat-tolerant ( $0.51–0.75$ ), moderately tolerant ( $0.76–1.00$ ), and heat-susceptible ( $HSI > 1.00$ ). Using two years of yield data, BRCHT-1, BRCHT-13, BRCHT-18, BRCHT-29, and IPC 11–20 consistently showed negative or very low HSI values, indicating minimal yield loss and confirming them as highly heat-tolerant and resilient genotypes (Table S5).

In the Plots pane, boxplots prior to normalisation (Fig. 1A) revealed limited interpretability: the components of several indices were compressed, making it difficult to distinguish meaningful variation across heat tolerance indices. Despite yield differences and significance tests suggesting variability, the unstandardised boxplots did not clearly display the patterns.

Following quantile normalisation in R, the variability across indices became more apparent (Fig. 1B). The boxplots of normalised data displayed easily distinguishable distributions, with nearly equalised medians across most indices, thereby demonstrating effective normalisation. Exceptions were observed in RSI, MRP, and HSI, which still exhibited some deviation, suggesting that these indices may inherently capture greater variation. Overall, the normalisation procedure successfully enhanced clarity and comparability among the indices, allowing more robust interpretation of their biological significance.



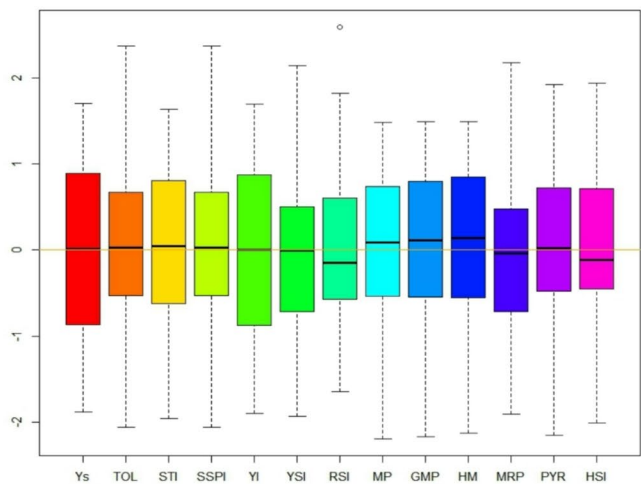
(A) Before normalization

**Fig. 1** Distribution of heat-tolerance indices; **A** Boxplots of the twelve heat-tolerance indices using raw (unnormalized) values, which shows variation among indices is compressed due to differences in scale, making it difficult to visually compare their distributions; **B** Boxplots of the same indices after quantile normalization, Normaliza-

## Correlation of grain yield with stress tolerance indices

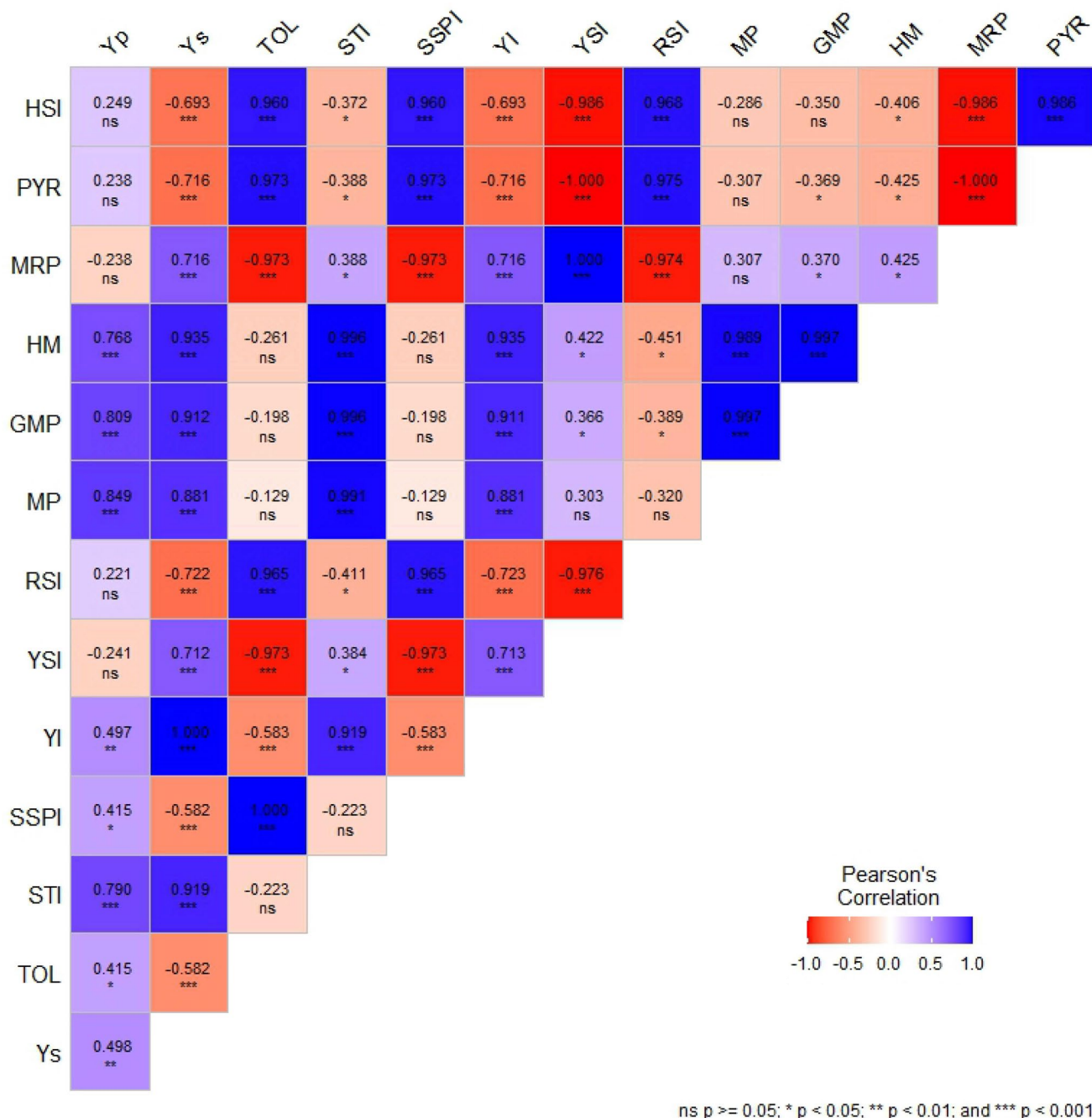
Correlation analysis revealed a significant positive association ( $r=0.498$ ) between grain yield under stress ( $Y_s$ ) and non-stress ( $Y_p$ ) conditions, indicating that genotypes performing well in optimal environments also tend to maintain yield under heat stress (Fig. 2; Table S6). This suggests the possibility of selecting broadly adapted genotypes that combine productivity with resilience (Fig. 2). Under heat stress,  $Y_s$  exhibited negative correlations with TOL ( $-0.582$ ), SSPI ( $-0.582$ ), RSI ( $-0.722$ ), and PYR ( $-0.718$ ). Higher values of these indices therefore correspond to greater yield reductions under stress, establishing them as indicators of susceptibility rather than tolerance. At the same time, these indices showed positive associations with  $Y_p$  ( $0.221–0.415$ ), meaning they may favour productivity under non-stress conditions but fail to predict stability under heat stress. SSPI was especially informative, showing a positive relationship with  $Y_p$  and a negative one with  $Y_s$ , effectively differentiating stress-sensitive genotypes, consistent with observations by Shirani Rad and Abbasian (2011).

The Yield Stability Index (YSI) showed a strong positive correlation with  $Y_s$  ( $0.712$ ) and a moderate negative correlation with  $Y_p$  ( $-0.241$ ), highlighting its effectiveness in identifying genotypes that maintain yield under stress, in agreement with Nouri et al. (2011). Indices such as STI, YI, MP, GMP, and HM demonstrated strong positive correlations with both  $Y_s$  and  $Y_p$ , confirming their reliability for selecting genotypes that perform consistently across environments. Correlation patterns identified BRCHT-1 and



(B) After normalization

tion removes scale-related distortion and allows clearer comparison of variability across indices, supporting interpretation of the ANOVA (Table 1). Each boxplot shows the median, interquartile range, and outliers for the respective index



**Fig. 2** Pearson correlation matrix among grain yield under timely-sown (Yp) and late-sown (Ys) conditions and twelve heat-tolerance indices across 30 chickpea genotypes. *Note:* Positive correlations are

shown in blue and negative correlations in red, with intensity reflecting the strength of association

BRCHT-10 as the most stable and productive genotypes. Additional promising entries included Sabour Chana-02, DCP 92-3, BRCHT-38, JG-14, and DEON, all showing strong yield potential and heat resilience. Very high correlations were observed among productivity-related indices: MP and GMP ( $r=0.997^{**}$ ), GMP and YI ( $r=0.911^{**}$ ), and MP and YI ( $r=0.881^{**}$ ). Likewise, TOL and SSPI were perfectly correlated ( $r=1.00^{**}$ ), reaffirming that they represent

susceptibility. Genotypes such as BRCHT-1, BRCHT-13, IPC-11-20, and BRCHT-29 combined low TOL and SSPI values with high MP, GMP, and STI scores, indicating their strong potential as parents for heat-tolerance breeding.

These results align with previous studies showing the reliability of productivity-based indices. Poudel et al. (2021) linked low TOL and SSI with higher stress-yield in wheat, while Farshadfar and Sutka (2002) and Nayyeripas (2019)

shown in blue and negative correlations in red, with intensity reflecting the strength of association

reported strong associations of MP, GMP, STI, and YSI with yield across environments. Anwar et al. (2011) similarly validated MP for identifying drought-tolerant cultivars.

### Multicollinearity analysis among stress tolerance indices

To assess the suitability of stress tolerance indices for regression-based prediction of grain yield under heat stress, multicollinearity was examined using the Variance Inflation Factor (VIF) and Tolerance statistics (Table S7). The analysis revealed extremely high multicollinearity among all indices, with VIF values ranging from  $1.24 \times 10^2$  to  $1.28 \times 10^7$ , far above the conventional threshold of 10, and Tolerance values between  $10^{-3}$  and  $10^{-7}$ , indicating substantial shared variance among predictors. This outcome is expected, as indices such as MP, GMP, HM, STI, YI, TOL, and SSPI are all computed from the same primary components yield under stress ( $Y_s$ ) and yield under non-stress ( $Y_p$ ) and are therefore inherently non-independent. Such structural multicollinearity led to singularity in the model matrix and resulted in an overfitted regression model, reflected by an inflated  $R^2 = 1.00$ , unstable coefficient estimates, and large standard errors (Table S9).

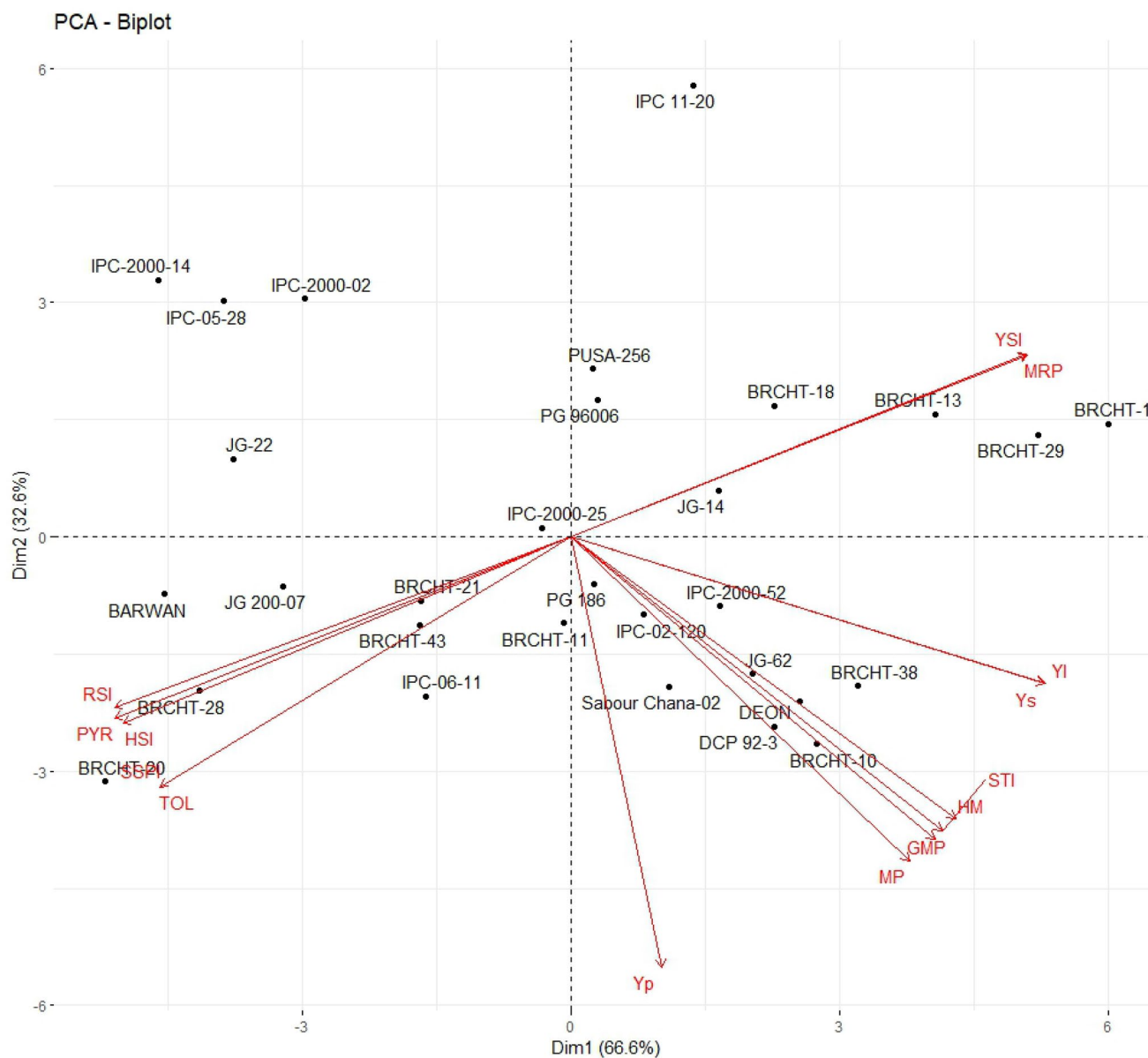
Zero-order correlations showed strong associations between several indices and yield; however, partial and part correlations were negligible, confirming that none of the indices contributed unique explanatory information (Table S8). As this multicollinearity is structural rather than incidental, it cannot be mitigated using standard remedial approaches (e.g., centering or variable removal). Consequently, the regression model is unsuitable for reliable parameter estimation or predictive inference.

### Principal component analysis and hierarchical cluster analysis

To identify heat-tolerant chickpea genotypes, Principal Component Analysis (PCA) was performed using several heat stress indices along with grain yield data. This multivariate approach provided a comprehensive understanding of genotypic performance under contrasting environmental conditions. The first two principal components (PC1 and PC2) were the most informative, explaining 66.64% and 32.64%, respectively, of the total variance, both with eigenvalues greater than one. Together, these components accounted for 99.28% of the variability (Table 2), indicating that they captured the majority of the total information embedded in the stress indices. As reported by Nouri et al. (2011) PCA offers a more reliable criterion for genotype selection under stress because it reduces a complex dataset

**Table 2** Calculation of total variation in terms of principal component analysis (PCA) for grain yield and stress tolerance indices

	Eigen-values	Variance %	Cumulative %	Yp	HSI	Ys	PYR	TOL	STI	SSPI	YI	YSI	RSI	MP	GMP	HM	MRP
PC1	9.330	66.640	66.645	-0.181	-0.942	0.818	-0.738	0.818	-0.942	-0.905	0.907	-0.673	-0.723	-0.765	-0.907	0.907	0.891
PC2	4.569	32.640	99.282	0.982	0.334	0.569	0.671	0.569	0.333	-0.417	0.389	0.739	0.691	0.643	-0.414	0.414	0.425



**Fig. 3** PCA biplot showing the relationships among twelve heat-tolerance indices and the distribution of 30 chickpea genotypes under timely-sown and late-sown conditions. *Note:* PC1 (66.64%) primarily represents heat-stress resilience, with strong loadings for Ys, YSI, HM, YI, and MRP. PC2 (32.64%) represents overall productivity and

yield potential, with high loadings from MP, GMP, STI, and Yp. Genotypes in the upper-right quadrant combine heat tolerance with high productivity, whereas those in the lower-left quadrant show greater susceptibility

into a smaller set of meaningful dimensions while simultaneously revealing interrelationships among traits.

The criterion used to retain components was based on eigenvalues greater than 1, indicating that each selected component contributed more variance than a single standardized variable. This principle, widely applied in plant stress research, helps in identifying the most biologically relevant dimensions. According to Kaya et al. (2002), genotypes with high PC1 and low PC2 scores demonstrate superior stability and yield performance, whereas those with low

PC1 and high PC2 generally exhibit poor performance and instability. These patterns were consistent with earlier findings by Jha et al. (2016), where PC1 and PC2 contributed 84.63% and 13.34% of total variation, respectively.

Biplot analysis based on PC1 and PC2 (Fig. 3) provided detailed insights into genotype responses and the relationships among heat tolerance indices. PC1 showed strong positive loadings for MRP, YI, YSI, Ys, and HM, indicating that genotypes with higher PC1 scores are more likely to maintain yield and express tolerance under heat stress.

Genotypes such as BRCHT-1, BRCHT-13, BRCHT-29, and IPC 11–20 exhibited high PC1 scores, suggesting superior stability under stress conditions.

In contrast, traits including GMP, STI, SSPI, MP, and Yp showed stronger associations with PC2, reflecting general productivity under both normal and stress conditions. Genotypes with high PC2 scores typically perform well across environments. However, several genotypes including BRCHT-20, BRCHT-28, JG 200-07, and BARWAN displayed high PC2 but low PC1 values, indicating susceptibility to heat stress despite good performance under optimal conditions.

Traits such as RSI, PYR, MRP, HSI, and TOL exhibited wide dispersion in the biplot, highlighting their role in distinguishing stress-tolerant versus susceptible genotypes. Genotypes such as BRCHT-1, BRCHT-13, BRCHT-18, BRCHT-29, and IPC 11–20 showed consistently high PC1 scores and favourable values for key tolerance indices (GMP, MP, HM, STI, YI), making them the most promising heat-tolerant entries in this study.

The biplot clarified trait relationships through vector angles; acute angles indicated positive correlations, obtuse angles negative associations, and right angles minimal relationships. Yp and Ys showed strong positive associations with YI, HM, STI, GMP, and MP, while TOL, SSPI, RSI, and PYR formed obtuse angles with Ys, confirming susceptibility roles consistent with findings in safflower (Bahrami et al., 2014), sunflower (Tyagi & Dhillon, 2019), wheat (Poudel et al., 2021), and mustard (Singh & Bhajan, 2016; Saroj et al., 2021).

A hierarchical cluster analysis based on 12 heat-tolerance indices and grain yield under normal and stress conditions grouped the chickpea genotypes into five distinct clusters (Figure S2A). Cluster 3 was the largest, containing 14 genotypes, while Clusters 1 and 4 comprised 5 and 6 genotypes respectively. Genotypes within a cluster exhibited similar heat-tolerance patterns, whereas those across clusters showed greater diversity.

Cluster 2 emerged as the most desirable group, characterized by the lowest values of TOL, SSPI, RSI, PYR, and HSI and the highest levels of Ys, STI, YI, YSI, MP, GMP, HM, and MRP, indicating strong heat tolerance, stability, and superior productivity. In contrast, Cluster 5 represented more susceptible genotypes, reflected by elevated HSI, PYR, and SSPI.

Cluster 1 contained five genotypes, among which IPC 11–20 and BRCHT-18 were highly tolerant, while the remaining three (JG-14, PUSA-256, PG 96006) showed moderate tolerance. In cluster 3 (JG-62, DEON, DCP 92–3, BRCHT-10, IPC-2000-52, PG 186, Sabour Chana-02) comprised moderately tolerant genotypes. Cluster 2 included

three highly tolerant genotypes (BRCHT-1, BRCHT-13, BRCHT-29).

These findings agree with earlier studies (Jha et al., 2018; Yücel & Mart, 2014), which also used cluster analysis to distinguish chickpea genotypes under stress and recommended genetically distant clusters as ideal parental sources. Likewise, Thana et al., (2019) reported that genotypes with high MP, GMP, HM, STI, and YSI consistently performed better under stress, supporting the present results.

## Discussion

The production of chickpeas is getting more and more affected by heat stress, especially in the case of late-sown systems where the flowering and pod-filling stages take place at the same time as the temperature rise. Even though different methods have been used to assess heat tolerance in chickpea plants, most of those methods had disadvantages like single-environment screening, limited trait coverage or small index sets. The current research builds upon this groundwork by utilizing a germplasm panel that has been rigorously curated, which was selected from a decade-long evaluation of 300 entries, together with a multidimensional analytical framework that employs twelve complementary heat-tolerance indices. This integrated approach not only confirmed the previously documented tolerant genotypes but also led to the discovery of new heat-resilient candidates that were consistent in their performance across environments and had promising traits. Hence, this study presents the following three points as significant advancements made over existing researches. To begin with, it issues multi-year field data that are indicative of the actual agronomic challenges faced in the Northeast Plain Zone (NEPZ) of India, specifically at Sabour, thus it targets late-sown rice-fallow production systems. Secondly, it utilizes a very comprehensive statistical framework engaging quantile normalization, twelve heat-tolerance indices, PCA accounting for 99.28% of total variation in the first two components, and hierarchical clustering to provide rigorous and biologically relevant classification of genotypes. Finally, it assesses the performance of regionally significant germplasm, including BRCHT series and Sabour Chana-02, thus providing directly applicable recommendations for breeding programs in heat vulnerable regions.

The entire process of analysis is fully transparent and reproducible as all R packages, workflows along with the raw data are available in Supplementary Files S1–S11. This provision enables researchers of the future to either rerun the method or do a comparison of the new germplasm panels with the existing results. All in all, the current study is a huge leap forward for chickpea heat tolerance improvement

using a data-driven, multivariate, and regionally contextualized approach.

Using a multi-dimensional analytical framework that combines yield traits, twelve heat tolerance indices, and multiple sophisticated multivariate techniques, the current study offers a thorough assessment of heat tolerance in chickpeas. Correlation analysis, PCA, biplot visualisation, multicollinearity diagnostics, and hierarchical clustering were integrated to provide a comprehensive and biologically significant interpretation of genotype performance under different temperature conditions. Because they capture intricate trait interactions and uncover underlying patterns that may not be apparent through univariate analyses alone, such integrative approaches are becoming more and more advised in abiotic stress-related crop improvement.

The correlation and PCA analyses consistently identified STI, MP, GMP, HM, and MRP as the most reliable indices for distinguishing heat-tolerant chickpea genotypes, supporting earlier findings in wheat, safflower, and chickpea. The present study strengthens previous evidence by showing that these indices maintain strong discriminating ability across two years and contrasting sowing environments, highlighting their stability. In contrast, TOL, SSPI, RSI, and PYR clustered along the susceptibility axis and displayed strong negative correlations with grain yield under stress (Ys), confirming their effectiveness in identifying genotypes prone to severe yield reductions under high temperatures. The close agreement between PCA loadings, hierarchical clustering, and correlation matrices enhances confidence in the robustness of these indices for breeding. Notably, yield under normal conditions (Yp) was positively correlated with Ys, indicating that high productivity often coincides with stress resilience, a trend consistent with earlier reports in chickpea (Devasirvatham et al., 2015).

Multicollinearity analysis played a crucial role in refining the methodological approach. Extremely high VIF values and very low tolerance confirmed structural multicollinearity among indices due to their shared dependence on Yp and Ys. Such multicollinearity, common in stress-tolerance research, can inflate coefficient variance and destabilize regression models (Dormann et al., 2013). The negligible partial correlations further indicated redundancy among indices, reinforcing the need for PCA and clustering, which remain more robust than parametric regression.

Principal Component Analysis effectively reduced the high-dimensional dataset into a smaller set of biologically meaningful components. PC1 and PC2 together accounted for more than 99% of the total variance an exceptionally high proportion consistent with earlier reports in chickpea and other crops (Nouri et al., 2011; Jha et al., 2016). PC1 represented yield stability and stress resilience, whereas PC2 reflected general productivity. Indices such as Ys, YSI,

YI, HM, and MRP contributed strongly to PC1, highlighting their central role in heat tolerance. In contrast, MP, GMP, STI, and Yp were dominant contributors to PC2, indicating their close association with baseline yield potential.

The PCA biplot further clarified relationships among traits and genotypes. Acute vector angles among MP, GMP, HM, STI, YI, and Ys indicated strong positive correlations, whereas obtuse angles between Ys and TOL, SSPI, RSI, and PYR revealed contrasting susceptibility effects. These patterns closely align with biplot-based analyses in wheat, sunflower, safflower, and mustard (Poudel et al., 2021; Bahrami et al., 2014; Tyagi & Dhillon, 2019; Saroj et al., 2021). Heat-resilient genotypes such as BRCHT-1, BRCHT-13, BRCHT-29, IPC 11–20, and BRCHT-10 consistently aligned with favourable tolerance indices. Hierarchical clustering supported the PCA results by grouping high-performing genotypes into distinct tolerant clusters, while susceptible lines exhibited high TOL, SSPI, and HSI values, reflecting stress vulnerability (Jha et al., 2015; Yücel & Mart 2014).

This study validates previously reported heat-tolerant genotypes such as BRCHT-1, BRCHT-13, PG-186, and Sabour Chana-02 (Kumari et al., 2024), while also identifying BRCHT-29, BRCHT-18, and IPC 11–20 as newly recognised, consistently superior performers. These genotypes hold strong potential for heat-prone rice-fallow systems, where their deployment can enhance chickpea productivity, improve resource-use efficiency, and strengthen livelihood stability in eastern India.

While the present work offers a comprehensive framework, future research should integrate physiological markers (e.g., membrane stability, pollen viability, canopy temperature depression), molecular markers, and genome-wide scans to refine the genetic basis of heat tolerance. Multi-location testing will further verify the stability of the identified lines across varying heat-stress patterns. By combining quantitative indices, physiological traits, and genomic tools, breeding programs can accelerate the development of cultivars adapted to climate-change-induced thermal extremes.

## Conclusion

This research has laid down a strong and reproducible system for the field-based screening of heat tolerance that would work parallel to the physiological and molecular methods. As for the future studies, besides recommending multi-location validation of the top candidates, it also suggests conducting targeted physiological assays (e.g., pollen viability, canopy temperature depression), and the incorporation of marker-assisted or genomic selection to make more rapid the breeding for climate resilience. The selection

of heat-resilient genotypes, in view of the ever-increasing importance of rice-fallow systems, presents an immediate chance to extend chickpea production to these mostly untapped areas resulting in higher productivity and farmer income.

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## Declarations

**Conflict of interest** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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