



# OPEN Assessing alpha lattice design for heat stress indices and yield stability in wheat genotypes

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Wheat (*Triticum aestivum* L.) faces increased susceptibility to terminal heat stress, a major yield-limiting factor, especially in subtropical regions. Despite India's anticipated record wheat production for 2024-25, high temperatures during the grain-filling stage threaten yield stability. This study assessed the relative efficiency of the alpha lattice design (ALD) against the randomized complete block design (RCBD) in quantifying fifteen heat stress indices (HIs) among 200 recombinant inbred lines (RILs) derived from WH711 × WH1021. The experiment was conducted at Chaudhary Charan Singh Haryana Agricultural University, during 2018-19 Rabi season over two sowing dates, capturing diverse environmental conditions. Results showed tolerance and yield stability indices with the largest (-56 to 614) and smallest (0.30 to 1.09) ranges, respectively, and most HIs exhibited medium to high heritability. Complementary gene interactions were noted for stress tolerance, yield index, yield stability, relative heat, and heat resistance indices, while duplicate gene interactions were observed for heat susceptibility index. Significant genetic variability among RILs was observed, identifying RILs 34, 59, and 106 as the most heat-tolerant with superior yield and stability. The ALD improves precision, achieving lower error mean squares and reduced coefficients of variation compared to RCBD, with a relative efficiency (RE) range up to 1.05. These findings underscore ALD's value in managing environmental heterogeneity in trials with large genotype sets and advancing genetic precision under stress, supporting its use in breeding programs focused on developing heat-resilient wheat cultivars amidst rising climatic challenges.

**Keywords** ALD, Heat, RCBD, Relative efficiency, RILs, Wheat

Globally, wheat (*Triticum aestivum* L.) is one of the main cereal crops, grown in an array of climatic conditions, including different soil types, temperatures, and water regimes. In 2024-25, approximately 31.80 million hectares (Mha) of wheat crop were harvested in the Indian subcontinent<sup>1</sup>. For the upcoming year, India anticipates a record wheat crop yield of 3.54 million tons/hectares and total production of 112.5 million metric tons (MMT), assuming favorable weather conditions<sup>1</sup>. Over the past decade, delayed monsoon withdrawals and early summer onset have caused wheat to face terminal heat stress during its reproductive stages, adversely affecting yield production. Daytime temperatures above 38–40 °C and nighttime temperatures over 18–20 °C during the grain filling stage can reduce yield prospects by 5–10 MMT<sup>1</sup>. Unpredictable temperature and rainfall patterns pose significant challenges by causing heat stress<sup>2</sup> which impacts approximately 40% of the global irrigated wheat area<sup>3</sup>. Each degree Celsius increase in temperature causes an estimated loss of 6 ± 2.9% wheat yield globally<sup>4</sup>. Heat stress accelerates the maturation phase by shortening the grain filling period<sup>5</sup> reducing the time for adequate starch and nutrient accumulation, leading to smaller, lighter grains<sup>6</sup>. Mitigating wheat yield losses due to climate

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variability, particularly from terminal heat stress in high-yielding regions, is critical to advancing global food security and necessitates resilient crop breeding strategies.

Wheat breeders conduct extensive field trials to identify heat-tolerant, high-yield cultivars, making both experimental design and statistical analysis vital to obtaining accurate, reliable results. Although there are different types of experimental designs used in agricultural research<sup>7</sup>. Selecting an optimal experimental design remains challenging, as the arrangement of plots must be carefully controlled to ensure precise error estimation. Commonly used designs, such as the randomized complete block design (RCBD), consider each block homogeneous and replicate treatments fully within each block. However, RCBD, Latin square, and other complete block designs struggle to manage soil heterogeneity when evaluating numerous treatments<sup>8</sup>. A major drawback of RCBD design is its suitability only for treatments fewer than ten per block<sup>9</sup>. As factors and treatment combinations increase, accommodating them in a single homogeneous block becomes impractical<sup>8</sup>. Consequently, the adoption of randomized incomplete block designs has increased as they better accommodate larger numbers of treatments while managing environmental variability across diverse conditions. Incomplete block designs include both partially balanced (alpha lattice) and fully balanced lattice designs. The alpha lattice design (ALD) is preferred for its flexibility and practicality, compared to balanced lattice designs, which are constrained by strict guidelines, high evaluation costs, and substantial seed requirements. Unlike balanced lattice designs, ALD accommodates any number of replicates and does not require a perfect square of genotypes<sup>7</sup>. Originally developed by Patterson and Williams<sup>10</sup> ALD is gaining prominence in global plant breeding for its greater efficiency relative to the RCBD<sup>11</sup>. ALD's structure in smaller, incomplete blocks with fewer varieties enhances precision and controls error effectively, making it ideal for large-scale studies and incomplete block sizes. Moreover, ALD designs are easy to construct, even when balanced or traditional lattice designs are impractical<sup>12</sup>. They are also adaptable, allowing treatment removal when entries do not fit exact block multiples. Typically, the efficiency of one analysis over another is assessed by expected error mean squares, reduced error variance, or the standard error of the difference between genotype means.

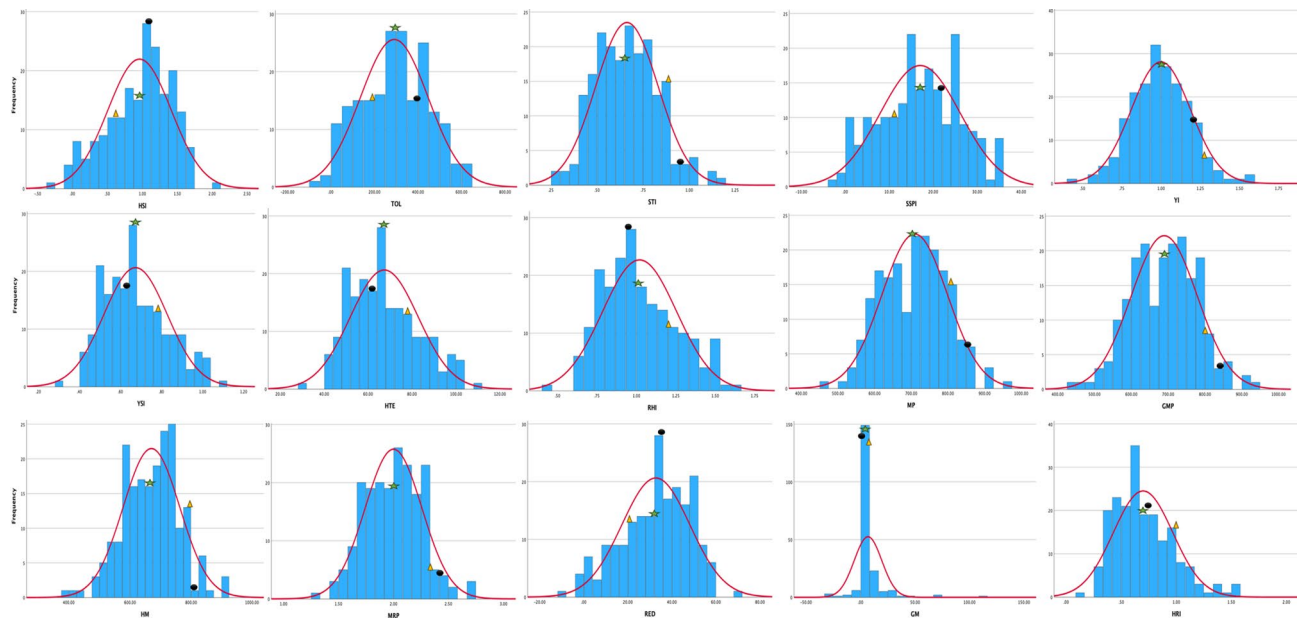
Research on the efficiency of alpha lattice designs compared to RCBD in large-scale wheat trials under heat stress conditions is limited, highlighting the need for more studies examining these approaches for improved experimental precision in breeding programs. Therefore, a large-scale study was planned on a population of 200 recombinant inbred lines (RILs) with the following objectives: (i) analyze various heat stress indices (HIs) for the selection of heat-resilient genotypes; and (ii) determine the efficiency of an alpha-lattice design relative to RCBD.

## Materials and methods

The present research was carried out at the Chaudhary Charan Singh Haryana Agricultural University (CCSHAU) research farm in Hisar during 2018–19 *Rabi* season. This place is located at 29.09° N, 75.43° E and at an altitude of 215.20 m above mean sea level. The genetic material was comprised of two hundred recombinant inbred lines (RILs) derived from WH711 X WH1021. The experiment was conducted in an alpha lattice design (ALD) with two replications. Each replication comprised five blocks, with each block containing 40 RILs under two different time periods, i.e., timely sown and late sown. The RILs were randomly assigned to the blocks within each replicate to minimize bias and ensure a balanced evaluation of their performance. Each RIL was planted in a paired row of 2.5 m length with 20 cm row-to-row spacing. Grain yield/plot (g) data was used to estimate various heat stress indices (HIs) (Table 1), enlisted in Fig. 1. The environmental conditions were recorded at CCSHAU meteorological station, located within a 1 km radius of the field experiments (Table S1).

| S. No. | Heat Indices                  | Abbreviations | Formulas                                                                                       | References |
|--------|-------------------------------|---------------|------------------------------------------------------------------------------------------------|------------|
| 1      | Heat susceptibility index     | HSI           | $(1 - Y_{si}/Y_{pi})/(1 - Y_s - Y_p)$<br>< 1.0 = stress tolerant<br>> 1.0 = stress susceptible | 13         |
| 2      | Stress tolerance index        | STI           | $(Y_{si} \times Y_{pi})/(Y_p)^2$                                                               | 14         |
| 3      | Stress tolerance              | TOL           | $Y_{pi} - Y_{si}$                                                                              | 15         |
| 4      | Stress susceptibility % index | SSPI          | $((Y_{pi} - Y_{si})/(2 \times Y_p)) \times 100$                                                | 16         |
| 5      | Yield index                   | YI            | $Y_{si}/Y_s$                                                                                   | 17         |
| 6      | Yield stability index         | YSI           | $Y_{si}/Y_{pi}$                                                                                | 18         |
| 7      | Heat tolerance efficiency     | HTE           | $(Y_{si}/Y_{pi}) \times 100$                                                                   | 19         |
| 8      | Relative heat index           | RHI           | $(Y_{si}/Y_{pi})/(Y_s/Y_p)$                                                                    | 20         |
| 9      | Mean productivity             | MP            | $(Y_{pi} + Y_{si})/2$                                                                          | 15         |
| 10     | Geometric mean productivity   | GMP           | $\sqrt{Y_{pi} \times Y_{si}}$                                                                  | 14         |
| 11     | Harmonic Mean                 | HM            | $2 \times (Y_{pi} \times Y_{si})/(Y_{pi} + Y_{si})$                                            | 21         |
| 12     | Mean relative performance     | MRP           | $(Y_{si}/Y_s) + (Y_{pi}/Y_p)$                                                                  | 22         |
| 13     | Reduction                     | RED           | $(Y_p - Y_s)/Y_p \times 100$                                                                   | 23         |
| 14     | Golden Mean                   | GM            | $(Y_{pi} + Y_{si})/(Y_{pi} - Y_{si})$                                                          | 24         |
| 15     | Heat resistance index         | HRI           | $Y_{si} \times (Y_{si}/Y_{pi})/Y_s$                                                            | 25         |

**Table 1.** List of heat stress indices (HIs) along with their formulas used in the study. Yp: Mean of grain yield under timely sown; Ys: Mean of grain yield under late sown; Ypi: grain yield under normal sown for “i<sup>th</sup>” RIL; Ysi: grain yield under late sown for “i<sup>th</sup>” RIL.



**Fig. 1.** Distribution patterns of various heat stress indices (HIs) in the wheat RILs population. Green stars, black circles, and yellow triangles denoted indices mean of RILs population, WH711, and WH1021 respectively. HSI: Heat susceptibility index; STI: Stress tolerance index; TOL: Stress tolerance; SSPI: Stress susceptibility % index; YI: Yield index; YSI: Yield stability index; HTE: Heat tolerance efficiency; RHI: Relative heat index; MP: Mean productivity; GMP: Geometric mean productivity; HM: Harmonic Mean; MRP: Mean relative performance; RED: Reduction; GM: Golden Mean; and HRI: Heat resistance index.

Descriptive statistics and analysis of variances (ANOVA) were performed for all heat stress indices using IBM SPSS Statistics for windows, version 29.0.1.0, Armonk, NY: IBM Corp. The significance of skewness and kurtosis was evaluated by dividing each of these values by their corresponding standard errors, resulting in t-values. These t-values were then compared to the critical values from the t-distribution table at  $n-1$  degrees of freedom to determine their significance<sup>26</sup>. The broad sense heritability was computed as a ratio of genotypic variance to phenotypic variance and expressed in percentage using the formula given by Falconer<sup>27</sup>. It was further classified into low (< 50%), medium (50–80%), and high (> 80%). The relative efficiency (RE 1 and 2) of ALD was compared with that of RCBD using coefficient of variations (CV) and error mean square (EMS), and computed with the following formulas:

Relative Efficiency 1 (RE1) =  $CV \text{ for RCBD} / CV \text{ for ALD}$ .

Relative Efficiency 2 (RE2) =  $EMS \text{ for RCBD} / EMS \text{ for ALD}$ .

If RE1 or RE2 > 1, it means ALD design is more efficient than RCBD comparatively; if RE1 or RE2 = 1, it means both designs are comparatively equal; and if RE1 or RE2 < 1, it means RCBD is more efficient than ALD comparatively<sup>12</sup>.

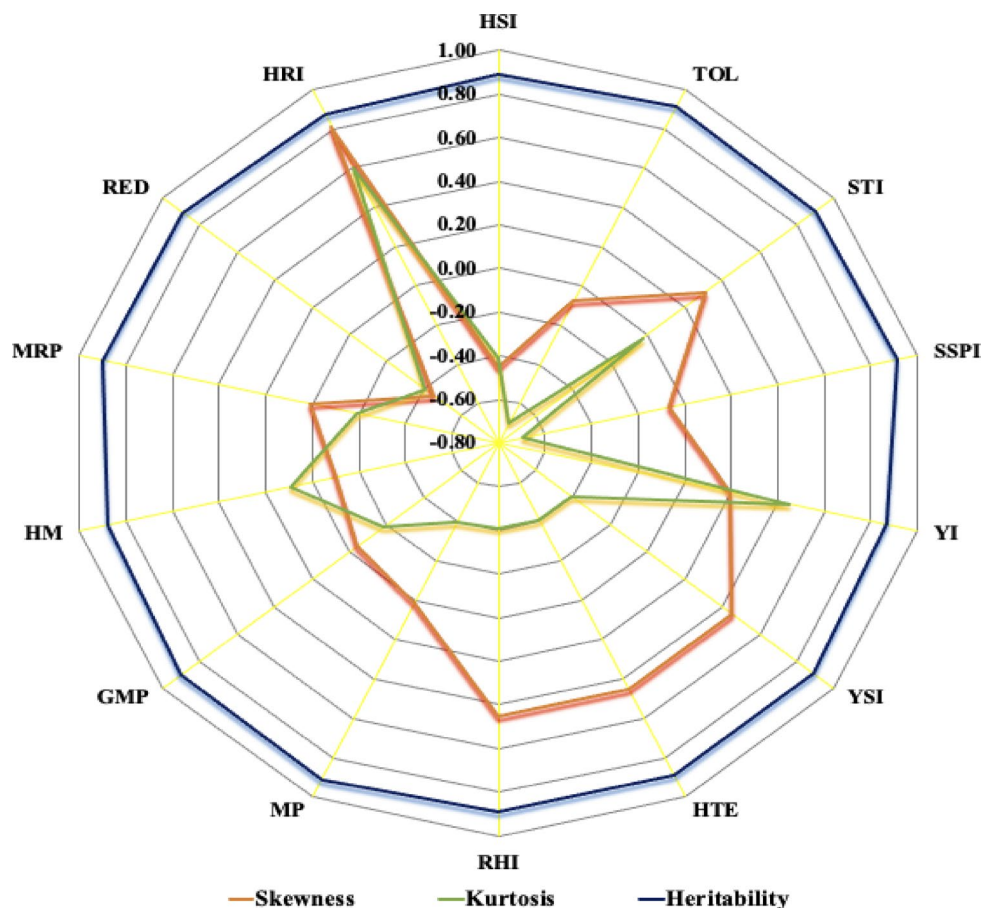
## Results & discussion

### Assessment of heat stress indices

Heat stress indices are quantitative tools, widely used to examine the impacts of heat stress on plant growth and development<sup>28</sup>. Stress indices are crucial tools for assessing physiological and yield-related traits under stress conditions, guiding the genotypic selection, and thereby aiding in the development of heat-resilient and adaptable wheat varieties<sup>29,30</sup>. Various weather parameters recorded during the experiment were presented in Table S1. During the anthesis and post-anthesis period, a continuous increase was noticed in the trend of temperature and evapotranspiration. Also, a low rainfall and decreasing humidity pattern were recorded, confirming the occurrence of heat stress coinciding with the post-reproductive stage of the wheat crop (Table S1). In this study, fifteen HIs were computed from the grain yield data obtained from the RILs population grown under different environmental conditions. All HIs were recorded with a wide range, indicating the presence of substantial genetic variability in the RILs population under heat stress conditions (Fig. 1). The longest range was recorded for tolerance (TOL, −56 to 614), followed by harmonic mean (HM, 383.91 to 911.63) and mean productivity (MP, 452.50 to 957.75), whereas yield stability index (YSI) and stress tolerance index (STI) were recorded with the shortest ranges (0.30 to 1.09 and 0.26 to 1.20). MP was recorded with the highest mean value ( $709.52 \pm 3.36$ ), followed by geometric mean productivity (GMP,  $690.02 \pm 3.42$ ) and harmonic mean (HM,  $671.45 \pm 3.57$ ), indicating the higher overall productivity of the RILs. The occurrence of a wide range for most of the HIs indicated the differential response of RILs under heat stress conditions. Even some of them performed better than the heat-tolerant parent. This indicated towards RILs structure with a unique combination of traits, conferring a high degree of heat tolerance and can be termed as transgressive segregants<sup>28</sup>. On the other hand, yield index (YI) was recorded with the lowest mean value ( $0.99 \pm 0.19$ ), followed by heat susceptibility index

(HSI,  $0.97 \pm 0.46$ ), heat resistance index (HRI,  $0.70 \pm 0.27$ ), and stress tolerance index (STI,  $0.66 \pm 0.21$ ). The results were matched with findings of Mohammadi et al.<sup>31</sup> that used MP, GMP, and STI as important criteria for screening wheat genotypes adapted to moderate stress conditions. Moreover, Bennani et al.<sup>32</sup> also found heat stress indices with high genetic diversity and an ability to discriminate between genotypes. The HIs are derived parameters from grain yield, which has a complex genetic architecture or environmental influence; therefore, broad-sense heritability was computed. Most HIs exhibited high heritability (> 86%), showing strong genetic control and reliable selection potential for wheat heat tolerance (Fig. 2). On the other hand, GM was recorded with medium heritability (61%). Although broad-sense heritability includes both additive as well as non-additive gene effects, the medium to high heritability can be effectively instrumental to improve further heat stress tolerance<sup>33</sup>.

The genetic nature of HIs was determined by analyzing the distribution pattern using third-degree statistics (Fig. 2). HSI exhibited a significant negatively skewed ( $-0.45^*$ ) with platykurtic distribution ( $0.41^*$ ), suggesting the involvement of duplicate (additive X additive) and polygenic interaction. Geometric mean showed a significant positively skewed ( $3.73^*$ ) with leptokurtic distribution ( $26.84^*$ ), suggesting involvement of a relatively smaller number of segregating genes. On other side, STI, YI, YSI, RHI, HRI were recorded with significant positively skewed ( $0.31^*$ ,  $0.20^*$ ,  $0.45^*$ ,  $0.45^*$  and  $0.81^*$ ) and platykurtic ( $-0.03^*$ ,  $0.45^*$ ,  $-0.41^*$ ,  $0.41^*$  and  $0.59^*$ ) distribution suggesting the involvement of complementary gene interaction with a large number of genes. The intense selection is required from existing variability for achieving maximum genetic gain in these parameters. Mean relative performance (MRP) recorded a non-significant, negatively skewed with significant platykurtic distribution ( $-0.19^*$ ), indicating the involvement of polygenic inheritance. The rest of the HIs recorded non-significant distributions, indicating the absence of additive epistasis interaction. Despite being derived parameters from grain yield component, HIs displayed distinct distribution patterns, highlighting the complexity of grain yield across various environmental conditions. This also offered an opportunity to select HIs that could be most effective in breeding programs aimed at improving heat tolerance.



**Fig. 2.** High-order (third-degree) statistics and heritability estimates of various heat stress indices (HIs). HSI: Heat susceptibility index; STI: Stress tolerance index; TOL: Stress tolerance; SSPI: Stress susceptibility % index; YI: Yield index; YSI: Yield stability index; HTE: Heat tolerance efficiency; RHI: Relative heat index; MP: Mean productivity; GMP: Geometric mean productivity; HM: Harmonic Mean; MRP: Mean relative performance; RED: Reduction, and HRI: Heat resistance index.



Selection of heat-tolerant lines

Heat susceptibility index (HSI) is one of the most used indices for screening genotypes under stress. In the present study, 91 RILs had HSI values less than 1.0, indicating potential tolerance. Among these, 47 RILs showed lower HSI values than the heat-tolerant parent WH1021 (HSI=0.63), highlighting their strong tolerance.

To refine the selection, 14 additional HIs were analyzed. Based on the performance better than the high yielding parent WH 711, indices such as STI, stress susceptibility % index (SSPI), MP, GMP, HM, and MRP identified 10, 59, 4, 10, 11, and 10 number of RILs with higher performance. Likewise, TOL and reduction (RED) identified 56 and 47 number of RILs with lower indices values than the heat-tolerant parent WH1021, indicating better heat tolerance. On the other hand, YI, YSI, heat tolerance efficiency (HTE), relative heat index (RHI), golden mean (GM), and HRI identified 14, 48, 47, 47, 39, and 26 number of RILs with higher indices values than the heat-tolerant parent, indicating superior performance.

Overall, eight RILs stood out based on multi-index selection (74, 102, and 192 selected by 9 HIs; 100 selected by 10 HIs; 106 selected by 12 HIs; 33 selected by 13 HIs and 34 and 59 selected by 14 HIs), which can be classified as heat-tolerant genotypes. RILs 34, 59, and 106 exhibited the highest grain yields (894.50 g, 895.00 g, and 855.00 g per plot); therefore, they can be called as most heat-tolerant and high-yielding genotypes, which can be directly used further in heat-tolerance wheat breeding programs. These results were in agreement with earlier studies, which used various indices such as SSPI, TOL, STI, YSI, YI, GM, HM, MRP, and RED to screen out genotypes for heat stress susceptibility and tolerance. These studies also identified these indices as most suitable parameters for evaluating genotypic responses to heat stress<sup>31,34</sup>. Similarly, Kumar and his colleagues<sup>12</sup> also identified 21 heat-tolerant genotypes using grain yield and heat stress indices.

ANOVA-RCBD for his

The variance attributed to replicates was not statistically significant for most indices, indicating minimal difference among replications (Table 2). Highly significant differences among genotypes ( $p < 0.01$ ) revealed considerable genetic variability in heat stress indices among wheat RILs, which can be vital for breeding programs aimed at improving heat stress tolerance, enabling the selection of genotypes with desirable traits for developing resilient wheat varieties. The error variance in RCBD was distributed across the entire dataset without any additional sub-structure. While it provided a straightforward analysis, the lack of an intermediate blocking factor led to higher residual error in comparison to ALD with significant variations. Similarly, higher residual error along with significant genotypic variance was reported previously<sup>12</sup>.

ANOVA-ALD for his

Like RCBD design, ALD was also not found with any statistically significant variance attributed to replications, with mean squares ranging from 2.25 for MP to 6541.64 for GM, indicating consistent performance across replicates and contributing to the reliability of both experiments (Table 2). The error variance in ALD was partitioned into error within blocks and residual error. It was noteworthy that the ALD had an additional layer of blocks within replicates that was significant for certain indices such as HSI (0.1\*), TOL (10063.48\*), SSPI (34.48\*), YSI (0.01\*), HTE (119.56\*), RHI (0.03\*), and RED (119.56\*). This indicated that block effects were present and accounted for, emphasizing the importance of block stratification in the experimental design to minimize environmental variability. Similar findings were reported by previous workers<sup>8,35</sup>. As block heterogeneity increases, precision in estimating variety effects typically declines because larger blocks, which face challenges in maintaining uniformity among experimental units, tend to produce elevated experimental error estimates and decreased accuracy<sup>35</sup>. Therefore, it clearly showed that the ALD better captured the variability among genotypes by accounting for the spatial heterogeneity within replicates. The genotype effect was highly significant ( $p < 0.01$ ) across all HIs. The mean squares for genotypes ranged from 0.05 (YI) to 22904.26 (GM), demonstrating substantial genetic variations in response to heat stress among the wheat RILs. This consistency demonstrated that the differences in HIs were primarily driven by genetic factors, reaffirming the validity of the findings across both experimental designs. Similarly, significant difference among genotypes for various stress indices was reported by previous studies<sup>36,37</sup>. Error mean squares in ALD were relatively lower in comparison to RCBD across all indices, with values ranging from 0.01 for several indices (YI, YSI, RHI, and MRP) to 8934.13 for GM. This reflected the effective error control provided by the ALD, enhancing the reliability and precision of the genotype comparisons. Also, the lower error variance indicated the reliability of HIs evaluating heat stress

| SOV   | DF  | MS of traits |            |        |          |              |        |          |              |            |            |            |              |          |            |        |
|-------|-----|--------------|------------|--------|----------|--------------|--------|----------|--------------|------------|------------|------------|--------------|----------|------------|--------|
|       |     | HSI          | TOL        | STI    | SSPI     | YI           | YSI    | HTE      | RHI          | MP         | GMP        | HM         | MRP          | RED      | GM         | HRI    |
| Rep.  | 1   | 2.33<br>E-05 | 4290.25    | 0.004  | 10.29    | 7.72<br>E-09 | 0.004  | 40.11    | 1.17<br>E-05 | 2.25       | 157.52     | 464.53     | 8.69<br>E-10 | 40.11    | 6541.64    | 0.002  |
| Geno. | 199 | 0.41**       | 48651.55** | 0.06** | 166.35** | 0.07**       | 0.05** | 478.29** | 0.11**       | 15995.22** | 16175.39** | 17242.34** | 0.13**       | 478.29** | 22904.26** | 0.15** |
| Error | 199 | 0.05         | 4787.12    | 0.01   | 16.36    | 0.01         | 0.01   | 56.08    | 0.01         | 1370.73    | 1639.16    | 2031.59    | 0.01         | 56.08    | 8934.71    | 0.02   |

**Table 2.** ANOVA-ALD for his in the wheat RILs population. \*significant at 0.05; \*\*significant at 0.01. SOV: Source of variations; Rep.: Replication; Geno.: Genotypes; HSI: Heat susceptibility index; STI: Stress tolerance index; TOL: Stress tolerance; SSPI: Stress susceptibility % index; YI: Yield index; YSI: Yield stability index; HTE: Heat tolerance efficiency; RHI: Relative heat index; MP: Mean productivity; GMP: Geometric mean productivity; HM: Harmonic Mean; MRP: Mean relative performance; RED: Reduction; GM: Golden Mean; HRI: Heat resistance index.

| SOV    | DF  | MS of traits |            |        |          |              |        |          |              |            |            |            |              |          |            |        |
|--------|-----|--------------|------------|--------|----------|--------------|--------|----------|--------------|------------|------------|------------|--------------|----------|------------|--------|
|        |     | HSI          | TOL        | STI    | SSPI     | YI           | YSI    | HTE      | RHI          | MP         | GMP        | HM         | MRP          | RED      | GM         | HRI    |
| Rep.   | 1   | 2.33<br>E-05 | 4290.25    | 0.004  | 10.29    | 7.72<br>E-09 | 0.004  | 40.11    | 1.17<br>E-05 | 2.25       | 157.52     | 464.53     | 8.69<br>E-10 | 40.11    | 6541.64    | 0.002  |
| Geno.  | 199 | 0.41**       | 48651.55** | 0.06** | 166.35** | 0.07**       | 0.05** | 478.29** | 0.11**       | 15995.22** | 16175.39** | 17242.34** | 0.13**       | 478.29** | 22904.26** | 0.15** |
| Blocks | 8   | 0.1*         | 10063.48*  | 0.01   | 34.48*   | 0.01         | 0.01*  | 119.56*  | 0.03*        | 1395.42    | 1778.32    | 2391.56    | 0.01         | 119.56*  | 8948.52    | 0.03   |
| Error  | 191 | 0.05         | 4566.13    | 0.01   | 15.6     | 0.01         | 0.01   | 53.43    | 0.01         | 1369.7     | 1633.33    | 2016.51    | 0.01         | 53.43    | 8934.13    | 0.02   |

**Table 3.** Relative efficiency (RE 1 and 2) of RCBD and ALD for his in the wheat RILs population. \*significant at 0.05; \*\*significant at 0.01. SOV: Source of variations; Rep.: Replication; Geno.: Genotypes; HSI: Heat susceptibility index; STI: Stress tolerance index; TOL: Stress tolerance; SSPI: Stress susceptibility % index; YI: Yield index; YSI: Yield stability index; HTE: Heat tolerance efficiency; RHI: Relative heat index; MP: Mean productivity; GMP: Geometric mean productivity; HM: Harmonic Mean; MRP: Mean relative performance; RED: Reduction; GM: Golden Mean; HRI: Heat resistance index.

| HI   | CV (RCBD) | CV (ALD) | RE 1 | EMS (RCBD) | EMS (ALD) | RE 2 |
|------|-----------|----------|------|------------|-----------|------|
| HSI  | 22.91     | 22.35    | 1.03 | 0.05       | 0.05      | 1.00 |
| TOL  | 23.77     | 23.22    | 1.02 | 4787.12    | 4566.13   | 1.05 |
| STI  | 11.73     | 11.69    | 1.00 | 0.01       | 0.01      | 1.00 |
| SSPI | 23.76     | 23.21    | 1.02 | 16.36      | 15.6      | 1.05 |
| YI   | 9.83      | 9.76     | 1.01 | 0.01       | 0.01      | 1.00 |
| YSI  | 11.12     | 10.86    | 1.02 | 0.01       | 0.01      | 1.00 |
| HTE  | 11.12     | 10.86    | 1.02 | 56.08      | 53.43     | 1.05 |
| RSI  | 11.13     | 10.87    | 1.02 | 0.01       | 0.01      | 1.00 |
| MP   | 5.22      | 5.22     | 1.00 | 1370.73    | 1369.7    | 1.00 |
| GMP  | 5.87      | 5.86     | 1.00 | 1639.16    | 1633.33   | 1.00 |
| HM   | 6.72      | 6.7      | 1.00 | 2031.59    | 2016.51   | 1.01 |
| MRP  | 5.75      | 5.74     | 1.00 | 0.01       | 0.01      | 1.00 |
| RED  | 22.92     | 22.37    | 1.02 | 56.08      | 53.43     | 1.05 |
| GM   | 1047.49   | 1047.46  | 1.00 | 8934.71    | 8934.13   | 1.00 |
| HRI  | 19.7      | 19.47    | 1.01 | 0.02       | 0.02      | 1.00 |

**Table 4.** Relative efficiency (RE 1 and 2) of RCBD and ALD for HIs in the wheat RILs population.

tolerance in the wheat RILs population. The use of the ALD design increased experimental precision in wheat by 24% compared to the RCBD, as ALD significantly partitioned the error mean square value, which usually doesn't happen in the RCBD design<sup>35</sup>. It can be concluded that as the number of blocks per replicate increases, the proportion of total variation attributed to blocking also rises, leading to a reduction in the coefficient of variation (CV) and overall enhanced effectiveness<sup>7</sup>.

**Relative efficiency (RE) analysis based on CV and EMS**

The coefficient of variations (CV) values for both RCBD and ALD were generally close, with ALD consistently showing slightly lower CV values across most indices (Table 3). The lower coefficient of variation and standard deviation in ALD design facilitate the detection of smaller differences when comparing means<sup>35</sup>. This also indicated that ALD has a marginally better capacity to control variability within the experiment, contributing to more precise estimates. Only MP showed the same value for CV. The relative efficiency (RE1), calculated as the ratio of CV in RCBD to CV in ALD, generally hovered around 1.00 to 1.03. Indices, HSI (1.03), TOL (1.02), SSPI (1.02), YI (1.01), YSI (1.02), HTE (1.02), RSI (1.02), RED (1.02), and HRI (1.01) reflected the ALD's slight advantage in reducing variability. The results were consistent with previous findings<sup>8,11,12</sup> which recorded significantly lower values for CV and EMS. Likewise, the EMS values for ALD were consistently lower than those for RCBD, indicating that ALD more effectively minimized experimental error.

This was particularly evident in indices like TOL, SSPI, HTE, and RED (1.05), where the relative efficiency (RE2) was found to be 1.05. The range of RE2 for all indices varied from 1.00 to 1.05. The higher RE2 values suggested that ALD was more efficient in terms of error reduction, making it a more reliable design for detecting genetic differences in heat stress indices. Despite the modest gains, these findings affirmed that ALD was a more efficient design for large-scale genetic studies, especially when dealing with complex traits such as heat stress tolerance. Masood et al.<sup>38,39</sup> found that the alpha lattice design improved efficiency by 9% and 14% compared to RCBD. They recommended RCBD for experiments with a small number of treatments, while ALD for large-scale variety trials on variable soils with moderate varietal differences. Khan et al.<sup>9</sup> observed an increase of 49% experimental efficiency while comparing ALD to RCBD.

## Conclusion

This study underscores the efficiency of alpha lattice design over the randomized complete block design for evaluating heat tolerance across a 200-recombinant inbred line wheat population. The alpha lattice design enhanced precision, reflected in lower error mean squares, reduced coefficients of variation, and higher relative efficiency than the randomized complete block design, making it advantageous for large-scale trials with diverse genotypes. Significant genetic variations in heat tolerance indices allowed the identification of promising heat-resilient genotypes (RILs 34, 59, and 106) for breeding programs. Moving forward, implementing ALD across multiple environments could validate its adaptability to various climates and soil types. Additionally, integrating genomic tools, such as quantitative trait loci (QTL) mapping, could refine the selection of heat-tolerant genotypes. This approach not only supports climate-resilient wheat breeding but also advances global food security by helping stabilize wheat yields amid escalating climate pressures.

## Data availability

The data that support the findings are available from the corresponding author upon a reasonable request.

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## Author contributions

This work was carried out in collaboration among all authors. Vikram Singh, Shikha Yashveer, Somveer Nimbal and Ram Niwas: Conceptualization, Methodology, Data curation, validation, project administration, review and editing. Mandeep Redhu: Methodology, data collection, formal analysis, visualization, original draft preparation. Ram Niwas, Sonu Langaya: Software and Formal analysis. Rukoo Chawla, Sachin Shehrawat, Mohit Dhukia and Aman: data collection, reviewing and editing. Mehdi Rahimi: visualization, Reviewing and Editing. All authors read and approved the final manuscript.

## Declarations

## Competing interests

The authors declare no competing interests.

## Additional information

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