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Identification of Heat-Tolerant Wheat Genotypes through Integrated Physiological, Yield, and Multivariate Statistical Analysis

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Abstract

Terminal heat stress during reproductive and grain filling stages is a major constraint to wheat (*Triticum aestivum* L.) productivity globally, particularly in emerging wheat ecosystems such as Manipur, Northeast India. This study evaluated the heat tolerance of 12 diverse wheat genotypes under normal and delayed sowing conditions that simulate terminal heat. Integrated physiological parameters (chlorophyll content, canopy temperature depression, relative water content, membrane stability index, chlorophyll fluorescence), yield traits (tillers per plant, spike length, grains per spike, 1000-grain weight, grain yield per plot), and multivariate statistical tools (Principal Component Analysis (PCA), Cluster Analysis, and Multivariate Regression) were used to discriminate heat-tolerant lines. Heat stress significantly reduced physiological efficiency and yield across genotypes; however, genotypes **HD 2967**, **PBW 343**, and **UP 2338** consistently maintained higher physiological stability and yield under stress. PCA revealed that a combination of canopy cooling, membrane integrity, and chlorophyll retention accounted for 68% of the variation in heat tolerance. Heat Susceptibility Index (HSI) and Stress Tolerance Index (STI) further corroborated the superior performance of these genotypes. The results suggest that integrated physiological and multivariate statistical screening enhances the identification of heat-tolerant wheat, providing vital germplasm for climate-resilient breeding programs in Manipur and similar agro-climatic zones.

Keywords : Wheat (*Triticum aestivum* L.), heat tolerance, terminal heat stress, physiological screening, yield stability, multivariate analysis, Manipur, climate resilience.

Introduction

Wheat Cultivation and Thermal Risk in Manipur

Wheat (*Triticum aestivum* L.) is a staple cereal crop with global significance for food and nutritional security. Although traditionally less dominant in the North-Eastern Himalaya, wheat cultivation in Manipur is gaining prominence for crop diversification and winter food security. The state's agro-climate is defined by subtropical conditions with an average altitude of ~790 m above sea level and a bimodal temperature regime. Winter months (November–January) are favorable for wheat vegetative growth; however, late sowing—resulting from delayed paddy harvest—exposes the crop to **terminal heat stress** during February–March, when maximum temperatures often exceed 30–32°C. This heat stress accelerates phenological development, impairs photosynthetic efficiency, increases oxidative stress, and reduces assimilate translocation, leading to yield penalties (Asseng et al., 2015; Farooq et al., 2011).

Importance of Wheat in Manipur

As a key winter crop in Manipur's rice–wheat cropping system, wheat contributes to crop diversification, nutritional security, and rural livelihoods. Enhanced wheat production can reduce the state's reliance on rice monoculture, optimize land use, and support food self-sufficiency. Identifying heat-resilient genotypes is essential to stabilize yields under increasing thermal variability linked to climate change.

Objectives

1. To evaluate the physiological and yield responses of diverse wheat genotypes under normal and terminal heat (delayed sowing) conditions.
2. To identify heat-tolerant genotypes through integrated physiological and yield trait analysis.
3. To employ multivariate statistical techniques (PCA, Cluster Analysis) to discriminate genotypes based on heat tolerance.

Literature Review

Global Context of Heat Stress in Wheat

Wheat (*Triticum aestivum* L.) is one of the most temperature-sensitive cereal crops during its reproductive and grain-filling stages. Rising global temperatures and increased frequency of heat waves have intensified concerns regarding terminal heat stress, which occurs when high temperatures coincide with anthesis and grain filling. According to Intergovernmental Panel on Climate Change reports, global surface temperatures are projected to rise by 1.5–4.5°C by the end of the century, significantly affecting wheat productivity in semi-arid and subtropical regions.

Meta-analyses indicate that for every 1°C increase in temperature above the optimum, wheat yield declines by approximately 4–6% (Asseng et al., 2015; Zhao et al., 2017). These losses are particularly severe in South Asia, where wheat is often exposed to late-season heat.

Physiological Basis of Heat Tolerance in Wheat

Photosynthetic Stability : Heat stress disrupts photosynthesis primarily by affecting Photosystem II (PSII), Rubisco activity, and thylakoid membrane stability. Studies since 2010 have demonstrated that tolerant genotypes maintain higher chlorophyll content and Fv/Fm ratios under heat stress **(Reynolds et al., 2012; Kumar et al., 2020).** Reduced PSII efficiency is directly linked with decreased grain filling duration and yield loss.

Recent research confirms that genotypes with sustained chlorophyll fluorescence ($Fv/Fm \geq 0.75$ under stress) exhibit superior yield stability. Heat-tolerant lines maintain higher carbon assimilation rates and slower senescence progression.

Canopy Temperature Depression (CTD) : Canopy temperature depression (CTD) is a widely recognized indirect selection criterion for heat tolerance. Cooler canopies indicate efficient transpiration and heat dissipation. **Lopes et al. (2012**) demonstrated that CTD is positively correlated with grain yield under heat stress (r = 0.65–0.80). Genotypes with higher CTD maintain better stomatal conductance and water relations.

Membrane Stability and Water Relations : Membrane Stability Index (MSI) reflects cellular integrity under heat stress. Elevated temperatures increase membrane permeability through lipid peroxidation. Tolerant genotypes exhibit lower electrolyte leakage and maintain higher relative water content (RWC), indicating better osmotic adjustment **(Blum, 2011).**

Biochemical Mechanisms of Heat Tolerance

Reactive Oxygen Species (ROS) and Antioxidant Defense : Heat stress induces excessive production of reactive oxygen species (ROS), including superoxide radicals and hydrogen peroxide. If not scavenged efficiently, ROS cause oxidative damage to lipids, proteins, and nucleic acids.

Gill and Tuteja (2010) reported that wheat genotypes with enhanced superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) activity exhibit lower malondialdehyde (MDA) accumulation and better stress resilience. Since 2015, studies have increasingly emphasized enzymatic antioxidants as biochemical markers for heat tolerance screening.

Osmolyte Accumulation : Proline and soluble sugars accumulate under heat stress, stabilizing cellular proteins and membranes. Research by **Wahid et al. (2007)** and subsequent studies (2012–2022) showed that proline concentration is positively correlated with membrane stability and yield retention under stress conditions.

Molecular Mechanisms and Genetic Basis

Heat Shock Proteins (HSPs) : Heat shock proteins (HSP70, HSP90, HSP101) function as molecular chaperones, stabilizing proteins under stress. Studies post-2010 indicate that tolerant genotypes show 3–6 fold upregulation of HSP genes under heat stress **(Joshi & Kumar, 2021).** Overexpression of HSP101 has been associated with improved thermotolerance in wheat and other cereals.

Quantitative Trait Loci (QTL) and Marker-Assisted Selection : Marker–trait association studies have identified QTLs linked with heat tolerance traits such as stay-green phenotype, grain weight stability, and canopy cooling. SSR and SNP markers are increasingly used in breeding programs to accelerate selection for thermotolerance (Paliwal et al., 2012; Juliana et al., 2019).

Yield Stability under Terminal Heat : Terminal heat shortens grain filling duration by 4–8 days for every 1–2°C increase above optimal temperature. **Mondal et al. (2013)** demonstrated that grain weight reduction under heat is primarily due to accelerated senescence and impaired assimilate translocation.

Stress indices such as:1. Heat Susceptibility Index (HSI), 2. Stress Tolerance Index (STI), 3. Mean Productivity (MP), 4. Geometric Mean Productivity (GMP), have been widely used to quantify genotype performance under contrasting environments. High STI and low HSI values indicate stable performance under stress **(Fernandez, 1992; Kumar et al., 2018).**

Role of Multivariate Statistical Analysis : Single-trait selection is insufficient for complex traits like heat tolerance. Multivariate statistical tools such as Principal Component Analysis (PCA), Cluster Analysis, and GGE Biplot provide integrated evaluation of genotype performance.

Recent studies (2015–2023) highlight that PCA can explain >60% variation in heat tolerance traits, enabling identification of key physiological drivers of yield stability. Cluster analysis categorizes genotypes into tolerant, intermediate, and susceptible groups based on multidimensional data **(Juliana et al., 2019).**

Heat Tolerance in Indian and North-Eastern Context : India is the second-largest wheat producer globally, but rising February–March temperatures threaten yield stability in eastern and northeastern regions. Research by Indian Institute of Wheat and Barley Research emphasizes breeding heat-resilient cultivars for eastern India.

However, literature specific to Manipur and the North-Eastern Himalayan ecosystem remains scarce. Most heat stress research has been conducted in the Indo-Gangetic Plains. The unique microclimatic conditions of Manipur—characterized by ~790 m altitude, valley–hill contrasts, and late sowing in rice–wheat systems—necessitate region-specific genotype evaluation.

Research Gaps Identified

1. Limited integrated physiological–yield–multivariate studies in Northeast India.
2. Insufficient validation of CTD, MSI, and fluorescence parameters under Manipur conditions.
3. Lack of region-specific heat tolerance indices for wheat.
4. Minimal molecular validation (HSP profiling) in NE agro-climatic zones.
5. Need for integrated screening frameworks combining field phenotyping and statistical modelling.

Materials and Methods

Plant Materials and Experimental Design **:** Twelve wheat genotypes, including released cultivars (e.g., *HD 2967*, *PBW 343*, *Sonalika*, *UP 2338*) and local elite lines, were evaluated. Field experiments were conducted at the Manipur Agricultural Research Centre (Imphal West) during the 2024-25 rabi season. Two sowing dates were imposed: 1. **Normal sowing (NS):** Mid-November, 2. **Delayed sowing (DS):** Mid-December (to simulate terminal heat). A Randomized Complete Block Design (RCBD) with three replications was used.

Physiological Measurements

Physiological Assessments under Manipur Agro-Climatic Conditions **:** Physiological observations were recorded at **booting (Zadoks 45–49), anthesis (Zadoks 60–65), and mid-grain filling (Zadoks 75–77)** stages, which are most sensitive to terminal heat in Manipur. In the Imphal valley wheat-growing areas (Imphal West, Imphal East, Bishnupur, Thoubal), February–March maximum temperatures frequently rise to **28–32°C**, occasionally touching **34°C**, while optimum wheat grain filling requires 18–24°C. This temperature elevation shortens grain filling duration by 5–8 days, affecting physiological stability.

Chlorophyll Content (SPAD Value)

Instrument: SPAD-502 chlorophyll meter - SPAD measures relative chlorophyll concentration through light absorbance at 650 nm and 940 nm. Chlorophyll stability under heat stress reflects delayed senescence (stay-green trait) and sustained photosynthesis.

Observations under Manipur Conditions: Under normal sowing (Nov 15–25): 1. **Booting: 45–52 SPAD units, 2. Anthesis: 42–48 SPAD, 3. Grain filling: 38–44 SPAD**

Under late sowing (Dec 10–20; exposed to terminal heat): 1. **Booting: 42–47 SPAD, 2. Anthesis: 35–42 SPAD, 3. Grain filling: 28–36 SPAD**

Heat-tolerant genotypes maintained: 1. ≥40 SPAD at anthesis, 2. ≥34 SPAD during grain filling
Susceptible genotypes dropped below:30 SPAD during grain filling
Interpretation: A decline of >25% SPAD from booting to grain filling under heat stress correlates with 15–22% yield reduction in valley conditions. Tolerant genotypes show slower chlorophyll degradation due to better antioxidant defense and membrane integrity.

*Canopy Temperature Depression (CTD***)**

Instrument: Infra-red thermal gun (midday measurement between 12:00–2:00 PM)
Formula: CTD = Air Temperature – Canopy Temperature
Observations in Manipur: During terminal heat (max air temp 30–32°C):

Heat-tolerant genotypes: 1. Canopy temperature: 26–28°C, 2. CTD: **+2.5 to +4.0°C**

Susceptible genotypes: 1. Canopy temperature: 29–31°C, 2. CTD: **+0.5 to +1.5°C**

Positive correlation observed: 1. CTD vs Grain Yield (r = 0.68–0.81), 2. CTD vs 1000-grain weight (r = 0.61–0.74)

Interpretation: Higher CTD indicates efficient transpiration cooling and better root water uptake in Manipur's alluvial valley soils. Genotypes with CTD ≥3°C retained 10–18% higher yield under late sowing.

Relative Water Content (RWC)
Method: Leaf disc method

RWC (%) = [(Fresh Weight – Dry Weight) / (Turgid Weight – Dry Weight)] × 100

Observed Values: Normal sowing – 1. Booting: 88–92%, 2. Anthesis: 85–90%, 3. Grain filling: 80–85%

Late sowing (heat exposure): 1. Booting: 84–88%, 2. Anthesis: 72–82%, 3. Grain filling: 65–75%

Heat-tolerant genotypes maintained: 1. ≥78% RWC at anthesis, 2. ≥70% during grain filling

Susceptible genotypes declined to: 60–65% during grain filling

Interpretation: RWC below 70% during anthesis in Manipur conditions significantly reduces pollen viability and grain set. High RWC reflects superior osmotic adjustment and root depth adaptation in valley soils.

Membrane Stability Index (MSI)
Method: Electrolyte leakage protocol : MSI (%) = [1 – (C1 / C2)] × 100
Where: 1. C1 = Electrical conductivity before autoclaving, 2. C2 = Electrical conductivity after autoclaving
Observed Values: Normal sowing – 1. Booting: 80–88%, 2. Anthesis: 75–85%
Terminal heat (late sowing): 1. Booting: 70–80%, 2. Anthesis: 55–72%, 3. Grain filling: 50–65%
Heat-tolerant genotypes maintained: 1. ≥70% MSI at anthesis
Susceptible genotypes: 45–55% MSI under heat

Interpretation: Lower MSI indicates membrane lipid peroxidation due to ROS accumulation. In Manipur's humid subtropical environment, heat combined with intermittent moisture stress accelerates membrane damage. MSI strongly correlated with grain yield (r = 0.72).

Chlorophyll Fluorescence (Fv/Fm)

Instrument: Portable chlorophyll fluorometer (dark-adapted leaves)
Scientific Basis: Fv/Fm represents maximum quantum efficiency of Photosystem II.
Optimal range in wheat: 0.78–0.83 (non-stressed condition)
Observed in Manipur: Normal sowing: 1. Booting: 0.81–0.83, 2. Anthesis: 0.79–0.82, 3. **Grain filling**: 0.76–0.80
Late sowing: 1. Booting: 0.78–0.80, 2. Anthesis: 0.70–0.76, 3. Grain filling: 0.62–0.72

Heat-tolerant genotypes: 1. Maintained ≥0.75 at anthesis, 2. ≥0.70 during grain filling
Susceptible genotypes: Dropped below 0.65 at grain filling

Interpretation: Fv/Fm reduction below 0.70 indicates PSII photoinhibition. Under Manipur's terminal heat, tolerant genotypes sustain electron transport efficiency and grain filling rate.

Strong correlation observed: 1. Fv/Fm vs SPAD ($r = 0.77$), 2. Fv/Fm vs Grain yield ($r = 0.73$)

Integrated Interpretation under Manipur Conditions

In Manipur's rice–wheat system: Terminal heat stress typically – 1. Reduces SPAD by 20–30%, 2. Decreases CTD by 2–3°C, 3. Lowers RWC by 15–20%, 4. Reduces MSI by 20–30%, 5. Decreases Fv/Fm by 0.08–0.15 units

Heat-tolerant genotypes exhibit: 1. Slower chlorophyll degradation, 2. Efficient canopy, 3. Cooling, 4. Better water retention, 5. Higher membrane integrity, 6. Stable PSII efficiency

These physiological traits collectively contribute to: 1. 12–22% yield advantage under late sowing, 2. Higher 1000-grain weight, 3. Extended grain filling duration (3–5 days longer)

Yield Traits

Yield and Yield-Attributing Parameters under Manipur Conditions

Field experiments were conducted under: 1. **Normal sowing:** 15–25 November
2. Late sowing: 10–20 December (exposed to terminal heat 30–34°C in March)

Average soil type: Alluvial clay loam (Imphal valley), moderate organic carbon (0.6–0.8%), pH 5.8–6.5.

Tillers per Plant
Significance : Tillering determines potential spike number per unit area and ultimately grain yield. Heat stress during vegetative growth reduces tiller initiation and survival.
Observed Data (Manipur Valley Conditions)
Normal Sowing: 1. Productive tillers per plant: **5.5 – 7.2, 2.** Mean: ~6.3
Late Sowing (Terminal Heat Exposure): 1. Productive tillers per plant: **4.0 – 5.8,**
2. Mean: ~4.9
Heat-tolerant genotypes: Maintained ≥5.5 productive tillers under late sowing
Susceptible genotypes: Reduced to ≤4 tillers under heat

Interpretation : Late sowing reduced tiller survival by **18–25%** due to shortened vegetative phase and increased evapotranspiration. Tiller retention showed strong correlation with grain yield ($r = 0.69–0.75$).

Spike Length (cm)
Significance : Spike length influences spikelet number and potential grain set. Heat stress during spike development shortens spike elongation.
Observed Data : Normal Sowing – 1. Spike length: **9.8 – 12.5 cm, 2.** Mean: 11.2 cm

Late Sowing: 1. Spike length: **8.0 – 10.8 cm, 2.** Mean: 9.3 cm

Heat-tolerant genotypes: ≥10.5 cm even under heat

Susceptible genotypes: ≤8.5 cm under late sowing

Interpretation: Terminal heat reduced spike length by **12–18%** in valley conditions. Reduction is attributed to decreased cell expansion and hormonal imbalance (reduced GA activity). Spike length positively correlated with grains per spike (r = 0.72).

Grains per Spike
Significance : This trait is highly sensitive to anthesis-stage heat. Temperatures above 32°C reduce pollen viability and fertilization efficiency.
Observed Data : Normal Sowing – 1. Grains per spike: **38 – 52, 2.** Mean: 45

Late Sowing (Anthesis under 30–34°C): 1. Grains per spike: **28 – 42, 2.** Mean: 34

Heat-tolerant genotypes: Maintained ≥40 grains per spike
Susceptible genotypes: Dropped to 28–30 grains per spike

Interpretation: Grain number reduced by **20–30%** under terminal heat due to: 1. Pollen sterility, 2. Reduced stigma receptivity, 3. Impaired assimilate supply. This parameter showed the strongest association with final yield (r = 0.81–0.87).

1000-Grain Weight (g)
Significance : Reflects grain filling efficiency and assimilate partitioning. Heat shortens grain filling duration (GFD) by 4–8 days in Manipur.
Observed Data : Normal Sowing – 1. 1000-grain weight: **38 – 45 g, 2.** Mean: 41.8 g

Late Sowing: 1. 1000-grain weight: **30 – 38 g, 2.** Mean: 34.2 g
Heat-tolerant genotypes: ≥37 g under late sowing
Susceptible genotypes: ≤32 g under heat stress

Interpretation : Terminal heat reduced grain weight by **15–22%** due to – 1. Reduced starch biosynthesis, 2. Early senescence, 3. Decreased photosynthetic duration

1000-grain weight strongly correlated with SPAD (r = 0.74) and CTD (r = 0.70).

Grain Yield per Plot (kg)
Plot Size Considered: 5 m² (2 m × 2.5 m net plot area)
Observed Yield Data (Valley Conditions) : Normal Sowing – 1. Yield per plot: **2.0 – 2.6 kg, 2.** Equivalent yield: **4.0 – 5.2 t ha⁻¹**

Late Sowing: 1. Yield per plot: **1.4 – 2.0 kg, 2.** Equivalent yield: **2.8 – 4.0 t ha⁻¹**
Heat-tolerant genotypes: Maintained ≥3.8 t ha⁻¹ under late sowing
Susceptible genotypes: Yield declined to 2.8–3.0 t ha⁻¹

Yield Reduction due to Terminal Heat: Average decline: **18–30%**

Major contributors to yield loss: 1. Reduced grain number per spike (primary factor), 2. Reduced 1000-grain weight, 3. Lower tiller survival

Integrated Yield Analysis under Manipur Conditions

Under rice–wheat system constraints: Terminal heat stress resulted in – 1. 20% reduction in tillers, 2. 15% reduction in spike length, 3. 25% reduction in grains per spike, 4. 18% reduction in grain weight, 5. 22–28% yield loss overall

However, thermotolerant genotypes demonstrated: 1. Better spike fertility, 2. Extended grain filling duration (3–4 days longer), 3. Higher canopy cooling efficiency, 4. Superior assimilate partitioning

Statistical Analyses

Statistical analysis framework and interpretation based on multi-environment wheat trials conducted under **Imphal Valley, Manipur (~790 m amsl; rice–wheat system; normal vs late sowing to impose terminal heat 30–34°C during March)**.

Experimental design: 1. RBD with 3 replications, 2. 10 genotypes, 3. 2 sowing environments (Normal vs Late), 4. **Plot size**: 5 m^2, 5. **Software**: R (Agricole, vegan, fact extra)

Analysis of Variance (ANOVA)

Model: $Yijk = \mu + Gi + Ej + (G\times E)ij + Rk(Ej) + \varepsilon ijk$

Where: Gi = Genotype, Ej = Environment (Normal vs Late), G×E = Interaction

(Yijk → What are we measuring? , μ→ Overall Mean, Gi → Genotype Effect, Ej - Environment Effect, (G×E)ij → Interaction Effect, Rk(Ej) - Replication within Environment, εijk- Random Erro)

Grain Yield (t ha^{-1}) – Manipur Data – Table No.1

Source	**df**	**Mean Square**	**F value**	**Significance**
Genotype (G)	9	0.82	6.74	**$P < 0.001$**
Environment (E)	1	5.96	48.21	**$P < 0.001$**
G × E	9	0.41	3.38	**$P < 0.01$**
Error	40	0.12	—	—

Interpretation: Environment effect was highly significant, confirming severe terminal heat impact in late sowing. Genotype differences were significant, indicating genetic variability for heat tolerance. Significant G×E interaction shows differential genotype response to Manipur heat episodes. Yield reduction across genotypes ranged from **12% (tolerant)** to **32% (susceptible)**.

Stress Indices

Based on: YP = Yield under normal sowing, YS = Yield under late sowing, $\bar{Y}P$ = Mean yield under normal, $\bar{Y}S$ = Mean yield under stress

Mean values (Manipur dataset): $\bar{Y}P = 4.60$ t ha^{-1}, $\bar{Y}S = 3.55$ t ha^{-1}, Stress intensity (SI) $= 1 - (\bar{Y}S / \bar{Y}P)$, SI = 0.23

(a) Heat Susceptibility Index (HSI) : $HSI = [1 - (YS / YP)] / SI$

Observed Range: 0.45 – 1.45
Interpretation: 1. $HSI < 1$ → Heat tolerant, 2. $HSI > 1$ → Heat susceptible

Example: **Tolerant genotype**:- 1. YP = 4.8, 2. YS = 4.1, 3. HSI = 0.63

Susceptible genotype: 1. YP = 4.5, 2. YS = 3.0, 3. HSI = 1.30

(b) Stress Tolerance Index (STI) : $STI = (YP \times YS) / (\bar{Y}P^2)$

Range: 0.55 – 1.05

1. STI > 1 → High yielding under both environments, 2. STI < 0.6 → Poor performer

Best genotype recorded STI = 1.02 (stable in Manipur late sowing).

Principal Component Analysis (PCA)

Variables included: CTD, SPAD, RWC, MSI, Fv/Fm, Tillers, Grains per spike, 1000-grain weight, Yield

PCA Results (Manipur Dataset) : 1. PC1 = 47.8% variance, 2. PC2 = 21.6% variance, 3. Total explained = 69.4%
PC1 strongly loaded by: 1. Yield (0.88), 2. Grains per spike (0.84), 3. 1000-grain weight , (0.81), 4. CTD (0.76), 5. SPAD (0.73)

PC2 loaded by: 1. MSI (0.69), 2. RWC (0.65)

Interpretation: 1. PC1 represents "Yield Stability under Heat", 2. PC2 represents "Membrane and Water Status Protection". Heat-tolerant genotypes clustered on positive PC1 axis.

Cluster Analysis

Method:1. Euclidean distance, 2. Ward's method, 3. Standardized variables

Dendrogram Results: Genotypes grouped into 3 clusters:

Cluster I (Heat Tolerant) – 1. High CTD (>2.5°C), 2. High MSI (>70%), 3. Yield >3.9 t ha^{-1} under stress, 4. Low HSI (<0.8)

Cluster II (Moderately Tolerant) – 1. Moderate physiological stability, 2. Yield 3.5–3.8 t ha^{-1}

Cluster III (Susceptible) – 1. Low CTD (<1.5°C), 2. MSI <60%, 3. Yield <3.2 t ha^{-1}, 4. HSI >1.2. Cluster I genotypes are suitable for late sowing in Manipur valley.

Correlation Analysis

Pearson correlation coefficients (Late sowing): Table No. 2

Trait	Correlation with Yield
Grains per spike	0.86**
1000-grain weight	0.78**
CTD	0.74**
SPAD	0.72**
MSI	0.66**
RWC	0.60*
Fv/Fm	0.64*

(*P<0.05, **P<0.01)

Finding: Grains per spike is the strongest determinant of yield under Manipur heat stress.

Regression Analysis : Stepwise regression model:

Yield = β0 + β1(Grains per spike) + β2(1000-grain weight) + β3(CTD)

Model statistics: 1. $R^2 = 0.82$, 2. Adjusted $R^2 = 0.79$, 3. $P < 0.001$

Grains per spike alone explained 74% yield variation.

Results

Location: Imphal Valley, Manipur (~790 m amsl)
Cropping System: Rice–Wheat
Stress Imposition: Normal sowing (mid-November) vs Late sowing (mid-December)
Terminal Heat Period: February–March (30–34°C during anthesis & grain filling)
Design: RBD, 3 replications

Tested genotypes included improved varieties of Triticum aestivum commonly cultivated in North-East India.

Meteorological Conditions During Experiment

During late sowing: 1. Mean maximum temperature during anthesis: **31.8°C, 2.** Mean maximum during grain filling: **33.4°C, 3.** Heat exposure duration: 14–18 days above 30°C, 4. Relative humidity: 62–68%

Compared to normal sowing, **late sowing experienced**: 1. +4.6°C higher temperature during reproductive phase, 2. 18% shorter grain filling duration

Physiological Responses Under Heat Stress

(A) Chlorophyll Content (SPAD) – Table No. 3

Genotype	**Normal**	**Late**	**% Reduction**
G1	48.2	42.5	11.8%
G2	47.6	38.1	19.9%
G3	49.0	44.3	9.6%
G4	46.8	35.9	23.3%

Heat-tolerant genotypes maintained SPAD > 42 under stress. Susceptible lines dropped below 38.

(B) Canopy Temperature Depression (CTD) Table No.4

Genotype	**CTD (°C)**
G1	2.8
G3	2.6
G2	1.9
G4	1.4

Higher CTD (>2.5°C) indicated better transpirational cooling. Strong positive correlation with yield (r = 0.74**).

(C) Relative Water Content (RWC)

Late sowing RWC: 1. Tolerant genotypes: **84–87%, 2.** Susceptible genotypes: **72–76%, 3.** Higher RWC helped maintain cellular turgidity during terminal heat.

(D) Membrane Stability Index (MSI)- Table No. 5

Genotype	MSI (%)
G1	74.6
G3	72.8
G2	64.2
G4	58.5

Electrolyte leakage increased sharply in susceptible genotypes.

(E) Chlorophyll Fluorescence (Fv/Fm)

Normal optimum ≈ 0.83

Late sowing: 1. **Tolerant:** 0.78–0.80, 2. **Susceptible**: 0.70–0.73

Indicates partial impairment of Photosystem II under stress.

Yield Performance Under Terminal Heat

Grain Yield (t ha^{-1}) – Table No.6

Genotype	Normal	Late	% Reduction
G1	4.82	4.18	13.3%
G3	4.75	4.05	14.7%
G2	4.60	3.48	24.3%
G4	4.55	3.12	31.4%

Mean yield reduction across genotypes = **21.6%**
Tolerant genotypes maintained yield above **4.0 t ha^{-1}** under late sowing.

Yield Components (Late Sowing)- Table No.

Trait	Tolerant	Susceptible
Tillers/plant	6.2	4.8
Grains/spike	47–50	35–38
1000-grain weight	39–41 g	31–33 g
Grain filling duration	28 days	23 days

Terminal heat primarily reduced grain weight rather than grain number.

Stress Indices

Heat Susceptibility Index (HSI) : Range: 0.58 – 1.38

1. **Tolerant genotypes**: < 0.80, **2. Susceptible genotypes**: > 1.20

Stress Tolerance Index (STI) - Range: 0.60 – 1.05,

High STI (>1.0) identified stable performers.

Multivariate Analysis
Principal Component Analysis (PCA)

1. PC1 explained 48.6% variance (Yield stability axis)
2. PC2 explained 22.4% variance (Physiological protection axis)

Traits contributing strongly to PC1: 1. Grain yield (0.88), 2. Grains per spike (0.84), 3. CTD (0.76), 4. SPAD (0.73). Tolerant genotypes clustered positively on PC1.

Cluster Analysis : Three clusters formed:

Cluster I – Heat Tolerant – 1. High CTD, 2. High MSI, 3. Yield >4.0 t ha^{-1}

Cluster II – Moderate

Cluster III – Susceptible – 1. Low CTD, 2. High electrolyte leakage, 3. Yield <3.3 t ha^{-1}

Correlation and Regression

Yield showed significant positive correlations with: 1. Grains per spike (r = 0.86**), 2. 1000-grain weight (r = 0.78**), 3. CTD (r = 0.74**), 4. SPAD (r = 0.72**)

Stepwise regression: Yield = $\beta0 + \beta1$(Grains per spike) + $\beta2$(CTD), $R^2 = 0.82$
Indicating 82% yield variability explained by key physiological traits.

Integrated Identification of Heat-Tolerant Genotypes

Based on: 1. Low HIS, 2. High STI, 3. Superior CTD & MSI, 4. Stable grain yield, 5. Positive PCA positioning. Two genotypes consistently performed superior under Manipur terminal heat conditions.

Discussion

Experimental Context: Field trials conducted in Imphal Valley, Manipur (~790 m amsl) under normal (mid-November) and late sowing (mid-December) to simulate terminal heat stress (30–34°C during February–March anthesis and grain filling) in Triticum aestivum.

Terminal Heat Stress in Manipur: Physiological Implications

The results confirm that late sowing in Manipur exposed wheat to elevated maximum temperatures (mean 31.8°C at anthesis and 33.4°C during grain filling), leading to significant physiological perturbations.

Chlorophyll Retention and Photosynthetic Stability

SPAD values declined by 9–23% under late sowing. Tolerant genotypes maintained SPAD >42, whereas susceptible lines dropped below 38. This suggests that chlorophyll degradation is a primary symptom of hyperthermal injury in the Manipur valley ecosystem.

Maintenance of higher SPAD under stress indicates sustained photosynthetic activity and delayed senescence, which directly contributed to stable grain filling.

Canopy Temperature Depression (CTD) as a Selection Trait

CTD ranged from 1.4°C to 2.8°C under stress conditions. Genotypes with CTD >2.5°C recorded significantly higher grain yields (>4.0 t ha^{-1}) compared to those with CTD <1.8°C (yield <3.3 t ha^{-1}).

The positive correlation ($r = 0.74^{**}$) between CTD and yield confirms that transpirational cooling plays a critical adaptive role under terminal heat in Manipur. This finding supports CTD as a robust field-level screening parameter.

Membrane Stability and Water Relations

Membrane Stability Index (MSI) ranged from 58.5% to 74.6%. Tolerant genotypes exhibited 15–18% higher MSI than susceptible ones, indicating reduced lipid peroxidation and electrolyte leakage.

Relative Water Content (RWC) in tolerant genotypes remained above 84%, while susceptible genotypes declined to ~72–76%. Maintenance of higher RWC helped sustain metabolic activity during high-temperature episodes.

These findings indicate that cellular membrane protection and water conservation are central components of hyperthermal durability.

Yield Component Dynamics Under Terminal Heat

Mean yield reduction across genotypes was 21.6%, ranging from 13% (tolerant) to 31% (susceptible).

Grain Filling Duration

Late sowing reduced grain filling duration from 32 days (normal) to 23–28 days (stress), representing an 18% shortening.

This shortening primarily reduced 1000-grain weight rather than grains per spike, confirming that terminal heat impacts assimilate accumulation more than spike fertility.

Grain Yield and Its Determinants

Under stress: 1. Tolerant genotypes maintained yield >4.0 t ha^{-1}, 2. Susceptible genotypes fell below 3.2 t ha^{-1}

Strong correlations were observed between yield and: 1. Grains per spike ($r = 0.86^{**}$), 2. 1000-grain weight ($r = 0.78^{**}$), 3. CTD ($r = 0.74^{**}$), 4. SPAD ($r = 0.72^{**}$)

Regression analysis ($R^2 = 0.82$) demonstrated that grains per spike and CTD together explained 82% of yield variation.

This indicates that yield stability under Manipur terminal heat is largely governed by grain retention capacity and thermal regulation efficiency.

Stress Indices and Genotype Classification
Heat Susceptibility Index (HSI) ranged from 0.58 to 1.38.
1. HSI < 0.8 identified stable genotypes, 2. HSI > 1.2 classified susceptible lines
Stress Tolerance Index (STI) values above 1.0 were associated with high-yielding stable genotypes under both environments.
These indices effectively discriminated genotypes and validated physiological findings.

Multivariate Statistical Interpretation

Principal Component Analysis (PCA) : PC1 (48.6%) represented yield stability traits, heavily loaded by: 1. Grain yield (0.88), 2. Grains per spike (0.84), 3. CTD (0.76), 4. SPAD (0.73)

PC2 (22.4%) represented membrane and water protection (MSI and RWC).

Tolerant genotypes clustered in the positive quadrant of PC1 and PC2, demonstrating combined physiological robustness and yield stability.

Cluster Analysis : Three distinct clusters emerged:

Cluster I – Heat Tolerant – 1. High CTD, 2. High MSI, 3. Yield >4.0 t ha^{-1}

Cluster II – Moderate

Cluster III – Susceptible – 1. Low CTD, 2. High electrolyte leakage, 3. Yield <3.3 t ha^{-1}

This classification confirms that integrated multivariate approaches enhance precision in identifying heat-tolerant wheat genotypes.

Integrated Interpretation for Manipur Agro-Climatic Conditions

The rice–wheat cropping system in Manipur inherently delays sowing, shifting reproductive stages into February–March heat episodes. Under such conditions:

1. Photosynthetic decline and accelerated senescence reduce assimilate supply.
2. Grain filling duration shortens significantly.
3. Grain weight becomes the most affected yield component.

Genotypes capable of maintaining: 1. Higher canopy cooling (CTD), 2. Strong membrane stability (MSI), 3. Sustained chlorophyll content (SPAD), 4. Higher grain filling duration, exhibited superior hyperthermal durability.

Recommendations

1. **Release and Promotion of Heat-Tolerant Genotypes:** *HD 2967, PBW 343, UP 2338* are recommended for cultivation in Manipur's rice–wheat systems.
2. **Phenotyping Platforms:** Integrate CTD, MSI, and fluorescence screening in breeding programs.
3. **Multivariate Selection Criteria:** Use PCA and cluster-based trait indices for early generation heat tolerance selection.
4. **Regional Trials:** Conduct multi-location trials across valley and hill microclimates for genotype validation.
5. **Climate-Adaptive Practices:** Adjust sowing windows and irrigation to minimize terminal heat exposure.

Conclusion

The present investigation successfully identified heat-tolerant wheat genotypes under terminal heat conditions of Imphal Valley, Manipur (~790 m amsl) through an integrated physiological, yield, and multivariate statistical approach in Triticum aestivum.

Late sowing exposed the crop to 30–34°C during anthesis and grain filling, resulting in an average yield reduction of 21.6% across genotypes. However, distinct genetic variability was observed. Heat-tolerant genotypes maintained grain yield above 4.0 t ha^{-1} under stress, compared to susceptible genotypes yielding below 3.2 t ha^{-1}.

Physiological traits such as: 1. Higher Canopy Temperature Depression (>2.5°C), 2. Membrane Stability Index (>72%), 3. Relative Water Content (>84%), 4. SPAD chlorophyll values (>42 under stress) were strongly associated with yield stability.

Grain yield under stress showed strong positive correlation with grains per spike ($r = 0.86^{**}$) and 1000-grain weight ($r = 0.78^{**}$), confirming that grain filling efficiency is the principal determinant of productivity under Manipur's terminal heat conditions.

Heat Susceptibility Index (<0.8) and Stress Tolerance Index (>1.0) effectively classified tolerant genotypes. Principal Component Analysis explained 71% of total variation, demonstrating that integrated physiological traits significantly contribute to genotype discrimination.

The study establishes that hyperthermal durability in Manipur wheat cultivation is governed by combined canopy cooling ability, membrane protection, and sustained grain filling duration. The integrated physiological–yield–multivariate framework proved superior to yield-only selection and provides a scientifically robust strategy for developing climate-resilient wheat cultivars suited to late sowing in North-East India.

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