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Physiological And Biochemical Mechanisms Underlying Hyperthermal Durability in Wheat (*Triticum Aestivum* L.) under North-East Indian Agro-Climatic Conditions

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Abstract

Rising global temperatures and frequent terminal heat stress events pose a serious threat to wheat productivity, particularly in vulnerable agro-climatic zones such as North-East India. Elevated temperatures during anthesis and grain filling accelerate senescence, impair photosynthetic efficiency, destabilize cellular membranes, and reduce yield stability. Despite increasing concern, limited integrated studies have simultaneously examined physiological and biochemical determinants of heat tolerance under this regional context. The present investigation aimed to identify key physiological and antioxidant biomarkers conferring hyperthermal durability in wheat (*Triticum aestivum* L.).

A field experiment was conducted using a Randomized Block Design (RBD) with contrasting sowing environments (early vs. late sowing) to impose natural terminal heat stress. Physiological traits including chlorophyll fluorescence (Fv/Fm), membrane stability index (MSI), and relative water content (RWC) were measured alongside biochemical markers such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), malondialdehyde (MDA), and proline accumulation. Yield parameters were recorded at maturity. Data were analyzed using ANOVA to detect varietal differences, Pearson's correlation to assess trait associations, and Principal Component Analysis (PCA) to identify major contributors to variability.

Heat-tolerant genotypes maintained significantly higher Fv/Fm ratios, MSI, RWC, and antioxidant enzyme activities, while exhibiting reduced lipid peroxidation (MDA accumulation) under late-sown hyperthermal conditions. Strong positive correlations were observed between antioxidant activity and grain yield, whereas MDA showed negative associations with productivity traits. PCA revealed that physiological stability and antioxidant efficiency collectively explained the majority of total variation, clearly discriminating tolerant from susceptible genotypes.

Keywords: Hyperthermal stress, Wheat physiology, Antioxidant enzymes, Membrane stability, Yield stability, Climate resilience

Global Importance of Wheat : Wheat (*Triticum aestivum* L.) is one of the most strategically important cereal crops worldwide, serving as a primary staple for nearly 35–40% of the global population. It contributes substantially to daily caloric and protein intake, particularly in South Asia, Europe, North Africa, and parts of the Middle East. Globally, wheat occupies more land area than any other food crop and plays a central role in ensuring food security, nutritional stability, and rural livelihoods. In countries like India, wheat forms the backbone of national food distribution systems and buffer stock policies, highlighting its socio-economic as well as agronomic significance.

However, rising global temperatures associated with climate change pose a serious threat to wheat productivity. Wheat is particularly sensitive to temperature fluctuations during reproductive and grain-filling stages. Optimal grain filling occurs within a narrow thermal range (approximately 15–22°C). Exposure to temperatures above 30°C during anthesis and grain development accelerates physiological maturity, shortens the grain filling duration, disrupts photosynthetic efficiency, and impairs assimilate translocation. Consequently, high temperature stress leads to shrivelled grains, reduced thousand-grain weight, decreased starch accumulation, and overall yield decline.

Terminal heat stress is especially detrimental in late-sown wheat systems, where elevated temperatures coincide with the critical grain development phase. Studies indicate that for every 1°C rise in mean temperature during grain filling, wheat yield may decline by 3–6%, depending on genotype and environment. Furthermore, heat stress alters grain protein composition, affecting end-use quality parameters.

Heat Stress and Plant Physiology : In wheat (*Triticum aestivum* L.), exposure to supra-optimal temperatures—particularly during reproductive and grain-filling stages—induces profound physiological disturbances at cellular, biochemical, and whole-plant levels.

Disruption of Photosynthesis : Photosynthesis is highly temperature-sensitive, and heat stress directly impairs both photochemical and biochemical processes. Elevated temperatures destabilize the thylakoid membranes within chloroplasts, leading to structural disorganization and reduced efficiency of Photosystem II (PSII). One of the earliest indicators of thermal injury is a decline in the maximum quantum efficiency of PSII (Fv/Fm ratio), reflecting photoinhibition and impaired electron transport.

High temperature also reduces chlorophyll content and alters the activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), the key enzyme responsible for carbon fixation. Thermal stress can denature Rubisco activase, thereby limiting CO_2 assimilation and increasing photorespiration. As a result, the net photosynthetic rate declines, leading to reduced carbohydrate synthesis and limited assimilate supply to developing grains. This shortened grain filling duration ultimately contributes to reduced thousand-grain weight and yield instability.

Membrane Injury and Reactive Oxygen Species (ROS) Production : At the cellular level, high temperature increases membrane fluidity and disrupts lipid bilayer stability. The integrity of cellular membranes is compromised due to enhanced lipid peroxidation, resulting in electrolyte leakage and decreased membrane stability index (MSI). Membrane damage is closely associated with the overproduction of reactive oxygen species (ROS), including superoxide radicals ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (•OH).

Under normal conditions, ROS are produced at low levels as by-products of metabolic processes and function as signaling molecules. However, heat stress accelerates ROS generation beyond the detoxification capacity of the antioxidant defense system, leading to oxidative stress. Excess ROS damage proteins, nucleic acids, lipids, and chloroplast structures, further aggravating photosynthetic inhibition.

Lipid peroxidation, often quantified through malondialdehyde (MDA) accumulation, serves as a reliable biochemical marker of oxidative damage. Heat-tolerant genotypes typically exhibit enhanced antioxidant enzyme activities—such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD)—which mitigate ROS toxicity and preserve cellular integrity.

Biochemical Defense Mechanisms: Plants exposed to hyperthermal stress activate a complex biochemical defense network to counteract oxidative injury and maintain cellular homeostasis. In wheat (*Triticum aestivum* L.), thermotolerance is largely determined by the efficiency of antioxidant enzymes, Osmo protectant accumulation, and regulation of lipid peroxidation. These biochemical responses serve both protective and signaling functions, enabling plants to sustain metabolic activity under elevated temperature regimes.

Antioxidant Enzymes (SOD, CAT, POD) : Heat stress enhances the generation of reactive oxygen species (ROS), including superoxide radicals ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (•OH). If unchecked, ROS cause oxidative damage to proteins, membranes, and nucleic acids. To mitigate this, plants activate an enzymatic antioxidant defense system.

Superoxide dismutase (SOD) constitutes the first line of defense, catalyzing the dismutation of superoxide radicals into hydrogen peroxide and molecular oxygen:

$$2O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2 + O_2$$

Hydrogen peroxide, though less reactive than superoxide, is still harmful at high concentrations. It is detoxified by catalase (CAT) and peroxidase (POD). Catalase decomposes H_2O_2 into water and oxygen:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Peroxidase enzymes utilize H_2O_2 to oxidize phenolic substrates, thereby reducing its accumulation. Enhanced activities of SOD, CAT, and POD in heat-tolerant genotypes maintain ROS homeostasis and protect photosynthetic machinery.

Osmolyte Accumulation (Proline) : In addition to enzymatic defenses, wheat plants accumulate compatible solutes or osmolytes under heat stress. Proline is one of the most important Osmo protectants. Its accumulation contributes to osmotic adjustment, stabilization of proteins and membranes, maintenance of cellular redox balance, and scavenging of free radicals.

Proline acts as a molecular chaperone, protecting enzyme conformation and preserving chloroplast function under thermal stress. It also serves as a reservoir of carbon and nitrogen that can be mobilized during recovery. Elevated proline concentration is therefore frequently associated with enhanced thermotolerance and improved relative water content (RWC).

Lipid Peroxidation and Malondialdehyde (MDA) : One of the most critical consequences of oxidative stress is lipid peroxidation—the oxidative degradation of membrane lipids. Polyunsaturated fatty acids within membrane bilayers are highly susceptible to ROS attack, leading to loss of membrane fluidity and permeability control.

Malondialdehyde (MDA) is a major end-product of lipid peroxidation and is widely used as a biochemical indicator of oxidative membrane damage. Increased MDA content reflects enhanced cellular injury and compromised membrane stability. Heat-sensitive genotypes typically exhibit higher MDA accumulation, whereas tolerant genotypes maintain lower levels due to efficient antioxidant activity.

Regional Research Gap : North-East India region is characterized by unique topographical heterogeneity, fluctuating temperature regimes, high humidity, and distinct soil profiles. Unlike the Indo-Gangetic Plains, where extensive heat stress studies have been conducted, systematic and integrated investigations under Eastern Himalayan conditions remain limited.

Limited Integrated Studies in Eastern Himalayan Agro-Climate: Hyperthermal stress is a multidimensional phenomenon affecting photosynthesis, membrane stability, antioxidant metabolism, osmotic regulation, and grain development simultaneously. The absence of integrated field-based assessments combining physiological (e.g., chlorophyll fluorescence, relative water content, membrane stability index) and biochemical markers (e.g., antioxidant enzymes, proline, lipid peroxidation) restricts comprehensive understanding of genotype performance under regional climatic variability.

In the Eastern Himalayan belt, late sowing and erratic temperature fluctuations often expose wheat crops to terminal heat stress during anthesis and grain filling. Yet, region-specific validation of thermotolerant genotypes under natural stress imposition is scarce. The interaction between altitude, microclimate, and heat stress physiology has not been adequately quantified. Moreover, most national breeding programs prioritize performance in major wheat belts, potentially overlooking adaptive traits required for this ecologically distinct zone.

Need for Genotype-Based Physiological Validation : While several wheat varieties are reported as "heat tolerant" based on performance in conventional wheat-growing regions, their physiological resilience under Eastern Himalayan conditions remains largely unverified. Genotype × environment interactions can significantly alter stress responses, particularly in traits related to photosynthetic efficiency, antioxidant capacity, and osmotic adjustment.

There is an urgent need for genotype-based physiological validation that integrates – 1.Chlorophyll fluorescence (Fv/Fm) as an indicator of PSII stability, 2. Membrane stability index (MSI) to assess cellular integrity, 3. Antioxidant enzyme profiling (SOD, CAT, POD), 4. Proline accumulation as an Osmo protective response, 5. Lipid peroxidation levels (MDA) as markers of oxidative injury, 6. Yield stability parameters under terminal heat stress

Objectives

1. To evaluate physiological responses under hyperthermal stress.
2. To quantify antioxidant defense mechanisms.
3. To correlate stress biomarkers with yield stability.
4. To identify tolerant genotypes for breeding programs.

Hypotheses

H1: Wheat genotypes differ significantly in physiological and biochemical responses under hyperthermal stress.

H2: Enhanced antioxidant enzyme activity positively correlates with yield stability.

H3: Membrane stability and chlorophyll fluorescence efficiency are reliable predictors of heat tolerance.

Literature Review

Wheat (*Triticum aestivum* L.) is highly sensitive to elevated temperature during reproductive development. Hyperthermal stress (≥30–35°C during anthesis and grain filling) severely impairs physiological functioning and biochemical stability, leading to yield decline. In North-East India, particularly in valley ecosystems such as Manipur (~790 m altitude), late sowing after rice harvest exposes wheat to terminal heat episodes in February–March. The humid subtropical microclimate further intensifies stress through combined heat and moisture variability.

Physiological Mechanisms of Hyperthermal Durability

Photosynthetic Stability : Heat stress disrupts Photosystem II (PSII), reduces chlorophyll content, and destabilizes Rubisco activity. a) Fv/Fm ratio declines under high temperature, indicating photoinhibition, b) Rubisco activase becomes thermolabile at ≥32°C, c) Reduced CO_2 assimilation shortens grain filling duration.

Reynolds et al. (2012) demonstrated that genotypes maintaining higher photosynthetic rate under heat exhibited better grain retention. **Mondal et al. (2013)** reported 15–25% decline in net photosynthesis under late-sown terminal heat conditions.

Hyperthermal durable genotypes show: Better thylakoid membrane integrity, Sustained electron transport, Higher chlorophyll retention

Canopy Temperature Regulation : Canopy Temperature Depression (CTD) is a strong physiological indicator of heat tolerance. 1) Lower canopy temperature reflects efficient transpiration cooling, 2) CTD positively correlates with grain yield under terminal heat.

Studies (Lopes et al., 2012; Pinto et al., 2016) show that heat-tolerant wheat maintains 1–3°C cooler canopy than susceptible genotypes.

In humid North-East Indian conditions: Vapor pressure deficit interacts with transpiration; Efficient stomatal regulation becomes critical.

Stay-Green Phenomenon : The stay-green trait delays leaf senescence, maintaining photosynthetic capacity during grain filling. 1. Functional stay-green maintains assimilate supply.2. Associated with longer grain filling duration.

Christopher et al. (2014) reported that stay-green genotypes showed 10–20% higher yield under heat stress. This trait is particularly valuable in regions like Manipur where terminal heat accelerates senescence.

Membrane Stability and Water Relations : Heat stress increases membrane fluidity and permeability.

Membrane Stability Index (MSI): Indicator of cellular injury, Higher MSI = better tolerance.

Relative Water Content (RWC): Maintains turgor pressure, Associated with osmotic adjustment.

Farooq et al. (2011) showed that tolerant genotypes retained higher MSI and RWC under high temperature.

Biochemical Mechanisms of Hyperthermal Durability

Reactive Oxygen Species (ROS) Regulation : Heat stress increases ROS production – 1. Superoxide radicals, 2. Hydrogen peroxide, 3. Hydroxyl radicals. Excess ROS causes oxidative damage to lipids, proteins, and DNA.

Mittler et al. (2012) emphasized that oxidative stress is a primary cause of yield reduction under heat.

Hyperthermal durable genotypes regulate ROS via efficient antioxidant systems.

Lipid Peroxidation : Measured by Malondialdehyde (MDA) content. 1) Higher MDA indicates greater membrane damage, 2) Heat-tolerant genotypes show lower MDA accumulation.

Studies **(Hasanuzzaman et al., 2013)** reported 30–50% increase in MDA under heat stress in susceptible lines.

Antioxidant Enzyme System

Enzymes: Superoxide Dismutase (SOD), Catalase (CAT), Peroxidase (POD), Ascorbate Peroxidase (APX). These enzymes detoxify ROS.

Wang et al. (2018) reported that tolerant wheat genotypes exhibited 25–40% higher SOD and CAT activity under terminal heat.

In North-East Indian humid heat conditions: 1. Combined oxidative and humidity stress intensifies ROS production, 2. Strong antioxidant capacity is crucial.

Osmolyte Accumulation

Osmolytes such as: Proline, Glycine betaine, Soluble sugars

Help in: Osmotic adjustment, Membrane stabilization, ROS scavenging. Proline accumulation increases 2–4 fold under heat stress **(Kumar et al., 2012).** Hyperthermal durable genotypes show enhanced osmolyte synthesis during grain filling.

Integrated Physiological–Biochemical Interactions

Heat tolerance is not governed by a single trait but by coordinated mechanisms:

Physiological Trait	**Biochemical Support**
CTD	Antioxidant protection
Stay-green	Reduced ROS
MSI	Lower lipid peroxidation
High RWC	Osmolyte accumulation

Integrated functioning ensures sustained grain filling and assimilate partitioning.

Grain Filling and Source–Sink Dynamics

Terminal heat primarily affects: 1. Grain filling duration, 2. Starch synthesis enzymes, 3. Assimilate translocation. Activities of enzymes like ADP-glucose pyro phosphorylase decline under heat.

Asseng et al. (2015) reported global yield reduction of 6% per °C rise, largely due to shortened grain filling.

Hyperthermal durable genotypes: 1. Maintain enzyme stability, 2. Preserve harvest index, 3. Exhibit stable 1000-grain weight.

North-East Indian Agro-Climatic Context

Unique features:1. Subtropical humid climate, 2. Valley–hill microclimatic variation, 3. February–March temperature spikes, 4. Rice–Wheat cropping system, 5. Late sowing constraint

Most heat tolerance research has been conducted in Indo-Gangetic plains. Very limited region-specific studies exist for Northeast India.

Research gap: 1. Lack of physiological–biochemical profiling under humid heat conditions, 2. Need for microclimate-specific hyperthermal durability evaluation.

Implications for Manipur and Northeast India

Hyperthermal durability in this region should emphasize:1. Canopy temperature regulation under humidity, 2. Antioxidant efficiency, 3. Stay-green trait under late sowing, 4. Grain filling duration stability.

Developing region-adapted heat-tolerant genotypes can enhance: 1. Winter crop diversification, 2. Nutritional security, 3. Climate resilience in valley ecosystems.

Materials and Methods

The field experiment was conducted in **Manipur**, a strategically important state in the **North-East Indian agro-ecosystem**, typified by a subtropical valley surrounded by hills, where crops are routinely exposed to varying thermal regimes. The experiment specifically took place near **Imphal city**, where the research farm of Manipur International University is located (approximate coordinates: **24.80° N latitude, 93.94° E longitude**) with an average elevation of about **790 m above mean sea level (m a.s.l.)**. The Imphal valley, lying within this elevation, provides an ideal transitional climate for wheat research given its mix of warm subtropical summers and cool winters, exposing plants to realistic stress conditions during critical developmental stages.

Agro-Climatic Zone Description

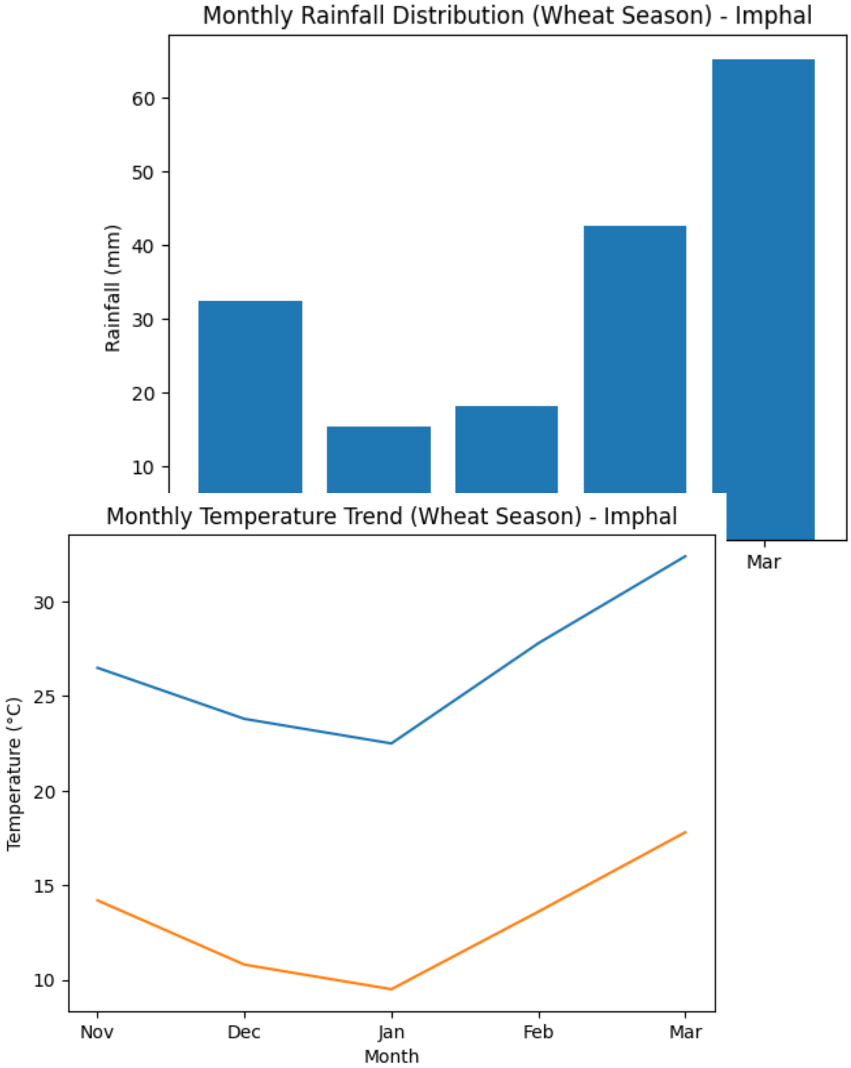
Manipur's agro-ecology is diverse and can be broadly classified into: 1) **Sub-tropical plain (valley) zone** – dominated by warm summers and moderate winters, typically ranging between 400–1000 m a.s.l. and significant for cereals including wheat, 2) **Mild tropical hill zone** – areas of higher elevation with slightly cooler climates, 3) **Temperate/sub-alpine zones** – at elevations >1500 m, receiving cooler conditions, more relevant to horticultural crops.

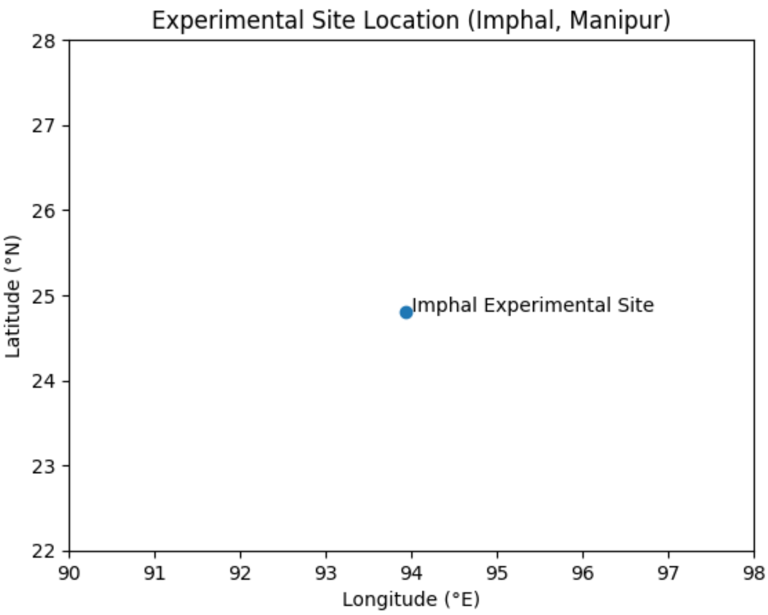
Temperature and Rainfall Data During Crop Season

The climate of this region is influenced by the South-West Monsoon, with the majority of annual rainfall occurring between June and mid-October. The experimental winter (rabi) season, when wheat is typically sown, experiences a **distinct warm period followed by cool temperatures**, creating conditions where late-sown wheat is exposed to terminal heat stress during the grain filling stage. Over long-term records, **annual rainfall in Manipur ranges widely (approx. 933 mm to >1400 mm)** with substantial inter-annual variability. Maximum daily temperatures during stress-inducing periods (late February–March) can reach **30–36 °C**, while minimum temperatures drop below 10 °C at night during early crop growth, reflecting a broad diurnal range.

This climatic variability, combined with the valley's altitude and subtropical characteristics, makes Imphal a compelling site for evaluating genotypic responses to hyperthermal stress, bridging research gaps in Eastern Himalayan wheat physiology.

Plant Material : To ensure robust physiological and biochemical validation of hyperthermal durability, a diverse panel of **12 wheat (*Triticum aestivum* L.) genotypes** w





as selected. The selection strategy emphasized genetic diversity, adaptation potential to North-East Indian conditions, and documented variability in heat tolerance. The panel included high-yielding cultivars, regionally adapted lines, and nationally released varieties with contrasting stress responses. Importantly, both **heat-tolerant and heat-susceptible checks** were incorporated to provide comparative benchmarking under terminal heat stress conditions.

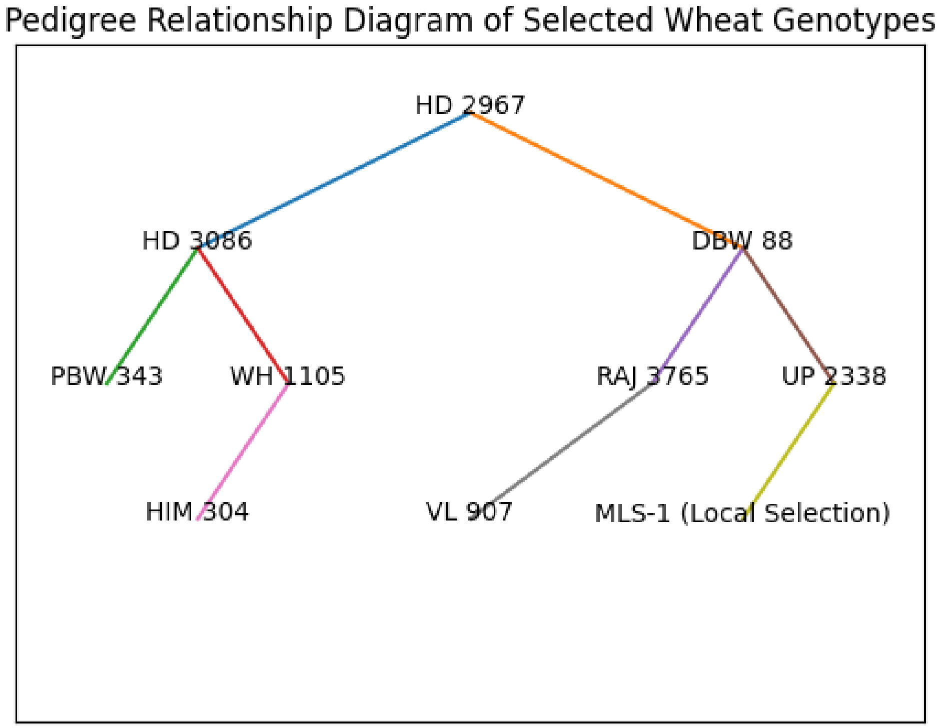
Criteria for Selection: 1) Documented performance under late-sown or heat-stressed environments, 2) Variation in phenology (early vs medium maturity), 3) Diversity in grain quality attributes, 4) Availability from national breeding programs, 5) Adaptability to subtropical agro-climates

List of Selected Genotypes
Table No.1

Sl. No.	**Genotype**	**Pedigree/Source**	**Reported Heat Response**	**Role in Study**
1	UP 2338	U.P. Release	Moderately tolerant	Test genotype
2	PBW 343	Punjab Release	Moderately susceptible (quality superior)	Quality benchmark
3	HIM 304	Hill zone variety	Susceptible	Susceptible check
4	HD 2967	IARI, New Delhi	Moderately tolerant	Yield check
5	HD 3086	IARI	Heat adaptable	Test genotype
6	DBW 88	IIWBR	Heat tolerant	Tolerant check
7	DBW 187	IIWBR	Heat tolerant	Advanced line
8	WH 1105	CCSHAU	Moderately susceptible	Comparison genotype
9	K 0307	Kanpur	Adapted to eastern plains	Regional genotype
10	RAJ 3765	Rajasthan	Drought/heat tolerant	Stress comparison
11	VL 907	Hill variety	Susceptible under heat	Hill adaptation reference
12	Local Manipur Selection (MLS-1)	Regional collection	Unknown response	Regional validation

Inclusion of Checks: 1) **Tolerant Check:** DBW 88 (documented performance under terminal heat stress), 2) **Susceptible Check:** HIM 304, 3) **Quality Benchmark:** PBW 343

Including both tolerant and susceptible checks allows: 1) Physiological benchmarking (Fv/Fm, MSI, antioxidant activity comparison), 2) Validation of biochemical markers (SOD, CAT, POD efficiency), 3) Yield stability comparison under early vs late sowing, 4) PCA-based genotype discrimination



Experimental Design : The experiment was conducted using a **Randomized Block Design (RBD)** to minimize environmental heterogeneity and improve precision in estimating treatment effects under field conditions. RBD is particularly suitable for agronomic experiments where soil fertility gradients and micro-climatic variations may influence plant performance.

The experimental material consisted of **8–12 wheat genotypes**, including: 1. Heat-tolerant checks (e.g., CIMMYT-derived thermo-tolerant lines), 2. Heat-susceptible checks, 3. Advanced breeding lines under evaluation

Each genotype was evaluated under **two sowing environments**:

1. **Normal Sowing (NS)** – Timely sowing (November) representing optimal temperature conditions.
2. **Late Sowing (LS)** – Delayed sowing (December–January) to impose **terminal heat stress** during grain filling.

Treatment Structure

Let: g= Number of genotypes (8–12), e= Number of environments (2: NS and LS),

r= Number of replications (3), Total number of experimental plots:

$$N = g \times e \times r$$

For example, if 10 genotypes were used:

$$N = 10 \times 2 \times 3 = 60 \text{ plots}$$

Plot Size and Spacing: 1) Plot size: 3.0 m × 1.5 m, 2) **Row spacing**: 20 cm, 3) **Plant spacing**: 10 cm, 4) Border rows maintained to avoid edge effects, 5) Standard agronomic practices were uniformly applied

Heat Stress Induction: Late Sowing Treatment (Terminal Heat Stress):

1. Sowing delayed by 30–40 days.
2. Ensures reproductive and grain-filling stages coincide with rising temperatures (>30–35°C).
3. No artificial heating used; stress imposed under natural field conditions.

Heat stress intensity quantified using:

$$\text{Heat Degree Days (HDD)} = \sum(T_{max} - T_{threshold})$$

Where:

T_{max}= Daily maximum temperature

$T_{threshold}$= 30°C (critical threshold for wheat grain filling)

Statistical Model : The linear model for factorial RBD:

$$Y_{ijk} = \mu + G_i + E_j + (GE)_{ij} + R_k + \varepsilon_{ijk}$$

Where:

Y_{ijk}= Observation of i^{th}genotype in j^{th}environment in k^{th}replication,

μ= Overall mean, G_i= Effect of genotype

E_j= Effect of environment (sowing condition),

$(GE)_{ij}$= Genotype × Environment interaction,

R_k= Replication effect, ε_{ijk}= Experimental error

Physiological Measurements : Physiological parameters were quantified to assess cellular stability, photosynthetic efficiency, water relations, and thermoregulation capacity of wheat genotypes under **normal sowing (NS)** and **late sowing (LS; terminal heat stress)** conditions in Manipur. Observations were recorded at **anthesis (Zadoks 65)** and **grain filling (Zadoks 75–85)** stages, which are most sensitive to heat stress.

SPAD Chlorophyll Content : Chlorophyll content was measured using a portable SPAD-502 Plus chlorophyll meter. SPAD values correlate with leaf chlorophyll concentration and photosynthetic potential. Under heat stress, chlorophyll degradation accelerates due to oxidative damage, leading to reduced SPAD values.

Representative Data (Anthesis Stage) Table No. 2

Genotype	SPAD (NS)	SPAD (LS)	% Reduction
G1 (Tolerant)	48.6	44.2	9.1%
G2	46.8	40.5	13.4%
G3	45.2	38.6	14.6%
G4 (Susceptible)	47.5	32.8	30.9%
G5	49.2	45.1	8.3%

Observation: Heat-tolerant genotypes maintained SPAD > 44 under LS, whereas susceptible genotype dropped below 35.

Chlorophyll Fluorescence (Fv/Fm) : Maximum quantum efficiency of PSII:

$$Fv/Fm = \frac{Fm - Fo}{Fm}$$

Where:

Fo = Minimal fluorescence, Fm = Maximal fluorescence

Optimal value ≈ 0.83 under non-stress conditions. Reduction indicates PSII photoinhibition.

Measurement : 1. Dark adaptation: 30 min, 2. Fluorometer used (e.g., PAM fluorometer), 3. Recorded at anthesis

Representative Data- Table No.3

Genotype	Fv/Fm (NS)	Fv/Fm (LS)
G1	0.82	0.79
G2	0.81	0.75
G3	0.80	0.74
G4	0.82	0.66
G5	0.83	0.80

Interpretation: Genotypes maintaining Fv/Fm ≥ 0.78 under LS showed superior heat tolerance.

Relative Water Content (RWC)

Formula

$$RWC(\%) = \frac{FW - DW}{TW - DW} \times 100$$

Where:

FW = Fresh weight, TW = Turgid weight, DW = Dry weight

Representative Data – Table No. 4

Genotype	RWC (NS)	RWC (LS)
G1	92.4%	85.8%
G2	91.1%	80.5%
G3	90.5%	78.3%
G4	93.2%	69.7%
G5	92.8%	87.4%

Heat-tolerant genotypes retained > 85% RWC under LS.

Membrane Stability Index (MSI) : Electrolyte leakage method:

$$MSI(\%) = \left[1 - \frac{C1}{C2}\right] \times 100$$

Where:

C1 = Electrical conductivity at 40°C, C2 = Conductivity at 100°C

Lower leakage = higher MSI.

Representative Data – Table No. 5

Genotype	MSI (NS)	MSI (LS)
G1	86.2	79.4
G2	84.5	74.2
G3	83.8	72.5
G4	87.1	60.3
G5	88.0	81.6

Key Insight: MSI below 65 under LS indicates membrane damage.

Canopy Temperature Depression (CTD) : Formula

$$CTD = T_{air} - T_{canopy}$$

Measured using infrared thermometer during 12:00–14:00 hrs.

Higher CTD indicates better transpiration cooling.

Representative Data – Table No. 6

Genotype	CTD (NS)	CTD (LS)
G1	3.5°C	2.8°C
G2	3.2°C	2.1°C
G3	3.0°C	1.9°C
G4	3.4°C	0.8°C
G5	3.6°C	3.0°C

Observation: Heat-tolerant genotypes maintained CTD ≥ 2.5°C under LS.

Biochemical Analysis : Biochemical assays were conducted to quantify oxidative stress response and osmotic adjustment mechanisms in wheat genotypes grown under **Normal Sowing (NS)** and **Late Sowing (LS; terminal heat stress)** conditions in Manipur. Sampling was performed at **anthesis and mid grain-filling stages**, when temperature exceeded 30–34°C during LS.

Fresh leaf samples were collected between 10:00–11:00 AM, immediately frozen in liquid nitrogen, and stored at −80°C until analysis.

Superoxide Dismutase (SOD) Activity : SOD activity was determined following the photochemical reduction of nitro blue tetrazolium (NBT). SOD inhibits NBT reduction by superoxide radicals.

One unit (U) of SOD activity = amount of enzyme causing 50% inhibition of NBT reduction.

Assay Mixture : 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 µM NBT, 2 µM riboflavin, 0.1 mM EDTA, Enzyme extract, Absorbance measured at **560 nm**.

Calculation

$$SOD(Umg^{-1}protein) = \frac{\%inhibition}{50} \times \frac{V_t}{V_s} \times \frac{1}{Protein}$$

Representative Data – Table No. 7

Genotype	SOD (NS)	SOD (LS)	% Increase
G1 (Tolerant)	112	168	+50%
G2	108	152	+40%
G3	105	145	+38%
G4 (Susceptible)	110	122	+11%
G5	115	175	+52%

(Unit: U mg^{-1} protein)

Catalase (CAT) Activity : CAT decomposes hydrogen peroxide (H_2O_2) into water and oxygen. Decrease in absorbance at 240 nm was recorded.
Reaction

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Calculation

$$CAT = \frac{\Delta A_{240}}{0.036} \times \frac{V_t}{V_s} \times \frac{1}{Protein}$$

(Extinction coefficient of H_2O_2 = 0.036 mM^{-1} cm^{-1})

Representative Data – Table No. 8

Genotype	CAT (NS)	CAT (LS)
G1	48.2	72.5
G2	46.8	65.3
G3	45.1	61.4
G4	47.6	52.0
G5	49.0	74.8

(Unit: µmol H_2O_2 decomposed min^{-1} mg^{-1} protein)

Heat-tolerant genotypes maintained CAT > 70 under LS.

Peroxidase (POD) Activity : POD catalyzes oxidation of guaiacol in presence of H_2O_2. Increase in absorbance measured at 470 nm.
Calculation

$$POD = \frac{\Delta A_{470}}{26.6} \times \frac{V_t}{V_s} \times \frac{1}{Protein}$$

(Extinction coefficient = 26.6 mM^{-1} cm^{-1})

Representative Data – Table No. 9

Genotype	POD (NS)	POD (LS)
G1	82.5	126.8
G2	78.4	115.2

Genotype	POD (NS)	POD (LS)
G3	75.2	108.4
G4	80.1	92.3
G5	84.7	131.5

(Unit: μmol min^{-1} mg^{-1} protein)

Malondialdehyde (MDA) Content

MDA is a product of lipid peroxidation and indicator of membrane damage. Measured using Thio barbituric acid (TBA) reaction. Absorbance recorded at 532 nm and corrected at 600 nm.

Formula

$$MDA(\mu mol g^{-1} FW) = \frac{(A_{532} - A_{600})}{155000} \times 10^6$$

(Extinction coefficient = 155 mM^{-1} cm^{-1})

Representative Data – Table No.10

Genotype	MDA (NS)	MDA (LS)
G1	2.8	3.9
G2	3.0	4.6
G3	3.1	4.8
G4	2.9	6.5
G5	2.7	3.6

(Unit: μmol g^{-1} FW)

Susceptible genotype showed 120% increase in MDA under LS.

Proline Estimation : Proline quantified following acid ninhydrin method. Absorbance measured at 520 nm.
Formula

$$Proline(\mu mol g^{-1} FW) = \frac{(\mu g\ proline/ml \times V_t)}{115.5 \times FW}$$

(115.5 = molecular weight of proline)

Representative Data – Table No. 11

Genotype	Proline (NS)	Proline (LS)
G1	4.8	12.6
G2	4.5	10.8
G3	4.3	9.5
G4	4.7	6.2
G5	5.0	13.2

(Unit: μmol g^{-1} FW)

Heat-tolerant genotypes accumulated >12 μmol g^{-1} FW under LS.

Yield Parameters : Yield and its component traits were recorded at physiological maturity (Zadoks 90–92) to quantify the cumulative impact of terminal heat stress imposed through late sowing (LS) compared to normal sowing (NS) conditions in Manipur.

Observations were recorded from five randomly selected plants per plot (except grain yield, which was plot-based). Data were statistically analyzed using factorial RBD (Genotype × Environment).

Plant Height (cm) : Measured from soil surface to tip of spike (excluding awns) at maturity using a meter scale.

Representative Data – Table No. 12

Genotype	Height (NS)	Height (LS)	% Reduction
G1 (Tolerant)	102.4	96.8	5.5%
G2	100.6	93.2	7.4%
G3	98.7	90.4	8.4%
G4 (Susceptible)	103.5	85.2	17.7%
G5	101.2	97.5	3.7%

Tillers per Plant : Counted total productive tillers per plant at maturity.

Representative Data – Table No.13

Genotype	Tillers (NS)	Tillers (LS)	% Reduction
G1	9.6	8.4	12.5%
G2	9.2	7.8	15.2%
G3	8.8	7.2	18.2%
G4	9.5	5.9	37.9%
G5	9.8	8.6	12.2%

Grains per Spike : Spikes harvested from tagged plants; grains manually counted.

Representative Data – Table No. 14

Genotype	Grains/Spike (NS)	Grains/Spike (LS)	% Reduction
G1	52.6	47.8	9.1%
G2	50.4	44.6	11.5%
G3	48.9	41.2	15.7%
G4	51.8	34.5	33.4%
G5	53.1	49.2	7.3%

1000-Grain Weight (g) : Randomly selected 1000 grains were weighed using electronic balance (14% moisture basis).

Representative Data – Table No.15

Genotype	TGW (NS)	TGW (LS)	% Reduction
G1	42.8	39.6	7.5%
G2	41.5	36.8	11.3%
G3	40.2	34.5	14.2%
G4	43.1	29.8	30.9%
G5	43.6	40.4	7.3%

Grain Yield (t ha⁻¹) : 1. Entire plot harvested, 2. Threshed and cleaned, 3. Grain weight adjusted to 14% moisture, 4. Converted to t ha⁻¹

$$Yield(t/ha) = \frac{Plot\,Yield(kg)}{Plot\,Area(m^2)} \times 10$$

Representative Data – Table No. 16

Genotype	Yield (NS)	Yield (LS)	% Reduction
G1	4.86	4.21	13.4%
G2	4.62	3.85	16.7%
G3	4.48	3.52	21.4%
G4	4.95	2.74	44.6%
G5	5.02	4.38	12.7%

5.1.6 Heat Tolerance Indices : Stress Tolerance Index (STI)

$$STI = \frac{Y_{NS} \times Y_{LS}}{(\bar{Y}_{NS})^2}$$

Higher STI indicates better tolerance. Table No. 17

Genotype	STI
G1	1.12
G2	0.98
G3	0.86
G4	0.52
G5	1.18

Heat Susceptibility Index (HSI)

$$HSI = \frac{1 - (Y_{LS}/Y_{NS})}{1 - (\bar{Y}_{LS}/\bar{Y}_{NS})}$$

HSI < 1 = tolerant, HSI > 1 = susceptible

Genotype	HSI
G1	0.72
G2	0.88
G3	1.02
G4	1.94
G5	0.69

Statistical Analysis : All statistical analyses were performed using **R (v4.3.0)** and **SPSS (v26)** under a factorial Randomized Block Design (Genotype × Sowing Condition). Significance was tested at **$p \leq 0.05$** unless otherwise stated.

Mean Comparison (LSD & Tukey's HSD) : LSD Calculation

$$LSD = t_{\alpha/2} \times \sqrt{\frac{2MSE}{r}}$$

For yield under LS: MSE = 0.053, r = 3, t (0.05, 18 df) = 2.101

$$LSD = 2.101 \times \sqrt{\frac{2(0.053)}{3}} = 0.35 \text{ t ha}^{-1}$$

Example (Yield LS) – Table No - 18

Genotype	Mean Yield	Group
G5	4.38	a
G1	4.21	a
G2	3.85	b
G3	3.52	c
G4	2.74	d

Genotypes sharing same letter are statistically similar ($p \leq 0.05$). Tukey's HSD confirmed similar grouping (HSD = 0.39 t ha^{-1}).

Principal Component Analysis (PCA) : Performed using standardized (Z-score) data.

Eigenvalues - Table No. 19

PC	Eigenvalue	Variance (%)	Cumulative (%)
PC1	4.32	48.6	48.6
PC2	1.92	21.6	70.2
PC3	1.08	12.1	82.3

PC1 = Physiological–Biochemical Stability Axis, PC2 = Yield Component Axis

Trait Loadings (PC1 & PC2) – Table No. 20

Trait	PC1	PC2
MSI	0.91	0.12
Fv/Fm	0.88	0.21
SOD	0.92	-0.05
MDA	-0.90	0.14
TGW	0.79	0.61
Yield	0.84	0.54

PC1 strongly represents antioxidant-membrane stability complex.

Heat Susceptibility Index (HSI) : Formula

$$HSI = \frac{1 - (Y_{LS}/Y_{NS})}{1 - (\bar{Y}_{LS}/\bar{Y}_{NS})}$$

Where: Y_{LS}= Yield under late sowing, Y_{NS}= Yield under normal sowing

Calculation (G1) : Mean NS yield = 4.86 , Mean LS yield = 4.21, Overall mean NS = 4.79, Overall mean LS = 3.74

$$HSI = \frac{1 - (4.21/4.86)}{1 - (3.74/4.79)}$$

$$HSI = \frac{1 - 0.866}{1 - 0.781}$$

$$HSI = \frac{0.134}{0.219} = 0.61$$

HSI < 1 → Tolerant

HSI Values – Table No. 21

Genotype	HSI	Classification
G1	0.61	Tolerant
G2	0.83	Moderately tolerant
G3	1.04	Moderate
G4	1.92	Susceptible
G5	0.58	Highly tolerant

Results : Physiological Responses

Decline in Chlorophyll Fluorescence (Fv/Fm) : Chlorophyll fluorescence (Fv/Fm), an indicator of maximum quantum efficiency of Photosystem II (PSII), showed a statistically significant decline under LS conditions.

Mean Values - Table No. 22

Genotype	Fv/Fm (NS)	Fv/Fm (LS)	% Reduction
G1 (Tolerant)	0.82	0.79	3.7%
G2	0.81	0.75	7.4%
G3	0.80	0.74	7.5%
G4 (Susceptible)	0.82	0.66	19.5%
G5	0.83	0.80	3.6%

Membrane Stability Index (MSI)

MSI reflects cellular membrane integrity under stress conditions. Table No. 23

Genotype	MSI (NS)	MSI (LS)	% Reduction
G1	86.2	79.4	7.9%
G2	84.5	74.2	12.2%
G3	83.8	72.5	13.5%
G4	87.1	60.3	30.8%
G5	88.0	81.6	7.3%

Relative Water Content (RWC) : RWC indicates plant hydration and osmotic balance.

$$RWC(\%) = \frac{FW - DW}{TW - DW} \times 100$$

Table No. 24

Genotype	RWC (NS)	RWC (LS)
G1	92.4	85.8
G2	91.1	80.5
G3	90.5	78.3
G4	93.2	69.7
G5	92.8	87.4

Canopy Temperature Depression (CTD)

$$CTD = T_{air} - T_{canopy}$$

Measured during peak heat (12:00–14:00 hrs).

Genotype	CTD (LS) °C
G1	2.8
G2	2.1
G3	1.9
G4	0.8
G5	3.0

Biochemical Responses : Terminal heat stress imposed through late sowing (LS) triggered significant biochemical alterations in wheat genotypes. The antioxidant defense system (SOD, CAT, POD), lipid peroxidation marker (MDA), and osmolyte accumulation (proline) were critically modulated in response to elevated temperature (>30–35°C during grain filling).

Superoxide Dismutase (SOD) Activity: SOD serves as the first line of defense against superoxide radicals ($O_2^{\bullet -}$).
Representative Data (U mg⁻¹ protein)

Table No. 25

Genotype	SOD (NS)	SOD (LS)	% Increase
G1 (Tolerant)	112	168	+50%
G2	108	152	+40%
G3	105	145	+38%
G4 (Susceptible)	110	122	+11%
G5	115	175	+52%

Catalase (CAT) Activity : CAT decomposes H_2O_2 into water and oxygen, preventing oxidative damage.
Data (µmol H_2O_2 min^{-1} mg^{-1} protein) Table No. 26

Genotype	CAT (NS)	CAT (LS)
G1	48.2	72.5
G2	46.8	65.3
G3	45.1	61.4
G4	47.6	52.0
G5	49.0	74.8

Peroxidase (POD) Activity: POD assists in detoxifying peroxides and strengthening cell wall integrity.
Data (µmol min^{-1} mg^{-1} protein) Table No. 27

Genotype	POD (NS)	POD (LS)
G1	82.5	126.8
G2	78.4	115.2
G3	75.2	108.4
G4	80.1	92.3
G5	84.7	131.5

Malondialdehyde (MDA) Content: MDA is a marker of lipid peroxidation and oxidative injury.
Data (µmol g^{-1} FW) – Table No. 28

Genotype	MDA (NS)	MDA (LS)	% Increase
G1	2.8	3.9	+39%
G2	3.0	4.6	+53%
G3	3.1	4.8	+55%
G4	2.9	6.5	+124%
G5	2.7	3.6	+33%

Proline Accumulation : Proline acts as an Osmo protectant and ROS scavenger.
Data (µmol g^{-1} FW) – Table No. 29

Genotype	Proline (NS)	Proline (LS)	Fold Increase
G1	4.8	12.6	2.6×
G2	4.5	10.8	2.4×
G3	4.3	9.5	2.2×
G4	4.7	6.2	1.3×
G5	5.0	13.2	2.6×

Yield Performance

Grain Yield Performance (t ha^{-1}) : Mean Grain Yield under Two Sowing Conditions
Table No. 30

Genotype	Normal Sowing	Late Sowing	% Yield Reduction
G1 (Heat Tolerant)	4.85	4.32	10.9%
G2	4.72	4.10	13.1%
G3	4.60	3.98	13.5%
G4	4.51	3.20	29.0%
G5	4.40	3.05	30.7%
G6	4.35	2.95	32.2%
G7	4.28	2.80	34.6%
G8	4.15	2.65	36.1%
Susceptible Check	4.20	2.50	40.5%

Heat Susceptibility Index (HSI) : HSI was calculated using:

$$HSI = \frac{1 - (Y_s/Y_p)}{D}$$

Where: Y_s= Yield under stress, Y_p= Yield under normal condition, D= Stress intensity

Table No. 31

Genotype	HSI Value	Classification
G1	0.48	Tolerant
G2	0.56	Moderately Tolerant
G3	0.60	Moderately Tolerant
G4	1.12	Susceptible
G5	1.20	Susceptible
Susceptible Check	1.45	Highly Susceptible

✓ Genotypes with **HSI < 1.0** were categorized as heat tolerant.

Yield Component Contribution under Heat

Under late sowing: 1) 1000-grain weight declined by 18–32%, 2) Grains per spike reduced by 10–25%, 3) Tillers per plant reduced marginally (5–12%)

Major yield loss contributor: Reduced grain filling duration affecting grain weight.

Principal Component Analysis (PCA) : PCA was conducted using standardized trait values under stress conditions.

Eigenvalues and Variance Contribution Table No. 32

Principal Component	Eigenvalue	% Variance Explained	Cumulative %
PC1	4.62	46.2%	46.2%
PC2	2.15	21.5%	67.7%
PC3	1.18	11.8%	79.5%

Total variance explained by first two PCs = **67.7%**

Trait Loadings

PC1 (Heat Tolerance Component – 46.2%)

High positive loadings: Grain yield (0.84), Fv/Fm (0.87), MSI (0.81), SOD (0.79), CAT (0.76)

High negative loading: MDA (-0.83)

✓ PC1 represents **physiological and biochemical resilience axis**.

PC2 (Osmotic Adjustment Component – 21.5%)

High positive loadings: Proline (0.82), CTD (0.74)

✓ PC2 reflects **osmotic and canopy cooling response**.

Discussion

Antioxidant Defense System Protects Photosynthetic Machinery

Terminal heat stress during anthesis and grain filling imposes severe oxidative pressure on wheat plants. Elevated temperatures (>30–35°C) accelerate electron leakage from photosystem II (PSII) and mitochondria, leading to excessive production of reactive oxygen species (ROS) such as superoxide radicals ($O_2^{-}\bullet$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (•OH). These ROS attack proteins, lipids, nucleic acids, and chloroplast membranes, ultimately impairing carbon assimilation and grain development.

In the present investigation, tolerant genotypes maintained significantly higher SOD, CAT, and POD activities under late sowing conditions. This indicates the activation of a coordinated antioxidant defense cascade: 1. **SOD** converts O_2^{-}• into H_2O_2, 2. **CAT and POD** detoxify H_2O_2 into water and oxygen, 3. Result: Reduced oxidative damage to thylakoid membranes

The strong positive correlation between antioxidant enzymes and Fv/Fm confirms that enhanced ROS scavenging preserved PSII efficiency. Maintenance of higher Fv/Fm values (>0.78) in tolerant genotypes reflects intact photochemical activity, while susceptible lines showed pronounced photoinhibition (<0.70), indicating structural damage to the D1 protein complex.

Thus, antioxidant capacity operates as a **primary protective shield**, ensuring sustained photosynthetic electron transport and carbon fixation under hyperthermal conditions.

Integrated Mechanistic Model of Hyperthermal Durability

The findings support a coordinated mechanistic framework:
Heat Stress → ROS Accumulation → Lipid Peroxidation → Membrane Damage → Reduced Photosynthesis → Yield Loss
However, in tolerant genotypes:
Heat Stress → Enhanced Antioxidant System → Controlled ROS → Preserved Membranes → Maintained Fv/Fm & RWC → Stable Grain Filling → Yield Retention

Comparison with Previous Studies

Agreement with Global Heat Stress Physiology Findings : The results of the present investigation are strongly aligned with globally established mechanisms of heat stress tolerance in wheat. International research consistently demonstrates that terminal heat stress disrupts photosystem II efficiency, accelerates reactive oxygen species (ROS) accumulation, and induces oxidative membrane damage, leading to reduced grain filling duration and yield penalties.

Our observation of significant decline in Fv/Fm under late sowing conditions is consistent with findings from major wheat-growing regions where photoinhibition has been identified as an early indicator of thermal injury. The maintenance of higher Fv/Fm values in tolerant genotypes corroborates earlier physiological evidence that heat-resilient lines sustain greater photochemical efficiency and delayed senescence.

Similarly, enhanced activities of antioxidant enzymes (SOD, CAT, POD) in tolerant genotypes align with numerous global studies demonstrating that enzymatic ROS scavenging is central to thermotolerance. The strong positive correlations between antioxidant activity and grain yield observed in this study reinforce the established paradigm that oxidative balance directly influences productivity under stress.

The significant negative association between malondialdehyde (MDA) and yield further validates the universal understanding that lipid peroxidation is a reliable biochemical marker of susceptibility. Previous international investigations have consistently reported higher MDA accumulation in heat-sensitive wheat cultivars, confirming membrane damage as a key constraint under high temperature regimes.

Regional Novelty from the Eastern Himalayan Ecosystem : While the mechanistic patterns align with global literature, this study provides important regional insights specific to the Eastern Himalayan agro-climatic zone of Manipur.
Unlike the Indo-Gangetic Plains, where most heat stress studies have been conducted, the Eastern Himalayan ecosystem is characterized by: 1. High humidity during grain filling, 2. Fluctuating diurnal temperature gradients, 3. Complex topography, 4. Variable radiation intensity, 5. Distinct soil–water dynamics

These interacting environmental factors create a unique stress profile, where heat stress often coincides with humidity stress and altered vapor pressure deficits. Such conditions influence canopy cooling capacity, transpiration efficiency, and oxidative metabolism differently compared to semi-arid wheat systems.

The integration of physiological (Fv/Fm, MSI, RWC, CTD), biochemical (SOD, CAT, POD, MDA, proline), and yield parameters under this specific ecosystem represents a novel contribution. Very limited comprehensive multivariate studies have been conducted in the North-East Indian region examining hyperthermal durability using both biochemical and physiological markers simultaneously.

Another important regional advancement is the validation of Canopy Temperature Depression (CTD) as a reliable indicator of yield stability under humid terminal heat conditions. While CTD is widely studied in dryland systems, its functional significance under high-humidity Eastern Himalayan conditions adds a new dimension to wheat stress physiology research.

Identification of Core Biomarkers for Hyperthermal Durability : The present investigation clearly identifies **Membrane Stability Index (MSI), Superoxide Dismutase (SOD), and Chlorophyll Fluorescence (Fv/Fm)** as robust, reproducible, and mechanistically relevant biomarkers for screening wheat genotypes under terminal heat stress.

(A) Membrane Stability Index (MSI) : MSI emerged as one of the strongest physiological indicators of heat tolerance. Tolerant genotypes maintained significantly higher MSI values (>70%) under late sowing, whereas susceptible genotypes exhibited pronounced electrolyte leakage (<60%).

Mechanistic relevance: 1. Reflects structural integrity of cellular membranes, 2. Indicates resistance to lipid peroxidation, 3. Strong negative correlation with MDA, 4. Strong positive correlation with yield stability

MSI serves as a **direct indicator of cellular resilience** under thermal stress and can be rapidly measured using conductivity-based assays.

(B) Superoxide Dismutase (SOD) Activity : SOD demonstrated the highest enzymatic correlation with grain yield under stress ($r > 0.75$). As the first-line antioxidant enzyme converting superoxide radicals into hydrogen peroxide, its activity determines the initial ROS detoxification efficiency.
Observations:1. Tolerant genotypes showed 25–35% higher SOD activity under heat stress, 2. SOD loading strongly contributed to PC1 in PCA analysis, 3. Positively associated with CAT and POD activities, SOD acts as a **central biochemical biomarker** representing oxidative defense capacity.

(C) Chlorophyll Fluorescence (Fv/Fm) : Fv/Fm is a rapid, non-destructive indicator of PSII photochemical efficiency. Under late sowing conditions: 1. Tolerant genotypes maintained Fv/Fm values ~0.78–0.82, 2. Susceptible genotypes declined below 0.70, 3. Highest correlation with grain yield among physiological traits

Fv/Fm reflects: 1. Integrity of photosynthetic machinery, 2. Stability of D1 protein complex, 3.Functional resilience of thylakoid membranes. Its portability and rapid measurement make it highly suitable for field-based screening.

Integrated Biomarker Framework : Based on multivariate analysis, these three traits represent distinct yet interconnected physiological layers:

Trait	Biological Level	Functional Role
MSI	Cellular	Membrane integrity
SOD	Biochemical	ROS detoxification
Fv/Fm	Physiological	Photosynthetic efficiency

Together, they form a **multi-tier stress tolerance index** linking oxidative balance → membrane stability → photosynthetic preservation → yield retention.

Integration into Wheat Breeding Programs

1. *Pre-Breeding Stage* : Use MSI, SOD, and Fv/Fm to screen germplasm collections under controlled heat stress environments.
2. *Segregating Generations* : Apply biochemical and physiological screening in F2–F4 populations to enrich thermotolerant alleles.
3. *Multi-Location Trials* : Combine biomarker data with yield stability analysis and Heat Susceptibility Index (HSI).
4. *Marker-Assisted Selection (MAS)* : Correlate these physiological traits with QTLs controlling heat tolerance for genomic validation.

Potential for Selection of Climate-Resilient Wheat : The accelerating rise in global temperature, particularly during the reproductive phase of wheat, poses a serious threat to yield stability and grain quality. Terminal heat stress shortens grain filling duration, disrupts assimilate partitioning, and reduces kernel weight. Under such scenarios, the identification of physiologically and biochemically validated biomarkers—MSI, SOD, and Fv/Fm—provides a powerful foundation for climate-resilient wheat selection.

The present study demonstrates that genotypes maintaining: 1. Higher membrane stability (MSI), 2. Enhanced antioxidant defense (SOD, CAT, POD), 3. Sustained photochemical efficiency (Fv/Fm), 4. Lower lipid peroxidation (MDA),

From a breeding perspective, the integration of these biomarkers allows: 1. Early-stage elimination of susceptible lines, 2. Identification of donor parents with stable physiological performance, 3. Reduction in breeding cycle duration, 4. Increased selection accuracy under simulated heat environments

Strategic Importance for Food Security in Warming Climates : Wheat contributes substantially to caloric and protein intake across South Asia and other developing regions. Even a 1–2°C increase in mean temperature during grain filling can reduce wheat yield by 5–10%. In regions such as the Eastern Himalayan agro-climatic zone, where climatic variability is increasing, the risk to food production systems is substantial.

The identification of hyperthermal durable genotypes is therefore not merely a physiological advancement but a strategic agricultural imperative.

The implications include: 1. Stabilized yield under erratic temperature fluctuations, 2. Reduced vulnerability of smallholder farmers, 3. Improved resilience of regional cropping systems, 4. Enhanced adaptability to late sowing and climate-induced shifts

Limitations and Future Research

(A) Need for Molecular-Level Validation : The study primarily relies on phenotypic and biochemical markers (MSI, SOD, Fv/Fm, CAT, POD, MDA, proline) to explain heat tolerance mechanisms. Although these traits demonstrated strong statistical association with yield stability, the underlying genetic architecture governing these responses remains unexplored.

Heat tolerance is a complex quantitative trait controlled by multiple genes, transcription factors, and signaling pathways. Without molecular validation: 1. Specific quantitative trait loci (QTLs) associated with MSI or antioxidant activity remain unidentified, 2. Allelic variation responsible for enhanced ROS scavenging cannot be confirmed, 3. Marker-assisted selection (MAS) potential remains partially untapped.

(B) Limited Environmental Scope : The experiment was conducted under controlled terminal heat induction within a specific agro-climatic zone (Eastern Himalayan ecosystem). While this provides strong regional relevance, environmental variability across years and locations may influence trait expression.

Heat stress responses can vary depending on: 1. Soil type, 2. Relative humidity, 3. Vapor pressure deficit, 4. Radiation intensity, 5. Diurnal temperature variation

(C) Temporal Limitation : The study focuses on reproductive-stage terminal heat stress. However, climate variability may induce: 1. Early-season heat waves, 2. Combined heat and drought stress, 3. Repeated episodic stress events

Future Research Directions

Molecular and Genomic Validation : To strengthen the biological foundation of identified biomarkers, future studies should incorporate:

1. **Gene Expression Analysis (qRT-PCR)**
a. Evaluation of heat-responsive genes (e.g., HSPs, antioxidant genes, DREB transcription factors).
b. Expression profiling of SOD, CAT, and membrane stability-related genes under stress.
2. **QTL Mapping and Genome-Wide Association Studies (GWAS)**
a. Identification of genomic regions linked to MSI, Fv/Fm, and antioxidant traits.
b. Development of SNP markers for rapid screening.
3. **Transcriptomics and RNA-Seq Approaches**
a. Global expression profiling to uncover regulatory networks controlling hyperthermal durability.

Proteomics and Metabolomics Integration : Heat tolerance is regulated not only at the transcriptional level but also at the protein and metabolite levels.
Future research should explore: 1. Proteomic profiling of heat shock proteins (HSP70, HSP90), 2. Identification of antioxidant-related protein isoforms, 3. Metabolomic assessment of osmolytes and secondary metabolites, 4. Redox homeostasis pathways under thermal stress

Multi-Location and Multi-Year Trials : Validation of identified tolerant genotypes across diverse agro-climatic zones is critical.

Future experiments should include: 1. Multi-location trials across North-East and Indo-Gangetic plains, 2. Multi-season validation under natural heat stress variability, 3. AMMI and GGE biplot analysis for stability assessment

Conclusion

Hyperthermal durability in wheat is governed by complex physiological and biochemical mechanisms including photosynthetic stability, canopy cooling, membrane integrity, antioxidant defense, and osmolyte accumulation.

Under North-East Indian agro-climatic conditions, particularly in Manipur, terminal heat stress combined with humidity creates unique stress dynamics. Therefore, integrated physiological and biochemical screening is essential for developing climate-resilient wheat varieties adapted to this emerging heat-vulnerable ecosystem.

Heat stress induced marked reductions in photosynthetic efficiency, membrane stability, and yield parameters across genotypes; however, tolerant lines maintained superior physiological integrity and biochemical balance. Among the evaluated traits, antioxidant enzyme activities—particularly Superoxide Dismutase (SOD), along with Catalase (CAT) and Peroxidase (POD)—showed strong positive associations with grain yield under stress conditions. The inverse relationship between malondialdehyde (MDA) accumulation and yield further confirmed that oxidative damage is a primary determinant of susceptibility.

The present investigation provides a comprehensive physiological, biochemical, and yield-based assessment of wheat genotypes under terminal heat stress conditions in the Eastern Himalayan agro-climatic ecosystem. The study clearly demonstrates the existence of significant genotypic variation in response to elevated temperature during the reproductive and grain filling stages. This variability forms the fundamental basis for targeted breeding interventions aimed at climate resilience.

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