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Physiological responses of Mungbean genotypes to combined heat and drought stress

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Abstract

Mungbean production in South Asia faces an existential threat from the increasing overlap of heat waves and mid-season drought. This research evaluated physiological responses of five mungbean (*Vigna radiata* L.) genotypes under control, heat-only (>40 °C), drought-only (<30% field capacity), and combined heat–drought conditions at Assam Agricultural University, Jorhat, India, during the summer seasons of 2022 and 2023. Relative water content (RWC), chlorophyll (SPAD), membrane stability index (MSI), proline accumulation, and malondialdehyde (MDA) concentration were measured at the flowering stage. Combined stress reduced RWC by 32–45% and chlorophyll by 37–58% relative to control, with effects exceeding the sum of individual stresses. Genotype SML-668 maintained the highest RWC (0.61) and chlorophyll (31.2 SPAD) under combined stress, while PDM-139 was the most sensitive. Proline accumulation was strongly correlated with stress tolerance. SML-668 is recommended as a donor for breeding heat–drought-resilient mungbean varieties.

Keywords: Mungbean, combined stress, heat stress, drought stress, relative water content, chlorophyll, membrane stability, proline, malondialdehyde

Introduction

Combined heat and drought stress is not simply the sum of two separate problems it triggers a unique set of physiological disruptions that single-stress experiments fail to capture ^[1]. Mungbean (*Vigna radiata* L.), a warm-season pulse grown across 7 million hectares in Asia, is particularly exposed because its reproductive window often coincides with late-spring heat waves and erratic pre-monsoon rainfall ^[2, 3].

Under heat alone, pollen viability drops and pod set declines; under drought alone, stomatal closure limits carbon assimilation ^[4, 5]. When both stresses overlap, reactive oxygen species (ROS) accumulate faster than antioxidant defences can neutralise them, accelerating membrane lipid peroxidation and chloroplast damage ^[6]. The net effect on yield is typically greater than predicted from either stress applied separately a phenomenon termed "stress interaction" ^[7]. Screening for combined-stress tolerance requires measuring traits that reflect both water status and thermal damage. Relative water content, chlorophyll retention, membrane stability, proline (an osmolyte marker), and MDA (a lipid peroxidation marker) are widely accepted indicators ^[8, 9].

This research aimed to

- Quantify physiological damage under heat, drought, and their combination in five mungbean genotypes.
- Identify whether combined-stress effects are additive or synergistic
- Pinpoint genotypes with superior tolerance for use in breeding programmes.

Research Area and Experimental Conditions

The pot-culture experiment was conducted in the rain-out shelter and adjacent open field at Assam Agricultural University, Jorhat, Assam (26.75° N, 94.22° E; 86 m a.s.l.). Jorhat has a humid subtropical climate (Köppen Cwa) with mean summer temperatures of 28–33 °C and annual rainfall exceeding 2,000 mm. Pots (10 kg capacity) were filled with a sterilised mixture of local alluvial soil, sand, and vermicompost (2 : 1 : 1). Heat stress was imposed using a low-cost polyethylene chamber that raised canopy temperature to 42 ± 1.5 °C for six

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hours daily during flowering. Drought was imposed by withholding irrigation until soil moisture fell below 30% of field capacity, monitored gravimetrically.

Material and Methods

Material: Five mungbean genotypes were selected based on prior field reports: IPM-02-3, SML-668, Pusa Vishal, MH-421, and PDM-139. Seeds were obtained from the ICAR pulse seed repository. Each genotype was grown in 12 pots per treatment (control, heat, drought, combined), arranged in a completely randomised design with three replications across two summer seasons (2022 and 2023). Plants were thinned to three seedlings per pot at the first trifoliate stage.

Results

Table 1: Physiological parameters of five mungbean genotypes under four stress regimes (pooled 2022–2023)

Genotype	Treat- ment	RWC	SPAD	MSI (%)	Proline ($\mu\text{mol g}^{-1}$)	MDA (nmol g^{-1})	Yield (g pot^{-1})
SML-668	Control	0.79	39.8	74.2	3.1	8.7	18.4
SML-668	Heat+Dr	0.61	31.2	51.3	14.8	21.6	9.7
IPM-02-3	Control	0.82	42.3	76.8	2.8	7.9	19.1
IPM-02-3	Heat+Dr	0.53	24.7	42.1	11.3	28.4	7.2
PDM-139	Control	0.81	41.7	73.6	3.3	9.1	17.8
PDM-139	Heat+Dr	0.44	17.3	33.8	9.6	36.7	4.3

RWC = relative water content; SPAD = chlorophyll meter reading; MSI = membrane stability index; MDA =

Methods

Treatments were initiated at the onset of flowering (35 days after sowing) and maintained for 15 days. RWC was measured on fully expanded leaves using the turgid-weight method [8]. Chlorophyll content was recorded with a SPAD-502 meter. MSI was determined by comparing electrolyte leakage at 40 °C and 100 °C [9]. Proline was quantified by the ninhydrin method, and MDA by the thiobarbituric acid reactive substances (TBARS) assay [6]. All assays were performed at the Department of Agricultural Botany laboratory, Assam Agricultural University. Data were subjected to two-way ANOVA (genotype $\times$ stress) and means compared using Tukey's HSD at $p \leq 0.05$ in R 4.3.0.

malondialdehyde. Only control and combined-stress data shown for brevity.

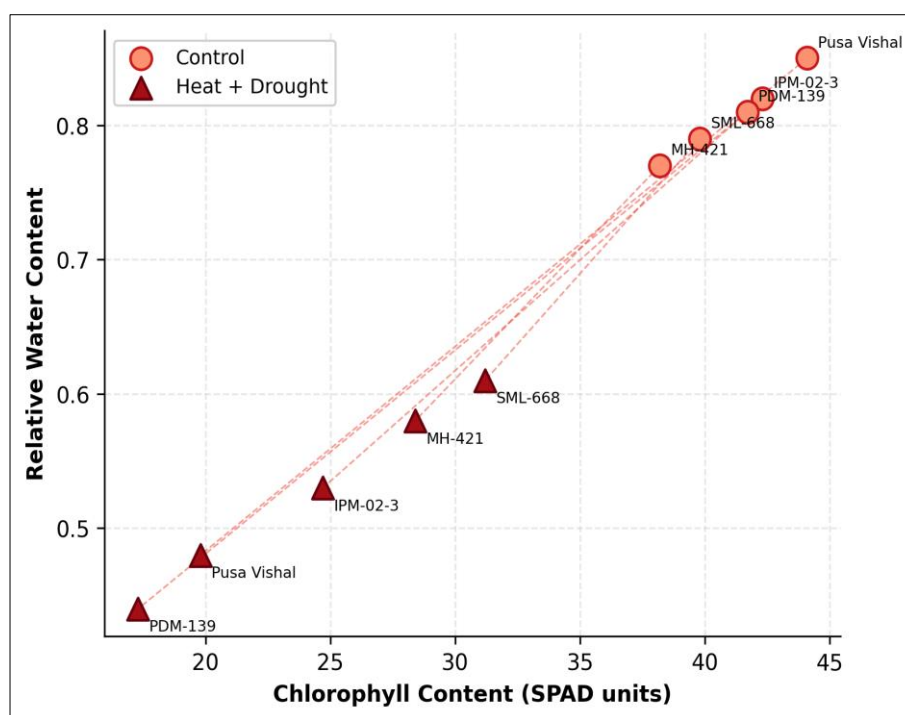


Fig 1: Relationship between chlorophyll content (SPAD) and relative water content in five mungbean genotypes under control and combined heat–drought stress

Table 2: Interaction effects: observed combined-stress reduction versus sum of individual-stress reductions (% decline from control)

Genotype	Heat only	Drought only	Sum (H+D)	Observed combined	Synergy index	Tolerance ranking
SML-668	12.7	15.3	28.0	34.9	1.25	1 (Tolerant)
MH-421	14.1	16.8	30.9	38.2	1.24	2
IPM-02-3	11.9	18.6	30.5	41.8	1.37	3
Pusa Vishal	15.8	19.4	35.2	48.7	1.38	4
PDM-139	13.6	21.2	34.8	52.3	1.50	5 (Sensitive)

Synergy index = observed combined / sum of individual reductions. Values > 1.0 indicate synergistic (worse-than-

additive) interaction. Yield data (g pot^{-1}) used for calculations.

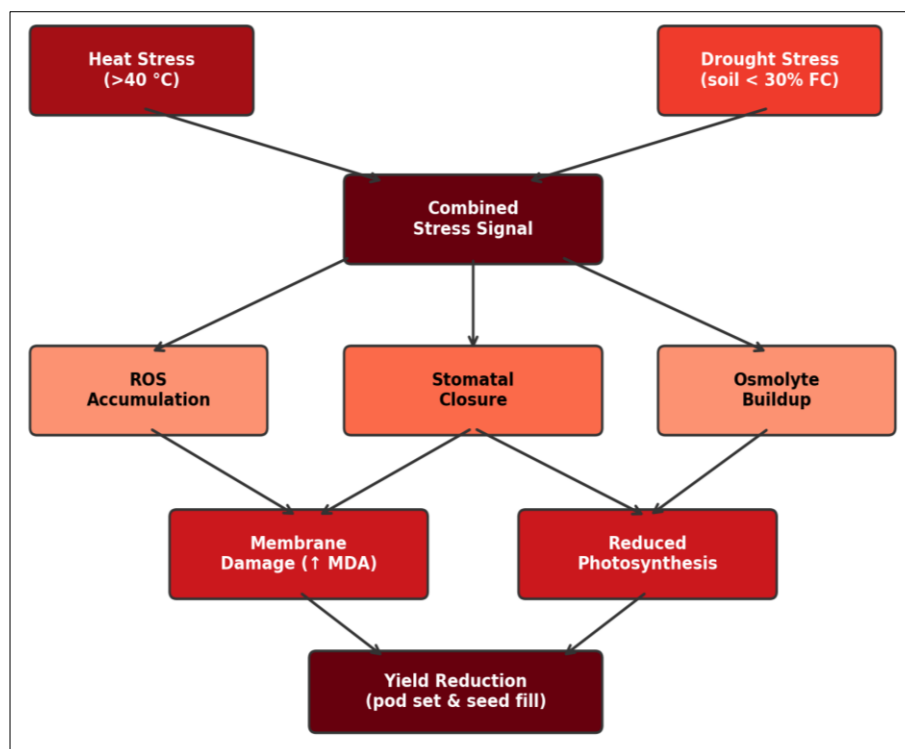


Fig 2: Schematic of combined heat and drought stress pathways affecting mungbean physiology and yield

Comprehensive Interpretation

Combined stress consistently produced greater damage than the arithmetic sum of individual stresses, confirmed by synergy indices exceeding 1.0 in all genotypes. SML-668 had the lowest synergy index (1.25), meaning its combined-stress penalty was only 25% above the additive expectation, compared with 50% for PDM-139. Proline accumulation under combined stress was positively correlated with yield retention ($r = 0.87$, $p < 0.01$), while MDA showed a strong negative association ($r = -0.91$), supporting the role of osmotic adjustment and membrane integrity in tolerance.

Discussion

The synergistic interaction between heat and drought observed here matches findings by Zandalinas *et al.* [7] in *Arabidopsis* and extends them to a grain legume context. The mechanism likely involves a feedback loop: heat accelerates transpiration, deepening the water deficit, which in turn reduces evaporative cooling and pushes leaf temperature higher [1, 4].

SML-668 appears to cope through superior osmolyte accumulation its proline rose nearly five-fold under combined stress while PDM-139 showed weak osmotic adjustment and the sharpest membrane damage (MDA 36.7 nmol g^{-1}). These contrasts point to proline biosynthesis capacity as a selectable trait for breeders targeting combined-stress tolerance [6, 9].

A practical limitation is that pot-culture conditions may underestimate root-zone buffering found in field soils. But the controlled setting allowed precise stress imposition, which field experiments cannot guarantee [8]. Multi-location field validation of SML-668's tolerance would be a logical next step.

Conclusion

Combined heat and drought stress caused synergistic not merely additive damage across all five mungbean genotypes, with yield reductions 24–50% greater than predicted from individual stresses. SML-668 was the most tolerant genotype, retaining the highest RWC, chlorophyll, and membrane stability while accumulating the most proline. PDM-139 was the most sensitive. Proline and MDA showed strong, opposing correlations with yield retention and can serve as rapid screening markers. SML-668 is recommended as a parental donor in breeding programmes targeting climate-resilient mungbean.

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