

Article

Elevated Respiration-to-Photosynthesis Ratio Under High Nighttime Temperature Constrains Mungbean Yield and Identifies Genotypic Targets for Climate Adaptation in Subtropical Farming Systems

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Abstract

Mungbean is a nitrogen-fixing break crop in subtropical broadacre cereal rotation, yet how high nighttime temperatures (HNTs) threaten its agronomic contributions remain poorly characterized. A controlled environment (CE) experiment (low nighttime temperature, LNT: 20 °C vs. high nighttime temperature, HNT: 25 °C) and a two-year staggered-sowing field trial at Rockhampton, Queensland, evaluated HNT impacts on two genotypes, Jade AU and Green Dragon. In the CE experiment, HNT produced a strong, time-dependent increase in the respiration-to-photosynthesis (Rn/A) ratio, evident within two days of treatment imposition, compressed the pod-filling duration by 15%, and reduced grain yield by 21% through fewer mature pods without impairing individual grain filling. The sowing dates generated a contrasting combination of nighttime heat, daytime heat, solar radiation, and seasonal conditions. The harvest index was lower across the 2024 environments than the 2025 environments, but these year-level contrasts reflect co-varying thermal and radiation conditions rather than isolated HNT and High daytime temperature (HDT) environments. Although unadjusted Rn/A–grain yield associations differed between years, split-plot mixed models showed that Rn/A did not explain additional grain yield variation after accounting for sowing date, genotype, and experimental blocking structure. Therefore, Rn/A remains a candidate physiological response indicator, requiring further validation across broader germplasms and independently replicated environments. Green Dragon maintained a larger pod pool and showed less observed Rn/A than Jade AU in selected field environments. These traits identify Green Dragon as a promising source of reproductive resilience for further multi-environment evaluation. To align the reproductive phase with fewer warmer nights, sowing date management is the most accessible strategy for sustaining mungbean yield in subtropical broadacre cereal rotation systems under asymmetric warming.

Keywords: high nighttime temperature (HNT); reproductive sink failure; respiration-to-photosynthesis (Rn/A) ratio; subtropical broadacre farming systems



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1. Introduction

Mungbean (*Vigna radiata* (L.) Wilczek) is a nitrogen-fixing, short-duration grain legume that underpins subtropical broadacre cereal rotation systems in Australia and across tropical Asia, contributing biological nitrogen fixation (BNF), dietary protein, and break-crop weed suppression to smallholder and broadacre farming systems alike [1,2]. In central Queensland, its adoption as a summer break crop in sorghum–wheat rotations has accelerated over the last decade, driven by its capacity to fix atmospheric nitrogen and reduce synthetic fertilizer demand within rotation sequences [3]. However, the thermal environment of Queensland summers is changing, with nighttime minimum temperatures rising disproportionately relative to the daytime maximum. The summer months, which are the most critical for the reproductive development of mungbean, are among the most affected by this asymmetric trend [4]. Because high nighttime temperatures during reproductive development can reduce grain yield and may alter carbon allocation to symbiotic nitrogen fixation, continued asymmetric warming could compromise the agronomic and ecological benefits that mungbean contribute to subtropical cropping systems [5,6].

High nighttime temperature (HNT) is a physiologically distinct stress category from classical daytime heat stress. Its mechanism of impact operates through increased nighttime respiration (R_n) without a proportional impairment of daytime photosynthesis (A), raising the respiration-to-photosynthesis ratio (R_n/A) and depleting the assimilate pool available for reproductive sink development [7,8]. In grain legumes, a recent synthesis concluded that HNT primarily affects both pod set and grain weight, while high daytime temperature (HDT) predominantly impairs pod set alone, indicating that the two stress types operate through partially distinct physiological pathways [6]. The R_n/A ratio has been proposed as a crop-agnostic index of this nocturnal carbon cost [9], validated in wheat [10], but its utility as a screening criterion for identifying HNT-tolerant mungbean genotypes under field-realistic conditions has not been evaluated. The evidence base for mungbean specifically remains limited to controlled-environment studies at the individual plant level [11,12]. Available mungbean studies show that heat stress can reduce photosynthetic capacity, rubisco activity, PSII function, assimilate supply, and pod retention during reproductive development [13,14]. However, no field-scale data exists that contrast HNT and daytime heat stress effects on mungbean yield under naturally varying subtropical conditions.

Mungbean occupies a biologically important position as a warm-season C_3 grain legume with a continued flowering habit and successive pod-setting flushes depending on the moisture availability, making it potentially more resilient to single thermal events than determinately structured cereals. Compensatory pod setting in later flowering and podding flushes can partially offset losses in early ones. At the same time, this extended reproductive window prolongs the crop's exposure to sustained HNT events, creating a compounded vulnerability that may ultimately limit the compensatory capacity [5,14,15]. Whether the magnitude and architecture of genotypic differences in reproductive plasticity and nocturnal respiratory efficiency are sufficient to confer meaningful HNT tolerance under field conditions, and whether the R_n/A ratio can differentiate these genotypic responses, are central questions for crop improvement programs.

This study combines a controlled environment experiment that isolates HNT effects from co-varying climatic variables with a two-year field trial using staggered sowing dates to generate contrasting nocturnal thermal environments across ecologically realistic conditions. Four specific questions are addressed: (a) does HNT independently reduce mungbean grain yield, and is the mechanism consistent with that documented for cereal crops?; (b) which thermal parameters under field conditions are the most strongly associated with yield and harvest index variation?; (c) do the two genotypes differ in their sensitivity to HNT, and is any differential response attributable to reproductive plasticity or

to leaf-level gas exchange traits?; and (d) whether the R_n/A ratio measured at pod filling is associated with grain yield, and can it help differentiate genotypic responses under the field environments sampled in this study?

2. Materials and Methods

This study consisted of two independent but complementary experiments: a controlled-environment (CE) experiment designed to isolate and quantify the direct physiological consequences of HNT during the reproductive stage, and a two-year field trial using a staggered sowing design to generate contrasting nocturnal thermal environments.

2.1. Experimental Site

Both experiments were conducted at the Central Queensland Innovation and Research Precinct (CQIRP) of Central Queensland University, Rockhampton campus (23.32° S, 150.51° E), Queensland, Australia. Rockhampton is in the subtropical belt of central Queensland, characterized by a seasonal climate with a summer-dominant rainfall pattern, hot and humid summers, and mild, frost-free dry winters. The site is representative of the broader central Queensland broadacre grain production environment and provides inter-seasonal variation in nighttime temperature that generates HNT-dominated and non-HNT-dominated field environments within a single experimental framework.

2.2. Controlled Environment (CE) Experiment

2.2.1. Plant Material and Growth Conditions

Two mungbean genotypes were evaluated: Jade AU, a high-yielding variety developed by Queensland DAF that is suited to both spring and summer planting in central Queensland, and Green Dragon, a variety developed by AgriVentis Technologies Pty Ltd., NSW, Australia for high-intensity cropping under warmer conditions. Three seeds of each genotype were hand-sown at a 5 cm depth into individual pots (15 cm diameter, 15 cm depth) filled with black cracking clay (Vertisol) soil sourced from the field trial site. The field soil had an organic carbon concentration of 1.15% and a pH of 5.5, determined from soil samples extracted from the top 20 cm layer. For each genotype, 12 pots were used in total, with 6 pots assigned to each nighttime temperature treatment. After emergence, pots were thinned to one plant per pot, resulting in 24 experimental plants in total. Each pot received 5 g of controlled-release fertilizer (Osmocote All Purpose, 21:0.5:6% N:P:K, Evergreen Garden Care Australia, Bella Vista, NSW, Australia) incorporated into the upper 5 cm of soil at sowing. All pots were maintained under ambient outdoor conditions until the appearance of the first flower, at which point they were treated with Aphidex insecticide at 3 g L⁻¹ before being transferred to the temperature-controlled chambers.

2.2.2. Temperature Treatment and Chamber Conditions

Two indoor growth chambers (CLIMATRON SL, Thermoline Scientific, Wetherill Park, NSW, Australia) were set at contrasting nighttime temperatures: 20 °C (low nighttime temperature, LNT) and 25 °C (high nighttime temperature, HNT). Daytime temperature and relative humidity in both chambers were maintained identically at 30 °C and 65%, respectively, thereby standardizing the programmed daytime temperature and relative humidity between treatments. Nighttime conditions were regulated from 18:30 to 06:00 h and daytime conditions from 06:30 to 18:00 h, with a 30 min gradual transition to avoid abrupt thermal shock. PAR was supplied during daytime hours at a photosynthetic photon flux density (PPFD) of 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Only two growth chambers were available; therefore, each temperature treatment was initially represented by one chamber. Chamber environments were exchanged 10 days after treatment imposition to reduce confounding

between chamber identity and nighttime temperature treatment. However, true chamber-level replication was not available, and the CE results should be interpreted with this limitation. Chamber temperature was continuously monitored at 30 min intervals using calibrated Hobo Pendant data loggers (Onset Computer Corporation, Bourne, MA, USA). Observed mean temperatures are summarized in Table 1. All pots were watered daily by weight to prevent soil moisture deficits.

Table 1. Observed daytime and nighttime temperatures in the CLIMATRON chambers throughout the treatment period.

Nighttime Temperature Treatment	Temperature (°C)	
	Daytime	Nighttime
Low nighttime temperature (LNT—20 °C)	30.1 ± 0.4	20.1 ± 0.6
High nighttime temperature (HNT—25 °C)	30.2 ± 0.5	24.9 ± 0.4

2.3. Field Trial

2.3.1. Infrastructure and Seedbed Preparation

The field trial was conducted using 24 raised garden bed structures (2100 mm × 1100 mm × 300 mm; Birdies Garden Products, Arundel, QLD, Australia) each season, filled with Vertisol soil sourced locally from a cotton farm (organic carbon 1.15%, pH 5.5). Raised beds allowed precise soil moisture control through surface drip irrigation while maintaining an open-air canopy environment representative of field conditions. Soil was maintained near field capacity throughout the growing season by supplemental irrigation, eliminating soil moisture deficit as a confounding variable. Fertilizer was applied at rates equivalent to 120 kg N, 27 kg P, and 93 kg K per hectare, incorporated by surface application one day before sowing.

2.3.2. Experimental Design and Sowing

The experiment was conducted over two consecutive growing seasons, 2023/24 and 2024/25 (hereafter referred to as 2024 and 2025, respectively). In both years, a randomized complete block design with a split-plot arrangement of four replications was used, with sowing date as the main plot factor and genotype as the subplot factor. Three sowing dates (early, mid, and late) were imposed in each season, commencing on 1 October. Sowing dates were separated by 45 days in 2024 and 60 days in 2025 to sample a wider range of naturally occurring reproductive thermal environments in the second season. The longer interval in 2025 extended the late sowing into a later seasonal period with altered thermal conditions, day length, and radiation. Consequently, corresponding sowing date labels were not assumed to represent identical environmental conditions between years, and year × sowing date interactions were retained in the statistical interpretation. The critical reproductive window (CRW) was defined as the 20-day period spanning 5 days before to 15 days after first flowering. This 20-day window was selected to capture the transition from late floral development and anthesis to early pod formation and retention of the first reproductive flushes. In mungbean, floral initiation, fertilization, early pod growth, and pod abortion overlap around flowering; therefore, exposure during this interval is expected to influence the establishment and retention of reproductive sinks [5,16]. The CRW was used as an operational phenological window for comparing thermal exposure among year × sowing date × genotype combinations rather than as a universal threshold for mungbean heat sensitivity.

Seeds of both genotypes were inoculated with a slurry of Class 1 *Bradyrhizobium* species (CB 1015) one hour before sowing to ensure uniform and effective nodulation, allowing nitrogen fixation to proceed across all treatments so that any treatment effect on agronomic performance could be attributed to thermal rather than nutritional stress.

Seeds were sown manually in rows spaced 30 cm apart with three seeds per hill, thinned to one seedling per hill three days after emergence to maintain a target plant population of 27 plants m^{-2} .

2.4. Data Collection

2.4.1. Weather Monitoring

In the field trial, weather conditions were recorded at five-minute intervals by an on-site automatic weather station. Daily minimum and maximum temperatures were derived from the five-minute records. Average nighttime temperature (ANT) was calculated as the means of all observations during the dark period. Diurnal temperature amplitude (DTA, $^{\circ}\text{C}$) was calculated as the difference between daily maximum and minimum temperatures. Photosynthetically active radiation (PAR, $\text{MJ m}^{-2} \text{ day}^{-1}$) was derived as the 400–700 nm fraction of incident solar radiation and calculated as half of the total incident radiation. Vapor pressure deficit (VPD, kPa) was calculated from air temperature and relative humidity using the Magnus–Tetens approximation. Cumulative daytime and nighttime heat stress during the 20-day critical reproductive window (CRW) was expressed as total hours above 38 $^{\circ}\text{C}$ and 26 $^{\circ}\text{C}$, respectively. Field environments were classified descriptively at the individual year $\times$ sowing date level rather than at the whole-season level. An HNT-dominant environment was defined as one with substantial nighttime heat exposure during CRW and limited daytime heat exposure, whereas an HDT-dominant environment was defined as one with substantial daytime heat exposure and limited nighttime heat exposure. Environments with substantial exposure to both daytime and nighttime heat, or with important co-occurring radiation constraints, were classified as mixed-stress environments.

2.4.2. Phenological, Growth, and Yield Measurements

In the CE experiment, phenological development was recorded as days from sowing to first flower, first pod, and physiological maturity. In the field trial, development was recorded as days to 50% emergence, 50% flowering, 50% podding, and physiological maturity. Vegetative growth duration was the sowing-to-flowering interval, while the pod-filling duration was the podding-to-maturity interval. Thermal time for each phase was expressed as heat units (HU, $^{\circ}\text{Cd}$) accumulated above a base temperature of 10 $^{\circ}\text{C}$.

At maturity, plant height was measured for each plant in the CE experiment and from five random plants per plot in the field trial. In the CE experiment, all mature and immature pods were separated, counted, and measured for pod length and grains per pod. In the field trial, pods from the central row were harvested, counted, and averaged per plant. Twenty filled pods per plot were measured for pod length and grains per pod. Pods were shade-dried for four days before threshing, and grain yield was determined. Haulm biomass was oven-dried at 65 $^{\circ}\text{C}$ for 96 h and weighed.

2.4.3. Leaf-Level Gas Exchange Measurements

Leaf-level daytime photosynthesis (A , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and nighttime dark respiration (R_n , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) were measured using a LI-COR 6800 portable photosynthesis system (LI-COR Biosciences, Lincoln, NE, USA) on the topmost fully expanded leaf. In the CE experiment, measurements were made on each individual plant at pre-treatment baseline and at 2, 7, and 14 days post-treatment. In the field trial, measurements were conducted during flowering (3 days after 50% flowering) and pod filling (10 days after the flowering measurement) on five random plants per plot. Daytime photosynthesis was measured between 10:30 and 13:00 h at a PPFD of 400 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ (CE experiment) and 1500 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ (field trial). Nighttime respiration was measured between 20:30 and 23:00 h. The CO_2 concentration was maintained at 400 $\mu\text{mol mol}^{-1}$. The R_n/A ratio was calculated as the quotient of the corresponding R_n and A values.

The photosynthesis values in the CE experiment reflect the $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ chamber's light environment (approximately 40% below field saturation) and were interpreted as relative LNT vs. HNT comparisons rather than absolute field equivalents. During nighttime respiration measurements in the field trial, short-term respiratory temperature sensitivity was assessed at three additional cuvette temperatures: ambient -4°C , ambient $+4^\circ\text{C}$, and ambient $+8^\circ\text{C}$. Stomatal conductance (g_{sw} , $\text{mol m}^{-2} \text{s}^{-1}$) and intrinsic water use efficiency ($i\text{WUE}$, $\mu\text{mol CO}_2 \text{mmol}^{-1} \text{H}_2\text{O}$) were recorded concurrently with photosynthesis measurements.

2.4.4. Grain Biochemical Analysis

Grain samples from the field trial were freeze-dried at -80°C , finely ground, and passed through a 0.5 mm sieve. Extracts were prepared by combining 0.5 g of ground flour with 7 mL of 90% (v/v) aqueous methanol, extracted by end-over-end mixing at 50 rpm for 60 min, and centrifuged at $1000 \times g$ for 10 min. Extraction was repeated on the residual pellet, and supernatants were combined to a final volume of 14 mL. Total phenolic content (TPC) was determined using the modified Folin–Ciocalteu procedure [17], expressed as mg gallic acid equivalents (GAE) per 100 g dry weight (dw). Antioxidant capacity was assessed by two complementary assays: the ferric reducing antioxidant potential (FRAP) [18] and the cupric reducing antioxidant capacity (CUPRAC) [19] assays, both expressed as mg Trolox equivalents (TE) per 100 g dw. Standard curves and detailed protocols are provided in Appendix A, Figure A1.

2.4.5. Statistical Analysis

All statistical analyses were performed in R (version 4.3.1). In the CE experiment, gas exchange measured repeatedly from the same individual plants was analyzed separately using repeated-measures mixed-effects models. Nighttime temperature treatment, genotype, measurement time, and all interactions were specified as fixed effects, while plant identity was included as a random effect to account for within-plant correction. Measurement time was treated as a categorical factor. Residual-versus-fitted and normal Q-Q plots were used to assess model assumptions, and the R_n/A ratio was log-transformed for analysis when required. Estimated marginal means were compared using multiplicity-adjusted pairwise contrasts. Traits measured once at final harvest were analyzed using two-way ANOVA, with nighttime temperature treatment and genotype as fixed effects.

Field trial data were analyzed using a mixed-model ANOVA with year (Y), sowing date (S), and genotype (G) as fixed factors, and replication within year as a random blocking factor, using a split-plot error structure. Assumptions of normality (Shapiro–Wilk test) and homogeneity of variance (Levene's test) were verified for all response variables. Treatment means were separated using Tukey's HSD test at $p < 0.05$. Pearson correlation coefficients were computed among all measured environmental, physiological, and agronomic variables across the full field trial dataset. Year-specific simple linear regressions were initially fitted to describe the unadjusted association between the R_n/A ratio at pod filling and grain yield. To test whether R_n/A explained grain yield variation beyond the field design, separate year-specific linear mixed-effects models were fitted with grain yield as the response; R_n/A , sowing date (S), genotype (G), and $S \times G$ interaction as fixed effects, and block (B) and $B \times S$ as random effects, to represent the split-plot design. Reduced models excluding R_n/A were compared with full models using likelihood-ratio tests fitted by maximum likelihood. The adjusted R_n/A coefficient and Wald 95% confidence interval were reported. Marginal and conditional R^2 , residual diagnostics, model singularity, and predictor collinearity were examined. The simple regressions were retained only as descriptive visualizations of the seasonal environment gradient. Principal component analysis (PCA) was performed on

the standardized dataset of all field trial variables using the factoextra and FactoMineR packages in R, with ggplot2 used for graphical outputs.

3. Results

3.1. Controlled Environment Experiment

3.1.1. Gas Exchange Dynamics and the Rn/A Ratio

Pre-treatment measurements showed comparable Rn/A ratios among the plants subsequently assigned to the low-nighttime-temperature (LNT) and high-nighttime-temperature (HNT) treatments (Figure 1). Repeated-measures mixed-effects analysis showed that the temporal response of net photosynthesis depended on nighttime temperature (NT), genotype (G), and measurement time (MT), as indicated by a significant $NT \times G \times MT$ interaction ($F_{3,60} = 3.33$, $p = 0.025$). Photosynthesis declined sharply by 2 days after treatment imposition in both temperature treatments, but the decline was generally greater under HNT. At 2 days post-treatment, mean photosynthesis under HNT was 4.48 and 5.43 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in Jade AU and Green Dragon, respectively, compared with 6.71 and 8.03 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ under LNT. Photosynthesis partially recovered under LNT at 7 days, whereas it remained lower under HNT throughout the 14-day measurement period (Figure 1a).

Nighttime respiration varied significantly with measurement time ($F_{3,60} = 15.71$, $p < 0.001$), and the $NT \times MT$ interaction was also significant ($F_{3,60} = 2.82$, $p = 0.047$; Figure 1b). Respiration was similar between temperature groups before treatment but increased under HNT relative to LNT after treatment imposition. At 2 days, mean respiration under HNT was 1.90 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in Jade AU and 1.86 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in Green Dragon, compared to 1.11 and 0.87 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, respectively, under LNT. Respiration declined towards LNT values by 14 days, and the $NT \times G \times MT$ interaction was not significant ($F_{3,60} = 0.08$, $p = 0.973$), indicating that the overall temporal respiration response to nighttime temperature was similar between genotypes.

The Rn/A ratio showed a pronounced $NT \times MT$ interaction ($F_{3,60} = 7.88$, $p < 0.001$; Figure 1c), indicating that HNT altered the temporal balance between respiratory carbon loss and photosynthetic carbon gain. Before treatment, the mean Rn/A ranged from 0.095 to 0.125 across genotypes $\times$ temperature groups. At 2 days after treatment imposition, Rn/A increased under HNT to 0.46 in Jade AU and 0.40 in Green Dragon, compared to 0.18 and 0.11 under LNT, respectively. The HNT-associated elevation persisted at 7 days; however, by 14 days, Rn/A under HNT had declined to 0.19 in Jade AU and 0.20 in Green Dragon but remained numerically above the respective LNT means of 0.12 and 0.11. The $NT \times G \times MT$ interaction was not significant ($F_{3,60} = 0.31$, $p = 0.816$), indicating that the overall time-dependent Rn/A response to HNT was similar between Jade AU and Green Dragon.

3.1.2. Reproductive Sink Penalty and Yield

Since the nighttime temperature treatments were imposed only after the appearance of the first flower, days to first flower and days to first pod were unaffected by subsequent chamber assignment (Table 2). Following the nighttime temperature treatment, HNT significantly shortened crop duration by approximately five days (71.9 vs. 77.1 days to maturity; $p < 0.001$), which is entirely attributable to a compressed pod-filling phase (29.8 vs. 35.1 days; 15% reduction). This compression was uniform across genotypes, with no significant nighttime temperature by genotype ($NT \times G$) interaction.

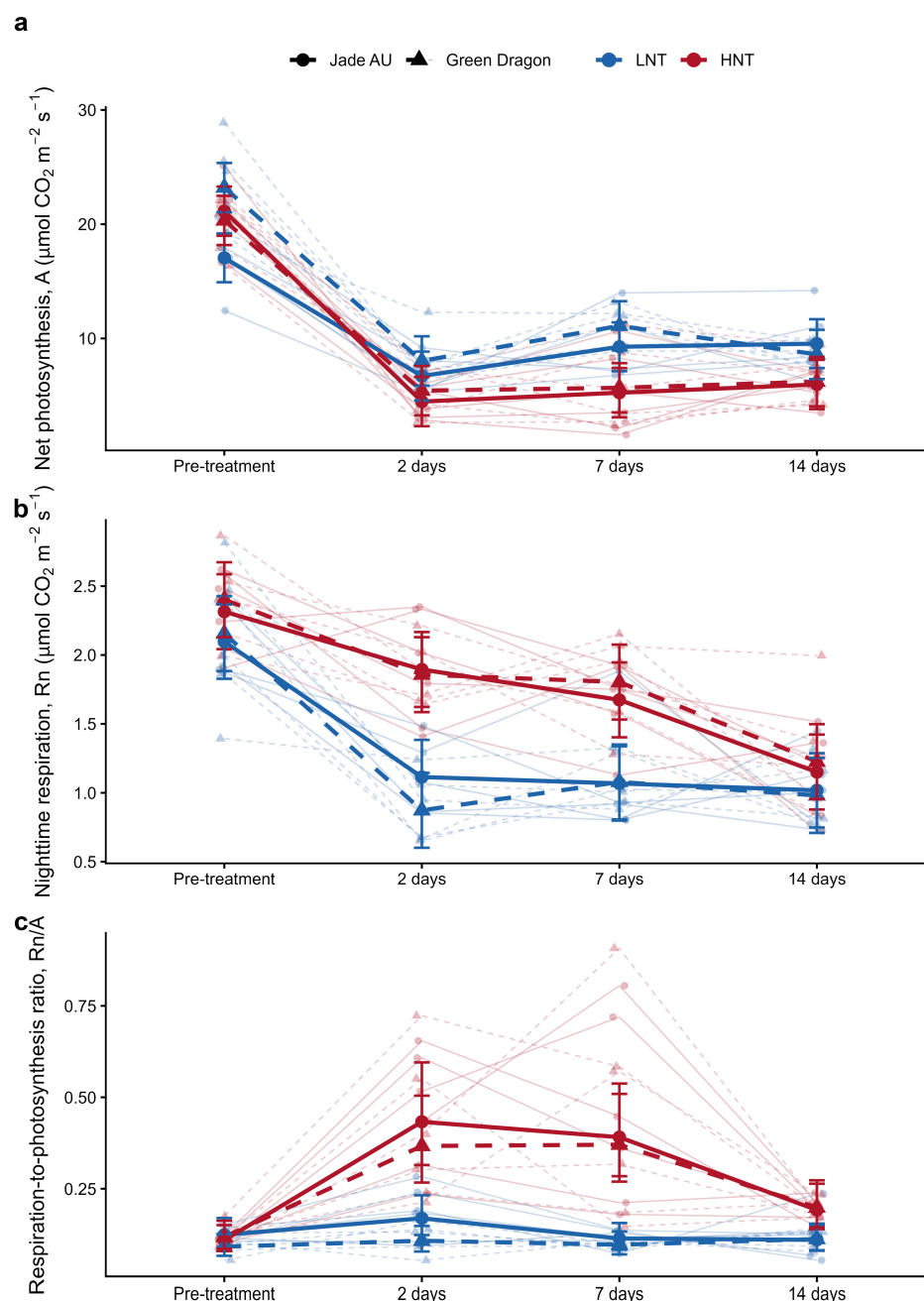


Figure 1. Temporal response of leaf gas exchange to nighttime temperature treatment in the controlled-environment experiment. (a) Daytime net photosynthesis (A , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$); (b) nighttime dark respiration (R_n , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$); and (c) the respiration-to-photosynthesis (R_n/A) ratio measured before treatment and at 2, 7, and 14 days after treatment imposition in Jade AU and Green Dragon genotypes. Blue lines represent low nighttime temperature (LNT, 20°C) and red lines represent high nighttime temperature (HNT, 25°C); circles and solid lines represent Jade AU, whereas triangles and dashed lines represent Green Dragon. Faint lines and small symbols show repeated observations from individual plants. Thick lines, larger symbols, and error bars show treatment means $\pm$ standard error ($n = 6$ plants per genotype $\times$ nighttime temperature treatment). Repeated observations were analyzed using mixed-effects models, with plant identity included as a random effect and measurement time treated as a categorical factor.

Table 2. Effect of nighttime temperature treatment on phenological observations recorded for the two mungbean genotypes in the CE experiment.

Treatment		Days to First Flower	Days to First Pod	Days to Maturity	Pod-Filling Duration	Plant Height (cm)
Nighttime Temperature (NT)						
Low NT—20 °C (LNT)		39.8 ± 0.3	42 ± 0.3	77.1 ± 0.4 a	35.1 ± 0.4 a	38.4 ± 1.3
High NT—25 °C (HNT)		39.8 ± 0.3	42.1 ± 0.3	71.9 ± 0.5 b	29.8 ± 0.7 b	38.3 ± 1.5
<i>p</i> -value		ns	ns	***	***	ns
Genotype (G)						
Jade AU		40 ± 0.3	42.1 ± 0.3	74.4 ± 1	32.3 ± 1	35.5 ± 1.1 b
Green Dragon		39.5 ± 0.3	42 ± 0.3	74.6 ± 0.9	32.6 ± 1	41.1 ± 1.1 a
<i>p</i> -value		ns	ns	ns	ns	**
NT × G						
LNT	Jade AU	40.4 ± 0.3	42.4 ± 0.3	77.2 ± 0.5	34.9 ± 0.5	35.3 ± 0.8
	Green Dragon	39.2 ± 0.4	41.7 ± 0.4	77 ± 0.6	35.4 ± 0.5	41.4 ± 1.7
HNT	Jade AU	39.7 ± 0.5	41.9 ± 0.5	71.5 ± 0.7	29.7 ± 1	35.7 ± 2.2
	Green Dragon	39.9 ± 0.4	42.4 ± 0.4	72.2 ± 0.7	29.9 ± 1	40.9 ± 1.4
<i>p</i> -value		ns	ns	ns	ns	ns

, and * signify $p < 0.01$, and $p < 0.001$; ns signifies non-significant. Same letters denote no statistical differences.

The number of mature pods at harvest was significantly lower under HNT than LNT (mean of 3.8 vs. 4.8 plant^{−1}; $p < 0.05$; Table 3), and this reduction was the primary driver of the 21% lower grain yield per plant under HNT (Figure 2c). The average pod length, grains per pod in mature pods, and thousand-grain weight were unaffected by the nighttime temperature treatment (Table 3; Figure 2b), confirming that HNT impaired pod initiation or early pod retention rather than the filling efficiency of established pods. Grains per plant declined by 23% under HNT (from 41 to 32), consistent with the pod number reduction. Above-ground biomass was not significantly affected by the nighttime temperature treatment (Figure 2d), confirming that the yield penalty operated through reproductive sink limitation rather than vegetative growth suppression.

Table 3. Effect of nighttime temperature treatment on yield and pod traits of two mungbean genotypes in the CE experiment.

Treatment		Mature Pod Traits per Plant				Immature/Late Pod Traits per Plant	
		Number	Weight (g)	Average Length (cm)	Grains per Pod	Number	Weight (g)
Nighttime Temperature (NT)							
Low NT (LNT)		4.8 ± 0.3 a	3.2 ± 0.2 a	7.7 ± 0.2	8.8 ± 0.3	3.1 ± 0.6	0.5 ± 0.1 b
High NT (HNT)		3.8 ± 0.4 b	2.5 ± 0.2 b	8.1 ± 0.2	8.8 ± 0.5	3.2 ± 0.5	1.1 ± 0.2 a
<i>p</i> -value		*	**	ns	ns	ns	**
Genotype (G)							
Jade AU		4 ± 0.4	2.6 ± 0.3	7.8 ± 0.2	8.6 ± 0.3	2.4 ± 0.5 b	0.7 ± 0.2
Green Dragon		4.5 ± 0.4	3.1 ± 0.2	8 ± 0.2	9 ± 0.5	4 ± 0.6 a	0.9 ± 0.2
<i>p</i> -value		ns	ns	ns	ns	*	ns
NT × G							
LNT	Jade AU	4.9 ± 0.5	3.1 ± 0.3	7.3 ± 0.2 b	8.6 ± 0.4	2.2 ± 0.9	0.3 ± 0.1
	Green Dragon	4.7 ± 0.4	3.3 ± 0.2	8 ± 0.2 a	9.1 ± 0.3	4 ± 0.8	0.6 ± 0.2
HNT	Jade AU	3.2 ± 0.4	2.2 ± 0.2	8.2 ± 0.1 a	8.7 ± 0.5	2.5 ± 0.5	1.1 ± 0.3
	Green Dragon	4.4 ± 0.6	2.8 ± 0.3	7.9 ± 0.4 ab	8.8 ± 0.9	3.9 ± 0.8	1.1 ± 0.3
<i>p</i> -value		ns	ns	*	ns	ns	ns

*, and **, indicate statistical significance at $p < 0.05$ and $p < 0.01$; ns indicates no significant difference. The same letters suggest no statistical difference between the treatments.

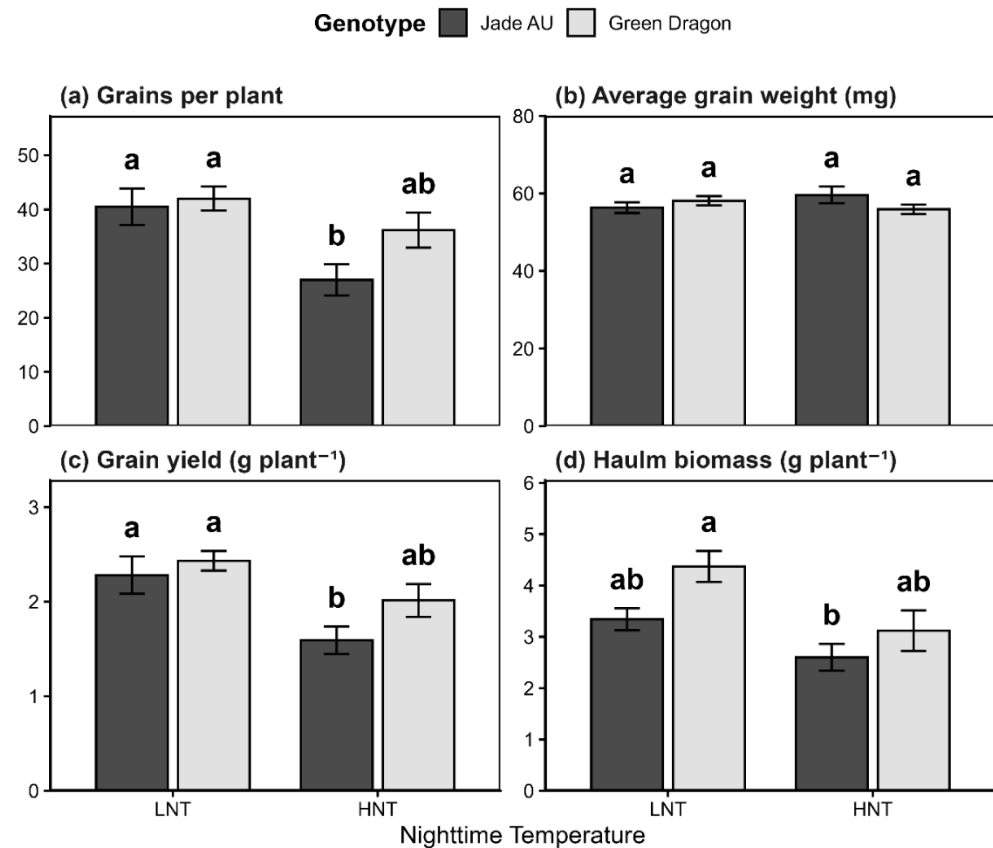


Figure 2. The effect of nighttime temperature treatment on the yield traits of two mungbean genotypes in the CE experiment. (a) Grains per plant; (b) Average grain weight (mg); (c) Grain yield per plant (g); and (d) Haulm biomass per plant (g). Same letters denote no significant difference. Bars represent treatment means ($n = 6$), and error bars indicate one standard error of the mean. Lowercase letters above bars indicate mean separation using the Tukey HSD test ($p < 0.05$); bars sharing the same letter within each panel are not significantly different. LNT, low nighttime temperature; HNT, high nighttime temperature.

Green Dragon maintained a significantly larger pool of immature pods at harvest than Jade AU under both temperature treatments (4.0 vs. 2.4 plant⁻¹; $p < 0.05$; Table 3). This advantage was more pronounced under HNT and translated into a consistent yield advantage for Green Dragon over Jade AU of 21% under HNT and 17% under LNT. Importantly, this genotypic divergence in yield occurred despite identical Rn/A ratios between genotypes at all measurement occasions, confirming that the yield advantage originated in reproductive architecture rather than leaf-level carbon exchange capacity.

3.2. Field Trial

3.2.1. Thermal Environment Classification Across Sowing Dates and Years

Weather parameters were characterized for the 20-day critical reproductive window (CRW) at each year $\times$ sowing date $\times$ genotype combination (Table 4).

The year $\times$ sowing date combinations produced distinct, but not fully isolated, thermal and radiation environments during the CRW (Table 4; Figure 3). The 2024 mid-sowing environment had the highest average nighttime temperature (ANT 25.4 °C), accumulated approximately 60–66 h above 26 °C, and had no daytime hours above 38 °C; it was therefore the clearest HNT-dominant environment in the experiment.

Table 4. Mean thermal and radiation conditions, and cumulative daytime and nighttime heat exposure, during the 20-day CRW (5 days pre-flowering to 15 days post-flowering) of mungbean across year × sowing date × genotype combinations.

Year	Sowing Date	Genotype	Average Daily Observation During Critical Period				Cumulative Observation During Critical Period			
			MaxT	MinT	ANT	DTA	SR	DH	NH	Cloudy/ Rainy Days
2024	Early	Jade AU	31.9	22.5	23.8	9.4	436.9	3	25.0	13.8
		Green	32.0	22.6	23.8	9.4	443.5	3	25.0	12.8
		Dragon	33.1	24.0	25.4	9.1	516.4	0	60.3	20
	Mid	Jade AU	33.1	24.0	25.4	9.1	516.4	0	60.3	20
		Green	33.0	24.1	25.4	9.0	518.3	0	59.8	20
		Dragon	34.2	23.8	25.2	10.4	449.5	2.6	66.2	10
	Late	Jade AU	34.2	23.8	25.2	10.4	449.5	2.6	66.2	10
		Green	33.7	23.7	25.1	10.0	463.5	1.1	59.2	9.5
		Dragon	33.7	23.7	25.1	10.0	463.5	1.1	59.2	9.5
2025	Early	Jade AU	36.2	20.7	23.7	15.5	508.6	12.6	42.8	3
		Green	36.2	20.7	23.7	15.5	510.1	12.6	42.7	3
		Dragon	36.5	22.2	24.7	14.3	533.7	25.0	66.7	9.3
	Mid	Jade AU	36.5	22.2	24.7	14.3	533.7	25.0	66.7	9.3
		Green	36.1	22.2	24.5	14.0	528.0	19.7	61.0	10
		Dragon	36.1	22.2	24.5	14.0	528.0	19.7	61.0	10
	Late	Jade AU	33.5	23.2	25.2	10.4	382.0	15.3	74.2	7
		Green	34.3	22.8	25.1	11.6	404.6	20.3	77.8	6.8
		Dragon	34.3	22.8	25.1	11.6	404.6	20.3	77.8	6.8

Abbreviations: MaxT—daily maximum temperature, °C; MinT—daily minimum temperature, °C; ANT—average nighttime temperature, °C; DTA—diurnal temperature amplitude, °C; SR—cumulative solar radiation during critical period, MJ m^{−2}; DH—daytime hours above 38 °C; NH—nighttime hours above 26 °C.

The 2024 early-sowing environment had fewer nighttime heat hours (25 h), the lowest cumulative solar radiation, and the greatest frequency of cloudy/rainy days; it was interpreted as a radiation-limited, mixed environment rather than pure HNT treatment. The 2024 late-sowing environment accumulated 59–66 nighttime heat hours but also had lower solar radiation than the mid-sowing environment and was classified as mixed late-season HNT stress.

In 2025, early sowing accumulated 12.6 h above 38 °C and approximately 43 h above 26 °C, indicating predominantly daytime heat with concurrent nighttime exposure. Mid sowing accumulated 19.7–25.0 daytime heat hours and 61–67 nighttime heat hours and was therefore a combined daytime and nighttime heat environment rather than a pure HDT treatment. Late sowing accumulated 15–20 daytime heat hours and 74–78 nighttime heat hours, together with lower solar radiation, and was classified as mixed late-season stress. Thus, the field trial generated a gradient of co-occurring daytime heat, nighttime heat, and radiation conditions rather than independent HDT-only and HNT-only treatments.

3.2.2. Pod-Filling Duration as a Phenological Indicator of HNT

The Y × S interaction was significant for pod-filling duration ($p < 0.001$; Appendix A, Table A1). In 2025, pod-filling duration ranged from 39 days under early sowing to 19 days under late sowing (a 20-day range within a single season). In 2024, the range was narrower (20–29 days). Early-sown crops in 2025 had a pod-filling period that was 10 days longer than their 2024 counterparts (39 vs. 29 days), reflecting the cooler nighttime conditions that prevailed during their pod-filling window. Conversely, the late-sown crops in the

two seasons did not differ in pod-filling duration (20.3 vs. 18.7 days), indicating that the dominant limitation under late sowing in both years was thermal compression of the reproductive window rather than season-specific radiation or photoperiod effects.

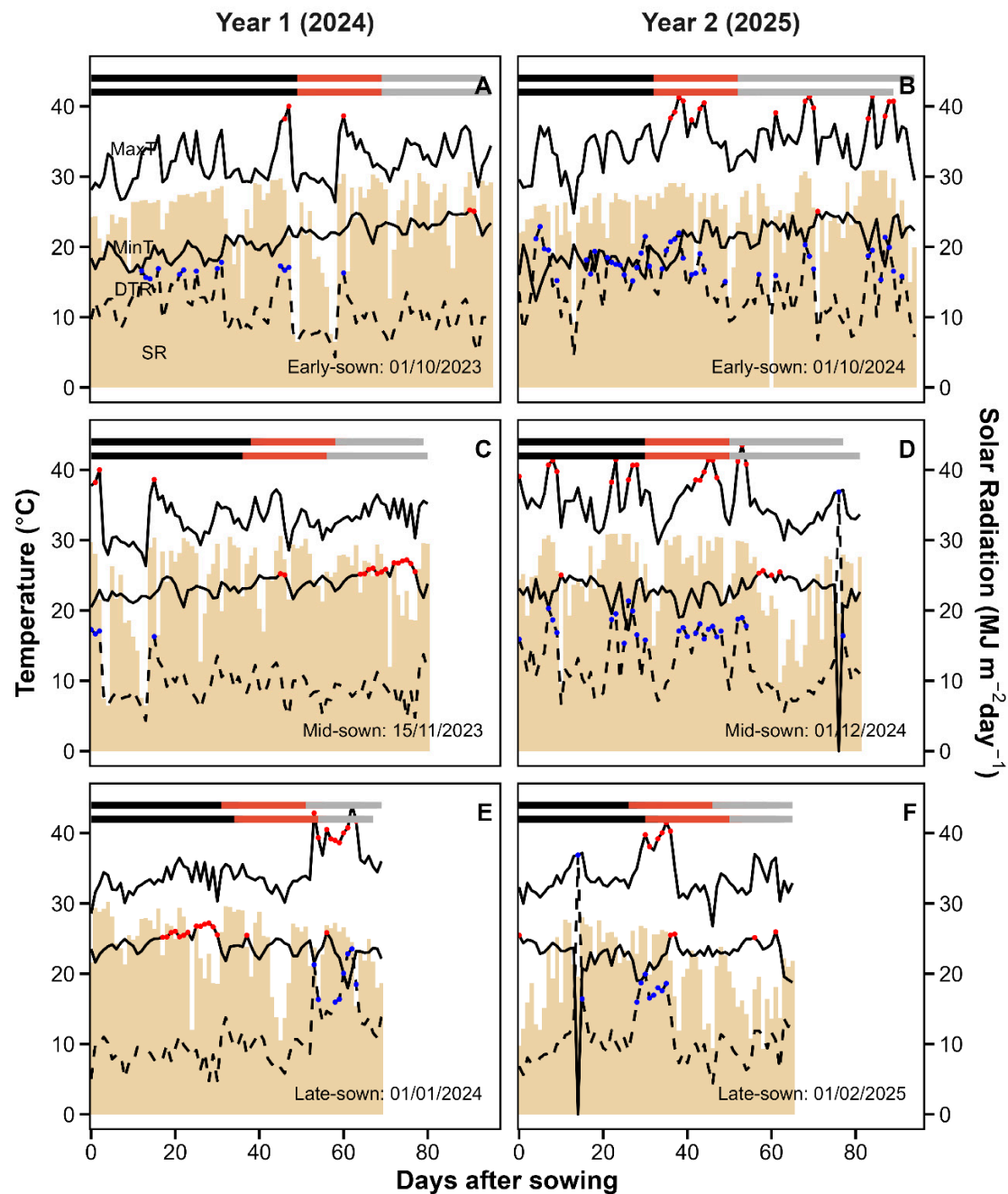


Figure 3. Seasonal dynamics of weather parameters measured during the mungbean field trial. SR—daily solar radiation ($\text{MJ m}^{-2} \text{day}^{-1}$); MaxT—daily maximum temperature ($^{\circ}\text{C}$); MinT—minimum temperature ($^{\circ}\text{C}$); and DTA—daily diurnal temperature amplitude ($^{\circ}\text{C}$) in six year $\times$ sowing date environments: (A) 2024 Early-sown, (B) 2025 Early-sown, (C) 2024 Mid-sown, (D) 2025 Mid-sown, (E) 2024 Late-sown, and (F) 2025 Late-sown. The red dots on MaxT and MinT indicate days with a maximum temperature above 40°C and nights with a minimum temperature above 26°C , respectively. The blue dots in DTA indicate a diurnal temperature amplitude above 15°C . Bars on the top indicate the time from sowing to 5 days pre-flowering (black), 5 days pre-flowering to 15 days post-flowering (red), and until maturity (gray) for the two mungbean genotypes: Green Dragon (top) and Jade AU (bottom).

3.2.3. Yield Components and Grain Yield

Grain yield was significantly lower in 2024 than in 2025 across all sowing dates and both genotypes (mean of 240 vs. 269 g m⁻²; Y × S interaction— $p < 0.001$). In 2024, early sowing produced a significantly higher yield than mid and late sowing. In 2025, the same sowing date direction was maintained but with a steeper decline (Figure 4d). The late-sown crop in 2025 produced the lowest yields recorded in the trial. The Y × G interaction ($p < 0.001$) revealed that the genotypes performed similarly in 2024 (~236–246 g m⁻²) but diverged in 2025, where Green Dragon (290.4 g m⁻²) significantly outyielded Jade AU (247.2 g m⁻²). Under late sowing in 2025, Green Dragon yielded 205.0 g m⁻² compared to 161.3 g m⁻² for Jade AU, a 27% advantage attributable to its larger mature pod pool.

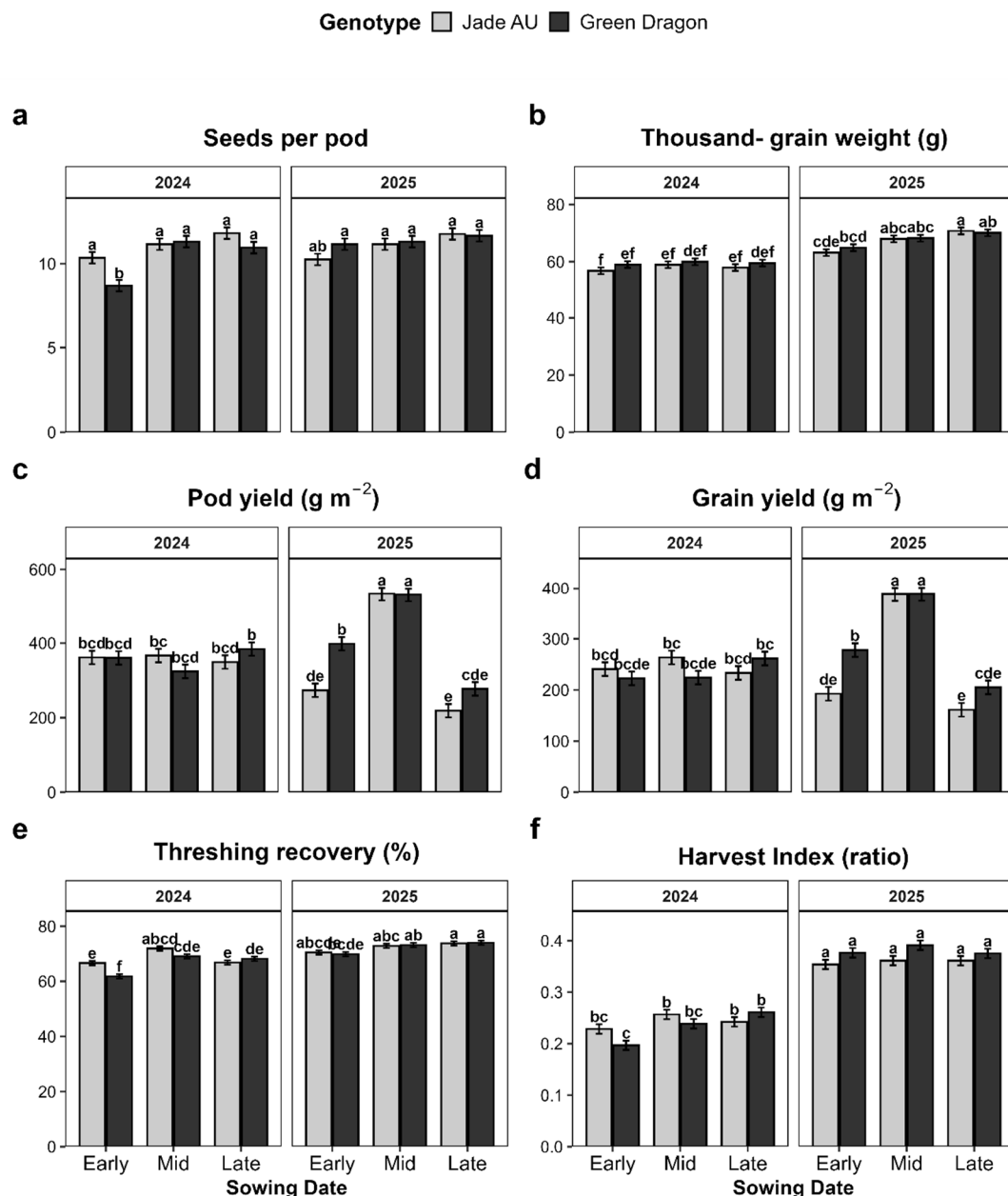


Figure 4. Effect of year (Y), sowing date (S), and genotype (G) on the yield and yield attributes of mungbean in the field trials. (a) Seeds per pod; (b) Thousand-grain weight (g); (c) Pod yield (g m⁻²); (d) Grain yield (g m⁻²); (e) Threshing recovery (%), and (f) Harvest index. Bars represent means across four replications and error bars indicate one standard error of the mean. Lowercase letters indicate mean separation by Tukey HSD ($p < 0.05$).

The harvest index (HI) exhibited the most analytically informative year-level contrast in the study ($Y \times S \times G, p < 0.05$). All 2024 treatments produced HI values of 0.20 to 0.27, while all 2025 treatments ranged from 0.36 to 0.40 (Figure 4f). Within 2024, Green Dragon under early sowing had the lowest HI (0.20), consistent with its higher immature pod pool and the radiation-limited environment of that treatment. Thousand-grain weight (TGW) was stable across 2024 sowing dates (57.9–59.5 g) but increased progressively from early to late sowing in 2025 (64.1, 68.1, and 70.5 g; $Y \times S, p < 0.01$) (Figure 4b), indicating that grain-filling efficiency per established pod improved under the shorter but less thermally demanding reproductive windows of 2025. Pods per plant followed a $Y \times S$ pattern in which 2024 early sowing produced the most pods (50 pods plant⁻¹) and 2025 late sowing the fewest (16 pods plant⁻¹) (Appendix A, Table A2), consistent with the year-level divergence in Rn/A ratios and pod-filling duration.

3.2.4. Respiration-to-Photosynthesis (Rn/A) Ratio Across Sowing Environments

Nighttime respiration (Rn) and the Rn/A ratio showed a highly significant $Y \times S$ interaction at both measurement stages ($p < 0.001$; Table 5 and Appendix A, Table A3). The Rn rate was substantially higher in 2024 than in 2025 across all sowing dates (pod filling: 5.7 vs. 2.5 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$). The 2024 mid-sown crop showed the highest Rn at both flowering (9.4 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and pod filling (7.5 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) of any treatment. The corresponding Rn/A ratio at pod filling reached 0.27–0.29 in the 2024 mid-sown treatment, which were the highest recorded values in the trial and directly comparable to the peak values in the CE experiment under the 25 °C HNT treatment. In contrast, the 2025 mid-sown crop recorded the lowest Rn/A of any treatment at pod filling (0.10), corresponding with its highest HI (0.40) and TGW. Furthermore, the response of nighttime respiration to a short-term elevation in temperature, achieved by raising the leaf cuvette temperature, showed a consistent and significant positive linear relationship between Rn and temperature (Appendix A, Figure A2). Across the years, Rn exhibited greater temperature sensitivity during 2024 compared to 2025, which coincided with the wider range of temperatures recorded during the measurements.

Daytime photosynthesis differed significantly between years (2024 > 2025 at both stages; Table 5) but was not a significant predictor of the Rn/A ratio or of yield differences between treatments within either year, consistent with the CE finding that HNT impairs yield through the nocturnal pathway rather than through daytime carbon fixation. The sowing date's main effect at pod filling showed Rn/A increasing progressively from early (0.15) to mid (0.20) and late (0.22) sowing ($p < 0.001$), indicating a compounding respiratory burden as crops were pushed into warmer nocturnal environments. The $S \times G$ interaction for Rn and Rn/A was significant at both stages ($p < 0.05$): Jade AU recorded higher Rn/A than Green Dragon under mid and late sowing (pod filling: 0.21 vs. 0.18 and 0.24 vs. 0.20, respectively), while the two genotypes were equivalent under early sowing (0.15 for both).

3.2.5. Seasonal Association Between the Rn/A Ratio and Grain Yield

Year-specific simple regressions showed contrasting unadjusted relationships between the Rn/A ratio at pod filling and grain yield (Figure 5). However, because the Rn/A ratio co-varied strongly with the sowing date and seasonal environmental gradient, these regressions were not interpreted as independent evidence of yield-predictive Rn/A effects. Therefore, we fitted year-specific split-plot mixed models that included sowing date (S), genotype (G), $S \times G$ interactions, block (B), $B \times S$, and Rn/A at pod filling. Detailed model comparison statistics, adjusted Rn/A coefficients, confidence intervals, R^2 values, collinearity diagnostics, and model-fit summaries are provided in Appendix A, Table A4.

Table 5. Effect of year, sowing date, genotype, and their interaction on photosynthesis (A), respiration (Rn), and Rn/A ratio measured during flowering and pod filling in mungbean during the field trial.

Treatments		Measurements During Pod Filling		
		A ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Rn ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Rn/A Ratio
2024		27.4 $\pm$ 1.14 a	5.7 $\pm$ 0.3 a	0.22 $\pm$ 0.02 a
2025		16.5 $\pm$ 0.46 b	2.5 $\pm$ 0.17 b	0.15 $\pm$ 0.01 b
p-value		***	***	***
Early		26.6 $\pm$ 2.02 a	3.7 $\pm$ 0.21 b	0.15 $\pm$ 0.01 c
Mid		20.5 $\pm$ 1.52 b	4.5 $\pm$ 0.79 a	0.2 $\pm$ 0.03 b
Late		18.7 $\pm$ 0.85 c	4.2 $\pm$ 0.34 a	0.22 $\pm$ 0.01 a
p-value		***	***	***
Jade AU		21.7 $\pm$ 1.49	4.3 $\pm$ 0.42 a	0.2 $\pm$ 0.02 a
Green Dragon		22.2 $\pm$ 1.37	3.9 $\pm$ 0.41 b	0.18 $\pm$ 0.02 b
p-value		ns	**	***
2024	Early	34.2 $\pm$ 0.83 a	4.3 $\pm$ 0.25 c	0.13 $\pm$ 0.01 e
	Mid	26 $\pm$ 0.91 b	7.5 $\pm$ 0.15 a	0.29 $\pm$ 0.01 a
	Late	22 $\pm$ 0.18 c	5.3 $\pm$ 0.27 b	0.25 $\pm$ 0.02 b
2025	Early	19.1 $\pm$ 0.55 d	3 $\pm$ 0.07 d	0.16 $\pm$ 0.01 d
	Mid	14.9 $\pm$ 0.49 e	1.4 $\pm$ 0.07 e	0.1 $\pm$ 0.01 f
	Late	15.5 $\pm$ 0.26 e	3 $\pm$ 0.13 d	0.2 $\pm$ 0.01 c
p-value		***	***	***
Y $\times$ G		ns	ns	ns
S $\times$ G		*	*	*
Y $\times$ S $\times$ G		ns	ns	ns

*, **, and *** indicate statistical significance at $p < 0.05$, $p < 0.01$, and $p < 0.001$; ns indicates no significant difference. The same letters suggest no statistical difference between the treatments.

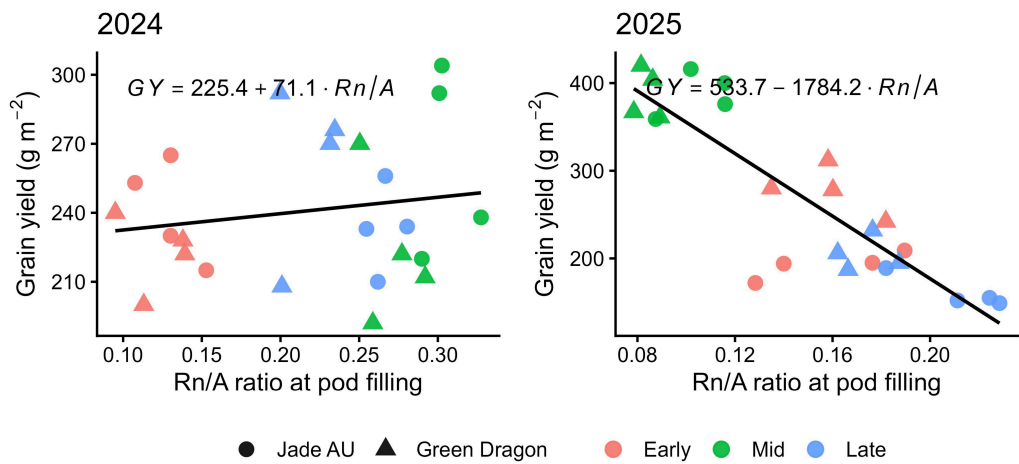


Figure 5. Unadjusted year-specific associations between the respiration-to-photosynthesis (Rn/A) ratio measured at pod filling and grain yield for two mungbean genotypes across three sowing dates in 2024 and 2025. Points represent individual plot means ($n = 4$ blocks per genotype $\times$ sowing date combination). Point colors denote the sowing date, and point shapes denote the genotype. Black lines represent ordinary least-squares regression fitted separately by year; equations are shown for descriptive visualization. Split-plot mixed-model analyses that accounted for sowing date, genotype, block, and block $\times$ sowing date found no additional independent contributions of Rn/A to grain yield variation in either year. The corresponding split-plot mixed-model statistics are reported in Appendix A, Table A4.

In 2024, adding Rn/A did not improve model fit relative to the reduced model containing sowing date, genotype, and the split-plot random structure ($\chi^2 = 0.456$, $p = 0.500$).

The adjusted Rn/A coefficient was -145.1 g m^{-2} per unit Rn/A (95% CI: -602.0 to 311.8 ; $p = 0.507$). Likewise, in 2025, adding Rn/A did not improve model fit ($\chi^2 = 0.162$, $p = 0.687$); the adjusted coefficient was 120.8 g m^{-2} per unit Rn/A (95% CI: -409.6 to 651.2 ; $p = 0.640$). The 2025 model explained a high proportion of yield variation through sowing date, genotype, $S \times G$ interactions, and the experimental structure, without evidence of an additional independent Rn/A contribution.

Collinearity diagnostics indicated substantial overlap between Rn/A and sowing date in both years (2024: VIF = 10.65 for Rn/A and 14.87 for sowing date; 2025: VIF = 7.72 for Rn/A and 12.39 for sowing date). Thus, the negative simple regression observed in 2025 reflected the combined environmental gradient across sowing dates rather than an independently supported Rn/A–yield relationship. The Rn/A ratio remains a responsive physiological trait under controlled HNT conditions, but its independent value for predicting field yield requires validation across a broader range of genotypes and independently replicated environments.

3.2.6. Genotypic Differences in Rn/A Ratio and Pod Set Under Field Stress

The $S \times G$ interaction for pods per plant ($p < 0.05$) revealed that genotypic differences in pod number were dependent on the sowing date. Under early and mid sowing, Jade AU and Green Dragon produced comparable pod numbers ($35\text{--}40$ pods plant $^{-1}$). However, under late sowing, Green Dragon maintained a significantly higher pod number than Jade AU (29 vs. 23 pods plant $^{-1}$), mirroring the CE experimental pattern where Green Dragon's larger immature pod pool conferred a greater capacity to sustain mature pod numbers under thermal compression. This pod set advantage, combined with Green Dragon's consistently lower Rn/A under mid and late sowing stress, suggests that the two components of HNT resilience—lower nocturnal respiratory cost and greater reproductive plasticity—were both expressed in Green Dragon under field conditions, but were absent in Jade AU under the same thermal environments.

3.2.7. Grain Biochemical Composition

Total phenolic content (TPC) was not significantly affected by year, sowing date, genotype, or any of their interactions ($\sim 100\text{--}119 \text{ mg GAE } 100 \text{ g}^{-1} \text{ dw}$ across all treatments), confirming that TPC accumulation in mungbean grain is under strong developmental and genetic control and was not induced by the thermal stress magnitudes imposed in this study. In contrast, both antioxidant capacity assays (CUPRAC and FRAP) were significantly higher in 2025 than in 2024 and highest under late sowing, an apparently counterintuitive result given that 2025 produced higher yields (Appendix A, Figure A3).

3.2.8. Principal Component Analysis

Principal component analysis (PCA) of all measured environmental, phenological, physiological, agronomic, and biochemical variables explained 62.1% of the total variance across the first two principal components (PC1 = 38.2%, PC2 = 23.9%; Figure 6). PC1 was characterized by positive loadings of nighttime heat hours above 26°C , Rn, and Rn/A at both measurement stages, and opposed by positive loadings of harvest index, filled pods per plant, grain yield, and thousand-grain weight. The near-zero loading of mean daytime VPD on PC1 confirmed that VPD was not a primary driver of the dominant variation axis. The year ellipses separated most distinctly along PC1: the 2024 ellipse was displaced toward the nighttime heat and Rn/A vectors, and the 2025 ellipse concentrated in the yield, HI, and filled pod count region. CUPRAC and FRAP loaded on PC2 alongside daytime heat hours above 38°C , maximum temperature, and DTA, consistent with both antioxidant assays being elevated under the HDT-dominant environment of 2025 rather than under HNT-dominant 2024 conditions. The genotype confidence ellipses overlapped on both PC

axes, consistent with the non-significant genotype effects on gas exchange and confirming that the genotypic yield advantage of Green Dragon was not reflected in any differentiation in physiological stress response at the leaf level.

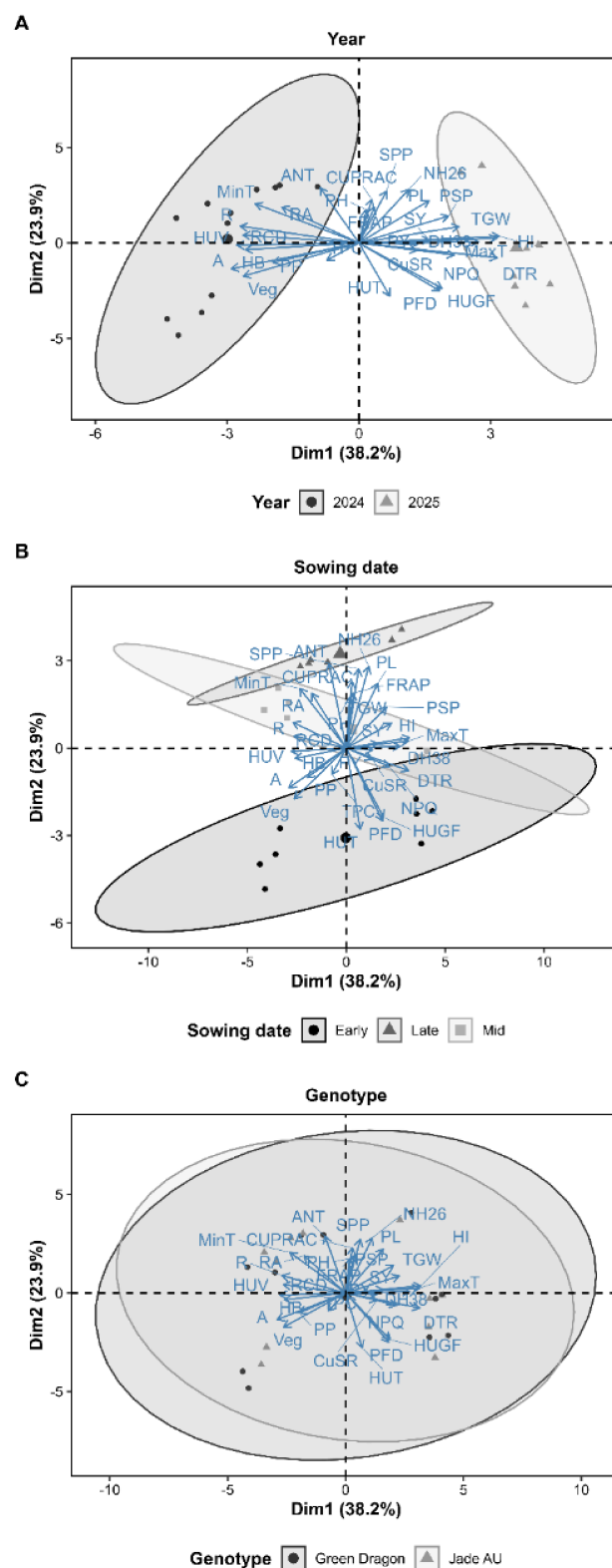


Figure 6. Principal component analysis (PCA) biplot of all measured environmental, phenological, physiological, agronomic, and biochemical variables from the mungbean field trials. Ellipses with 95% confidence are grouped by (A) year, (B) sowing date, and (C) genotype. Arrows represent variable loadings; Dim1 (PC1) and Dim2 (PC2) explain 38.2% and 23.9% of the total variance, respectively.

Variable abbreviations: DH38 and NH26, cumulative hours of daytime and nighttime temperature of 38 and 26 °C, respectively; RCD, frequency of rainy or cloudy days during the critical period; CuSR, cumulative solar radiation during the critical period; MaxT, MinT, ANT, and DTA, average daily maximum, minimum, nighttime, and diurnal temperature amplitude/amplitude, respectively; Veg, vegetative phase duration; GF, pod-filling duration; HUVeg, vegetative heat units; HUGF, grain-filling heat units; HUT, total heat units; A, photosynthesis at pod filling; R, nighttime respiration at pod filling; RA, Rn/A ratio at pod filling; PH, plant height; PP, pods per plant; PL, pod length; SPP, seeds per pod; PY, pod yield; SY, grain yield; PSP, threshing recovery; TGW, thousand-grain weight; HI, harvest index; TPC, total phenolic content; CUPRAC, cupric reducing antioxidant capacity; FRAP, ferric reducing antioxidant potential.

4. Discussion

4.1. HNT Impairs Carbon Balance and Truncates Pod-Filling Duration

High nighttime temperatures directly impaired carbon balance in mungbean and shortened pod-filling duration, confirming the first research question. In the CE experiment, a 5 °C increase in nighttime temperature above 20 °C shortened pod filling by 15% (5.2 days), equivalent to approximately 1.06 days per °C, a magnitude comparable to the 0.8 days per °C reported for peas, which was attributed to the thermal acceleration of development [20].

The mechanistic signature of HNT in mungbean was a sustained elevation of the Rn/A ratio driven by a two-phase response. The initial phase (within 2 days) was characterized by a Q_{10} -driven respiratory surge that elevated Rn/A to 0.44–0.46, while A simultaneously declined. The second phase (7–14 days) showed partial Rn recovery while A remained persistently suppressed, the reverse of what was expected under simple thermal acclimation. The persistence of lower photosynthesis after the initial respiratory response had moderated may reflect several temperature-induced processes, including altered carbohydrate status, feedback regulation of photosynthesis, stomatal limitation, or biochemical acclimation [21,22]. Because non-structural carbohydrate concentrations, carbon export, and source–sink fluxes were not measured, carbohydrate depletion remains a plausible but untested hypothesis.

Legume nodulation and symbiotic nitrogen fixation require substantial photosynthetically derived carbon and respiratory energy [23,24]. Therefore, high nighttime temperatures may influence both reproductive sink development and the carbon economy associated with nodules. However, the present experiment cannot determine whether HNT-induced changes in leaf carbon balance compromised nodule activity or nitrogen fixation because direct measurements of nodule traits and nitrogen fixation activity were not collected.

The carbon imbalance translated into yield loss by reducing the number of reproductive sinks, consistent with the established sensitivity of male and female reproductive processes, fertilization, and early embryo development in legumes to heat stress [5,16]. HNT reduced mature pods per plant by 21% and grains per plant by 23%, while pod length, grains per pod, and thousand-grain weight remained unaffected. This positions the impairment upstream at the pod initiation or early pod retention stage rather than at seed filling, consistent with observations in soybean and cowpea where HNT primarily disrupts fertilization and early embryo development before endosperm filling is initiated [25,26]. The higher immature pod weight under HNT (1.1 vs. 0.5 g plant^{−1}) indicates that some pods had initiated development but had not reached maturity by harvest. The timing and physiological basis of their incomplete development cannot be resolved from the present measurements.

4.2. Contrasting Thermal Stress Types Drive Divergent Yield Formation Pathways

The staggered-sowing field trial generated contrasting combinations of nighttime heat, daytime heat, solar radiation, and seasonal conditions. The PCA separated the sampled year $\times$ sowing date environments primarily along axes associated with nighttime heat exposure, Rn/A ratio, harvest index, and yield components; however, these relationships should be interpreted as multivariate field associations rather than evidence of fully isolated HNT and HDT effects (Figure 6). Despite contrasting thermal regimes, average grain yields were broadly comparable between seasons but were achieved through opposing yield–component compensation. Across the 2024 environments, yield formation was characterized by a relatively high pod number (up to 50 pods plant^{−1} under early sowing) but lower TGW and HI (0.20–0.27). Across the 2025 environments, higher TGW and HI partly compensated for a lower pod number. These differences should be interpreted in the context of contrasting combinations of temperature, radiation, and seasonal development. This compensation pattern closely parallels long-term observations in rice, where yield is strongly and negatively correlated with minimum temperatures [27], consistent with mungbean’s ability to maintain total pod yield stability through compensation between pod number and individual pod weight [28].

From a farming systems perspective, the comparable total grain yields achieved under HNT-dominant and HDT-dominant seasons confirm that mungbean retains its productive value as a break crop under both thermal stress types. However, the markedly lower harvest index in 2024 indicates substantially higher vegetative biomass under HNT conditions.

4.3. Genotypic Responses: Reproductive Architecture as a Priority Breeding Trait

The genotypic divergence in this study identifies two functionally distinct trait categories as priority targets for HNT tolerance improvement in mungbean: reproductive sink redundancy and nocturnal respiratory efficiency. Green Dragon’s superior maintenance of pod number under HNT, demonstrated in the CE experiment (4.0 vs. 2.4 immature pods plant^{−1}) and confirmed under the most compressed pod-filling duration in the field trial (29 vs. 23 pods plant^{−1} under late sowing in 2025), constitutes a quantifiable advantage in reproductive buffer capacity. This mechanism of offsetting yield loss through a larger pool of initiated pod sinks is documented in other indeterminate legumes, including soybean, where secondary flowering flushes partially compensate for early pod-set failure under stress [29,30]. Moreover, this reproductive advantage was not accompanied by any leaf-level Rn/A ratio advantage between genotypes at any measurement occasion in the CE experiment. In the two genotypes examined, reproductive pod retention and leaf-level Rn/A did not show the same pattern under controlled conditions. This phenotypic contrast suggests that the trait categories, namely carbon economy and reproductive architecture, may be separable. However, their genetic relationship cannot be inferred from two genotypes. Segregating populations and genomic analyses will be required to determine whether pod retention and Rn/A are independently inherited or regulated by shared pathways.

However, in the field trial, Jade AU recorded a consistently higher Rn/A than Green Dragon under mid- and late-sowing stress (0.21 vs. 0.18 and 0.24 vs. 0.20 at pod filling, respectively), while the genotypes were equivalent under early sowing. This field divergence in Rn/A, absent in the CE experiment, suggests that Green Dragon showed a lower observed Rn/A under selected field environments; the physiological stability and genetic basis of this difference remain to be determined.

4.4. Grain Biochemical Quality Under Contrasting Thermal Environments

The stability of total phenolic content (TPC) across all thermal environments (~100–119 mg GAE 100 g⁻¹ dw) indicates that phenylpropanoid pathway carbon allocation in mungbean grain is under strong genetic and developmental control, insensitive to the stress magnitudes imposed in this study. Studies in wheat have indicated that TPC is strongly influenced by genetic factors and controlled by specific genes in the phenolic acid biosynthesis pathways [17,31].

TPC was stable across the environments examined, whereas CUPRAC and FRAP varied between years and sowing dates. These assays indicate differences in extractable reducing and antioxidant capacity but do not identify the cellular source of reactive oxygen species or the molecular pathways responsible. The stability of TPC in mungbean, in contrast with responses reported in adzuki bean (*Vigna angularis*), may reflect the species-specific regulation of phenylpropanoid, flavonoid, or anthocyanin pathways, developmental timing, or differences in stress intensity and duration [32]. These explanations remain hypotheses because individual phenolic compounds, gene expression, and enzyme activities were not measured.

4.5. The Rn/A Ratio as a Candidate Physiological Indicator for HNT Response in Mungbean

Heat tolerance screening in grain legumes commonly combines reproductive traits, including pollen viability, flower retention, and pod and seed set, with physiological traits such as canopy temperature, membrane stability, chlorophyll fluorescence, photosynthetic performance, and antioxidant responses, along with molecular approaches using heat-responsive genes, heat-shock proteins, transcriptional markers, or genomic loci [5,33,34]. In this context, the Rn/A ratio may provide complementary information because it integrates nighttime respiratory carbon cost with daytime carbon assimilation.

The CE experiment showed that Rn/A responded rapidly and strongly to HNT, indicating its potential value as a non-destructive physiological response trait. However, the field analyses showed that Rn/A did not explain additional grain yield variation after sowing date, genotype, and the split-plot blocking structure were considered. The unadjusted 2025 regression therefore reflected the shared environmental gradient across sowing dates rather than an independently supported yield-predictive effect.

Accordingly, Rn/A should not yet be treated as a yield prediction tool or breeding selection criterion for mungbean. Instead, it is best regarded as a candidate physiological indicator of HNT response that warrants validation in a broader germplasm panel, across locations and seasons, and under independently replicated HNT treatments. Its eventual value for breeding will depend on whether it predicts performance independently of phenology, sowing environment, radiation, and other co-varying factors.

A longer-term breeding strategy could combine phenotypic assessments of pod retention and Rn/A with genomic approaches that identify heat-responsive loci and regulatory pathways. Evidence from rice suggests that naturally occurring regulatory variation can contribute to heat resilience and grain quality maintenance. However, candidate mechanisms must be validated specifically in mungbean before they can guide selection [35].

4.6. Limitations and Future Directions

Several limitations constrain the interpretation of these results. In the CE experiment, only two growth chambers were available; although chamber environments were swapped at 10 days post-treatment to minimize confounding, true chamber replication was absent. These results should therefore be treated as strong indicators requiring independent validation in a fully replicated CE design. In the field trial, nighttime temperature was not directly manipulated, and its effects are inferred from natural seasonal variation and multivariate

statistical association. Co-varying factors such as solar radiation, rainfall, and VPD were not fully disentangled despite multivariate analysis, and the 2024 radiation–Rn/A confounding identified in the regression analysis is an important reminder of this limitation.

Mechanistic interpretation is further constrained by the absence of direct NSC measurements in source and sink tissues, direct measurement of nitrogen fixation activity (acetylene reduction assay or ^{15}N natural abundance), and phenotypic characterization of individual flowering and podding flushes. Future research should prioritize: (i) field-based automated nighttime warming systems that independently elevate nighttime temperatures while preserving ambient DTR and daytime conditions; (ii) combined Rn/A screening and NSC quantification in segregating mungbean populations to confirm the substrate limitation hypothesis; (iii) parallel measurement of nodule activity and seasonal BNF under HNT-dominant and HDT-dominant environments; and (iv) broader germplasm screening, including exotic accessions and wild relatives, to expand the genetic pool for Rn/A-based selection [35].

Future work could also integrate single-cell and spatial transcriptomic approaches to resolve the tissue- and cell-type specificity of HNT responses [36]. Profiling floral abscission zones and developing pod tissues and nodule cell types could help identify where carbon balance disruption, reproductive abortion, and potential nodule dysfunction are initiated. Such approaches would complement leaf-level gas exchange measurements by distinguishing whole-plant association from cell-specific regulatory responses and could guide targeted candidate gene discovery.

5. Conclusions

This study demonstrated that HNT during the reproductive phase reduces mungbean grain yield through a two-component pathway: an immediate elevation of the Rn/A ratio associated with fewer mature pods and a shortened pod-filling duration. The CE experiment confirmed that a 5 °C increase in nighttime temperature above 20 °C reduced grain yield by 21% through fewer mature pods and grains, without impairing individual pod or grain-filling characteristics. The field trial showed lower harvest indices across the 2024 environments than across the 2025 environments, but the contrasting responses occurred under co-varying combinations of nighttime heat, daytime heat, radiation, and seasonal conditions.

The Rn/A ratio should be considered a candidate physiological indicator of HNT response rather than a validated yield predictor or breeding selection criterion. Validation across germplasm, locations, seasons, and independently replicated HNT experiments is required. Green Dragon showed traits consistent with greater reproductive resilience under selected stress environments and should be evaluated further across locations, seasons, and genetically diverse comparison sets before any grower recommendation is made. Aligning sowing dates to position the mungbean reproductive window within cooler nighttime thermal conditions remains the most immediately accessible management strategy for growers to sustain mungbean yield within subtropical broadacre cereal rotation systems facing continued increases in nighttime temperature.

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Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A

Table A1. Effect of year, sowing date, genotype, and their interaction on the duration and heat units ($^{\circ}\text{Cd}$) accumulated during the vegetative growth, pod filling, and total crop period of mungbean during the field trial.

Treatments		Duration (Days)		Heat Units Accumulated ($^{\circ}\text{Cd}$)		
		Vegetative Growth	Pod Filling	Vegetative Growth	Pod Filling	Total Crop Duration
Year (Y)						
2024		41 $\pm$ 1.24 a	24.8 $\pm$ 0.9 b	696.3 $\pm$ 8.41 a	451.2 $\pm$ 14.29 b	1278.4 $\pm$ 19.87
2025		32.7 $\pm$ 0.58 b	30.1 $\pm$ 1.84 a	577.8 $\pm$ 10.8 b	545.8 $\pm$ 35.69 a	1282.8 $\pm$ 38.48
<i>p</i> -value		***	***	***	**	ns
Sowing Date (S)						
Early		41.5 $\pm$ 1.91 a	34.2 $\pm$ 1.45 a	638.2 $\pm$ 26.15	612 $\pm$ 28.08 a	1419 $\pm$ 13.67 a
Mid		35.5 $\pm$ 1.09 b	28.7 $\pm$ 1.18 b	644.6 $\pm$ 14.66	534.8 $\pm$ 22.37 b	1320.3 $\pm$ 15.61 b
Late		33.4 $\pm$ 0.76 c	19.5 $\pm$ 0.5 c	628.4 $\pm$ 14.99	348.8 $\pm$ 10.87 c	1102.4 $\pm$ 19.63 c
<i>p</i> -value		***	***	ns	***	***
Genotype (G)						
Jade AU		37.1 $\pm$ 1.21	27.1 $\pm$ 1.6	641.8 $\pm$ 14.47	493 $\pm$ 29.28	1275.7 $\pm$ 30.36
Green Dragon		36.6 $\pm$ 1.39	27.8 $\pm$ 1.5	632.3 $\pm$ 16.76	504.1 $\pm$ 28.51	1285.5 $\pm$ 30.86
<i>p</i> -value		ns	ns	ns	ns	ns
Y $\times$ S						
2024	Early	48.7 $\pm$ 0.63 a	29.2 $\pm$ 0.52 b	734.3 $\pm$ 9.12 a	512.7 $\pm$ 9.27 c	1378.6 $\pm$ 9.43 b
	Mid	39.3 $\pm$ 0.6 b	24.8 $\pm$ 1.2 c	689.4 $\pm$ 11.08 ab	460.9 $\pm$ 21.96 c	1286.8 $\pm$ 21.62 c
	Late	35 $\pm$ 0.66 c	20.3 $\pm$ 0.73 d	665.1 $\pm$ 11.76 b	380.1 $\pm$ 12.93 d	1169.8 $\pm$ 14.29 d
2025	Early	34.4 $\pm$ 0.78 c	39.2 $\pm$ 1.24 a	542.1 $\pm$ 14.4 c	711.3 $\pm$ 21.78 a	1459.5 $\pm$ 15.64 a
	Mid	31.8 $\pm$ 0.84 c	32.5 $\pm$ 0.5 b	599.8 $\pm$ 14.98 c	608.7 $\pm$ 10.15 b	1353.8 $\pm$ 16.02 bc
	Late	31.8 $\pm$ 1.12 c	18.7 $\pm$ 0.57 c	591.7 $\pm$ 20.96 c	317.6 $\pm$ 7.77 d	1035.1 $\pm$ 12.24 e
<i>p</i> -value		***	***	***	***	***
Y $\times$ G		ns	ns	ns	ns	ns
S $\times$ G		ns	ns	ns	Ns	ns
Y $\times$ S $\times$ G		ns	ns	ns	Ns	ns

, and * indicate statistical significance at $p < 0.01$, and $p < 0.001$; ns indicates no significant difference. Same letters suggest no statistical difference between the treatments.

Table A2. Effect of year, sowing date, genotype, and their interaction on the growth and podding traits of mungbean during the field trial.

Treatments		Plant Height (cm)	Height of Lowest Pods (cm)	Pods per Plant	Pod Length (cm)	Haulm Biomass (g m ⁻²)
Year (Y)						
2024		77.2 ± 2.11 a	48.3 ± 1.96 a	41.9 ± 1.62 a	9.4 ± 0.14	665.7 ± 19.17 a
2025		74.5 ± 1.7 b	38.5 ± 1.05 b	26.3 ± 2.09 b	10.8 ± 0.72	353 ± 24.48 b
<i>p</i> -value		*	***	***	ns	***
Sowing Date (S)						
Early		74.1 ± 2.72	39.3 ± 1.15 c	37.6 ± 3.53 a	10.3 ± 1.13	521.4 ± 57.81 a
Mid		76.3 ± 1.1	41.9 ± 2.36 b	38.3 ± 1.15 a	9.8 ± 0.1	573 ± 27.27 a
Late		77.2 ± 2.89	49 ± 2.42 a	26.3 ± 2.91 b	10.1 ± 0.14	433.7 ± 48.63 b
<i>p</i> -value		ns	***	***	ns	***
Genotype (G)						
Jade AU		74.5 ± 1.72 b	41.9 ± 1.87 b	32.6 ± 2.69 b	9.7 ± 0.11	502.6 ± 39.69
Green Dragon		77.2 ± 2.1 a	44.9 ± 1.82 a	35.6 ± 2.21 a	10.5 ± 0.74	516.1 ± 38.9
<i>p</i> -value		*	***	*	ns	ns
Y × S						
2024	Early	64.8 ± 1.97 d	36.7 ± 1.55 de	50.5 ± 1.31 a	8.7 ± 0.16	736.4 ± 28.41 a
	Mid	79.4 ± 1.03 b	50.7 ± 0.91 b	38.4 ± 2.13 b	9.6 ± 0.16	643.7 ± 37.03 ab
	Late	87.4 ± 1.17 a	57.5 ± 1.49 a	36.8 ± 1.89 b	9.9 ± 0.1	616.9 ± 17.85 b
2025	Early	83.4 ± 1.74 ab	41.8 ± 1.18 c	24.7 ± 2.05 c	11.9 ± 2.15	306.3 ± 17.24 d
	Mid	73.3 ± 1.23 c	33 ± 0.78 e	38.3 ± 1.08 b	10 ± 0.09	502.3 ± 19.68 c
	Late	66.9 ± 2 d	40.6 ± 1.48 cd	15.8 ± 1.02 d	10.4 ± 0.22	250.4 ± 14.85 d
<i>p</i> -value		***	***	***	Ns	***
Y × G		*	ns	ns	Ns	ns
S × G		ns	ns	*	Ns	ns
Y × S × G		ns	ns	ns	Ns	ns

* and *** indicate statistical significance at $p < 0.05$, $p < 0.01$, and $p < 0.001$; ns indicates no significant difference. Same letters suggest no statistical difference between the treatments.

Table A3. Effect of year, sowing date, genotype, and their interaction on photosynthesis (A), respiration (Rn), and Rn/A ratio measured during flowering in the mungbean field trial.

Treatments	Measurements During Flowering		
	A (μmol m ⁻² s ⁻¹)	Rn (μmol m ⁻² s ⁻¹)	Rn/A Ratio
2024	31.6 ± 1.26 a	6.9 ± 0.4 a	0.22 ± 0.02 a
2025	15.9 ± 0.33 b	2.2 ± 0.13 b	0.14 ± 0.02 b
<i>p</i> -value	***	***	***
Early	25.3 ± 2.69 a	4.2 ± 0.39 b	0.18 ± 0.02 ab
Mid	26.1 ± 2.48 a	5.8 ± 0.96 a	0.2 ± 0.02 a
Late	19.8 ± 1.17 b	3.6 ± 0.52 c	0.17 ± 0.02 c
<i>p</i> -value	***	***	*
Jade AU	23.6 ± 1.8	4.6 ± 0.61	0.18 ± 0.02
Green Dragon	23.9 ± 1.96	4.5 ± 0.54	0.18 ± 0.02
<i>p</i> -value	ns	ns	ns

Table A3. Cont.

Treatments		Measurements During Flowering		
		A ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Rn ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Rn/A Ratio
2024	Early	35.3 $\pm$ 1.24 a	5.7 $\pm$ 0.18 b	0.17 $\pm$ 0.01 bc
	Mid	35.6 $\pm$ 0.43 a	9.4 $\pm$ 0.27 a	0.27 $\pm$ 0.01 a
	Late	23.9 $\pm$ 1.05 b	5.6 $\pm$ 0.2 b	0.24 $\pm$ 0.01 a
2025	Early	15.4 $\pm$ 0.9 c	2.8 $\pm$ 0.21 c	0.19 $\pm$ 0.03 b
	Mid	16.5 $\pm$ 0.25 c	2.1 $\pm$ 0.09 cd	0.13 $\pm$ 0.01 cd
	Late	15.8 $\pm$ 0.21 c	1.6 $\pm$ 0.08 d	0.11 $\pm$ 0.01 d
p-value		***	***	***
Y $\times$ G		ns	ns	*
S $\times$ G		ns	*	*
Y $\times$ S $\times$ G		ns	ns	ns

* and *** indicate statistical significance at $p < 0.05$ and $p < 0.001$; ns indicates no significant difference. Same letters suggest no statistical difference between the treatments.

Table A4. Year-specific split-plot mixed-model assessment of the independent association between respiration-to-photosynthesis (Rn/A) ratio measured at pod filling and grain yield in mungbean.

Year	Model Comparison for Adding Rn/A	Rn/A Coefficient (g m ⁻² per Unit Rn/A)	95% CI	p-Value	Marginal R ²	Conditional R ²	VIF: Rn/A	VIF: Sowing Date	Model Fit Diagnosis
2024	$X^2 = 0.456$, $p = 0.500$	−145.1	−602.0 to 311.8	0.507	0.307	0.773	10.65	14.87	No singular fit
2025	$X^2 = 0.162$, $p = 0.687$	120.8	−409.6 to 651.2	0.640	0.955	0.973	7.72	12.39	No singular fit

Grain yield was modeled separately for 2024 and 2025. Reduced models included sowing date, genotype, and the sowing date $\times$ genotype interaction as fixed effects, with block and block $\times$ sowing date included as random effects to represent the split-plot structure. Full models additionally included Rn/A at pod filling. Reduced and full models were compared using likelihood-ratio tests fitted by maximum likelihood. Coefficients and Wald 95% confidence intervals are from the full model. Marginal R² represents variance explained by fixed effects, whereas conditional R² represents variance explained by fixed and random effects. High VIF values indicate substantial collinearity between Rn/A and sowing date; therefore, Rn/A did not provide an independent yield contribution in either year.

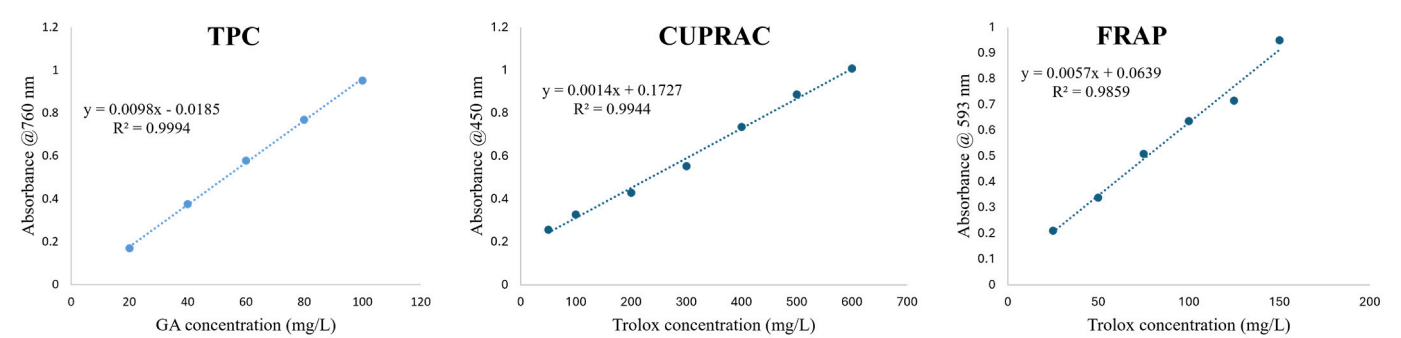


Figure A1. Standard curves for TPC, CUPRAC, and FRAP biochemical assays. Each standard curve was run in duplicate on each analysis day and used to calculate sample concentrations. R² values exceeded 0.985 for all assays across all analysis batches. TPC, total phenolic content; CUPRAC, cupric reducing antioxidant capacity; FRAP, ferric reducing antioxidant potential.

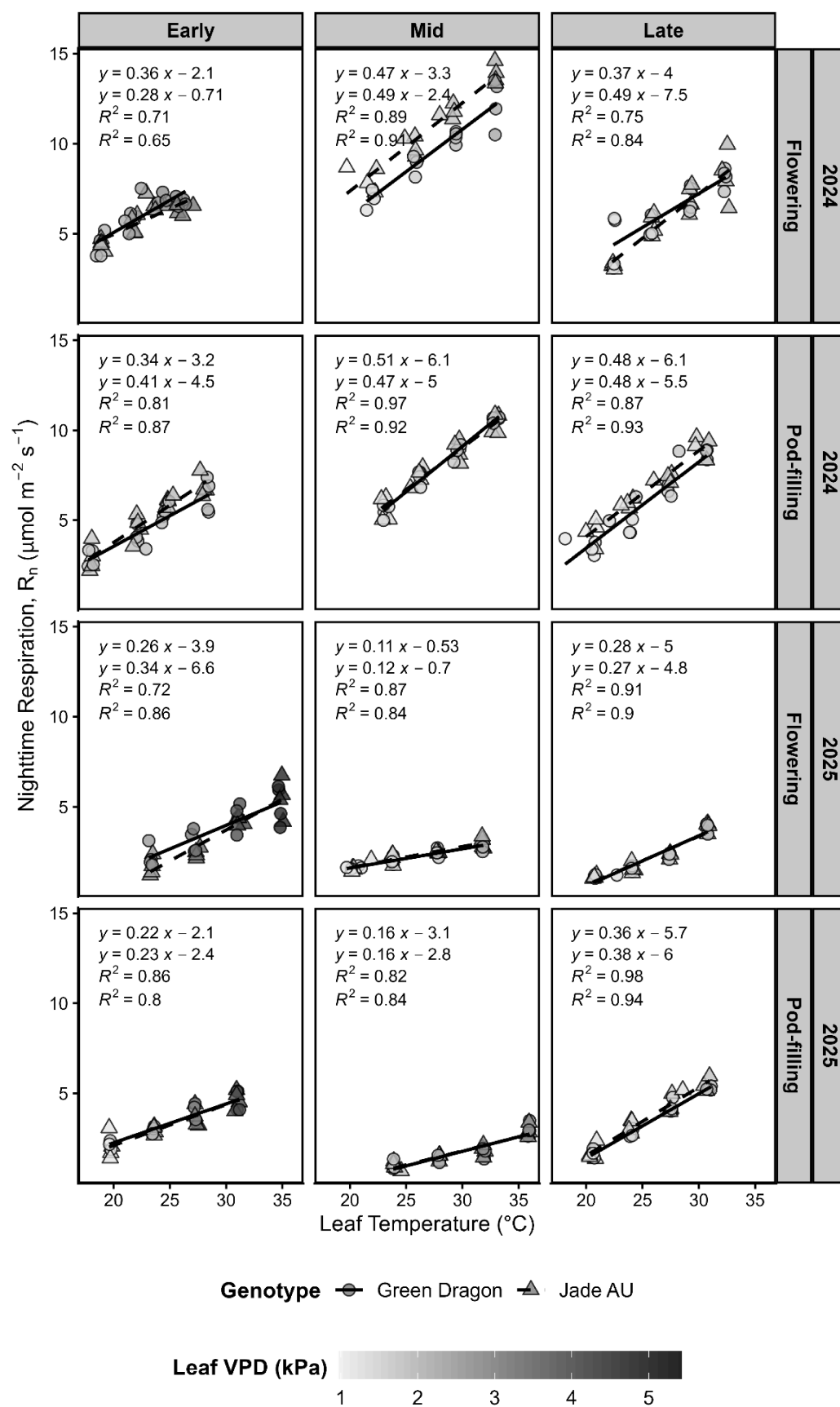


Figure A2. Response of nighttime respiration (R_n) to leaf temperature inside the cuvette during flowering and pod filling in Jade AU (triangles) and Green Dragon (circles) across three sowing dates in 2024 and 2025. Temperature was varied across four set-points: ambient $- 4^{\circ}\text{C}$, ambient, ambient $+ 4^{\circ}\text{C}$, and ambient $+ 8^{\circ}\text{C}$. Lines represent the fitted ordinary least-squares regressions; regression slope

($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and R^2 are shown within each panel for each genotype. Each point represents the average of five plants per plot across four replications. Steeper slopes in 2024 are consistent with higher absolute temperatures during measurements, while near-identical slopes between the genotypes indicate a largely conserved respiratory temperature sensitivity between Jade AU and Green Dragon.

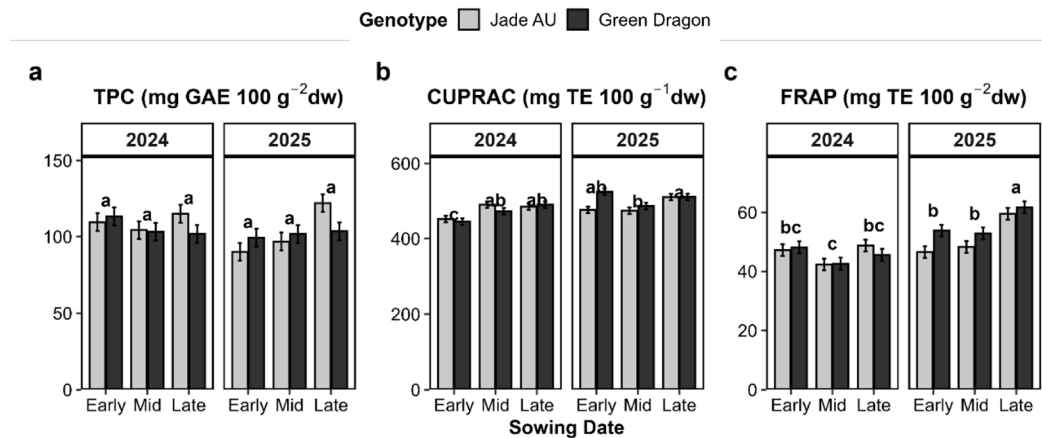


Figure A3. Effect of year, sowing date, and genotype on grain biochemical composition in mungbean. (a) Total phenolic content (TPC, mg GAE 100 g⁻¹ dw); (b) CUPRAC-based antioxidant capacity (mg TE 100 g⁻¹ dw); and (c) FRAP-based antioxidant capacity (mg TE 100 g⁻¹ dw). Bars represent means; error bars indicate one standard error of the mean. Lowercase letters indicate mean separation by Tukey's HSD ($p < 0.05$). Gray and dark bars represent Jade AU and Green Dragon, respectively. TPC was stable across the sampled environments, whereas antioxidant capacity (CUPRAC and FRAP) differed between years and sowing dates.

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