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Genotypic Variability in Response to Heat Stress and Post-Stress Compensatory Growth in Mungbean Plants (*Vigna radiata* [L.] Wilczek)

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Abstract: Understanding genotypic variability in tolerance to heat stress during flowering, a critical growth stage, and post-stress recovery remains limited in mungbean (*Vigna radiata*) genotypes. This study investigates the genetic variability in in vitro pollen viability, seed set, and grain yield among mungbean genotypes in response to transient high temperatures. Thirteen genotypes were evaluated in a glasshouse study, and four in a field study, subjected to high temperatures (around 40 °C/22 °C day/night) imposed midday during flowering. Across all genotypes, the pollen viability percentage significantly decreased from 70% to 30%, accompanied by reductions in the pod size and seed number per pod, and increases in unfertilized pods and unviable seeds. However, the seed yield per plant significantly increased for four genotypes (M12036, Celera-II AU, Crystal, and M11238/AGG325961), attributed to elevated shoot growth and pod numbers under high-temperature treatment in the glasshouse study. Conversely, Satin II, which exhibited the highest stress tolerance index, recorded a greater seed yield under optimum conditions compared to high temperatures. Similar genotypic variability in post-heat-stress recovery and rapid growth was observed in the field study. Under non-limiting water conditions, mungbean genotypes with a relatively more indeterminate growth habit mitigated the heat stress's impact on their pollen viability by swiftly increasing their post-stress vegetative and reproductive growth. The physiological mechanisms underlying post-stress rapid growth in these genotypes warrant further investigation and consideration in future breeding trials and mitigation strategies.

Keywords: grain legume; climate change; abiotic stress; high temperature; genotypic variability; pollen viability; grain yield; stress tolerance index

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1. Introduction

The mungbean (*Vigna radiata* [L.] Wilczek) stands as a significant tropical grain legume crop with the potential to contribute substantially to managing soil fertility through nitrogen fixation in crop rotation systems [1,2]. Its importance extends to being a staple food and a cash crop in the rice-based farming systems of South and Southeast Asia. Recently, interest in cultivating mungbean has expanded to regions like Australia due to its short growth duration, minimal input requirements, suitability for rotation in cereal-based systems, and resilience to heat and drought stress [3]. However, to meet the increasing market demand, improvements are needed in mungbean production reliability and profitability, especially considering the challenges posed by changing climatic conditions. Current mungbean yields in most regions hover between 0.5 and 1.5 t/ha, significantly below its potential yield of up to 3 t/ha [4].

Various challenges, particularly abiotic stresses, confront reliable mungbean production in major growing regions as climate patterns shift [5,6]. High-temperature stress, especially during flowering, poses a significant threat to seed set and grain yields in legumes by impairing pollen viability, as observed in numerous studies [7–18]. With global temperatures projected to rise by 1.5 °C by 2030 [19], legume crops will face more frequent and severe heat-stress episodes in the future [20,21]. Liu et al. [17] emphasized the irreversibility of yield losses due to heat stress's adverse effects on pollen viability and seed setting during early reproductive growth.

Pollen viability, a key determinant of plant reproductive potential, reflects factors crucial for successful fertilization, such as seed setting and grain yield [15,22]. Heat stress during flowering disrupts pollen viability in grain legumes, manifesting as reduced pollen quantity, abnormal morphology, poor germination, and impaired tube elongation (12 for chickpeas (*Cicer arietinum*), and 14, 23 for mungbeans). Such anomalies undermine fertilization and seed setting [11,12,17,20,23–26]. Additionally, heat-stress severity's impact on pollen viability may be influenced by low levels of heat-shock protein (HSP) transcript accumulation, as observed in maize and reviewed across various crop species [27,28]. Furthermore, pollen's extreme sensitivity to low humidity exacerbates heat-stress effects, potentially causing male sterility or reduced seed setting [20,22,26].

Recent research has primarily focused on mainstream grain legumes like soybeans (*Glycine max*), common beans (*Phaseolus vulgaris*), chickpeas, field peas (*Pisum sativum*), and cowpeas *(Vigna unguiculata)* regarding abiotic stresses, particularly heat stress. However, there is a scarcity of information regarding diverse stress responses and tolerance mechanisms in the mungbean or black gram (*Vigna mungo*) [1,29,30]. Priya et al. [18] underscored high-temperature stress as a growing threat to mungbean yield, necessitating urgent identification of heat-tolerant genotypes. Limited studies have reported decreased mungbean production due to heat stress, with varying degrees of tolerance being observed in a few genotypes under late-sown conditions [14,18,29–32]. Further investigation is warranted to understand the impacts of heat stress on additional morphological traits, such as leaf growth, branching, podding, and seed development, and to ascertain if indeterminacy in mungbean growth plays a role in recovering from short stress periods, akin to mechanisms observed in other legumes like chickpeas [33,34].

Therefore, this study aims to understand the variability among Australian mungbean cultivars and selected genotypes concerning the effects of high-temperature stress during early flowering on in vitro pollen viability and seed set. Additionally, it seeks to explore any associations between pollen viability and morphological traits, pod development, and grain yields, and to understand if genotypic tolerance to heat stress correlates with the post-stress recovery of certain yield components.

2. Materials and Methods

One glasshouse experiment and one field experiment were conducted.

2.1. Genetic Material

The genotypes used in this study comprised thirteen mungbean genotypes and one black gram genotype (Table 1). These genotypes represented a diverse range of germplasm used in mungbean breeding programs in Queensland, Australia. This included the parents of the mapping populations and some commercial lines (Crystal, Berken, Jade-AU, Celera II-AU and Satin II). Onyx-AU is also a commercial cultivar for black gram; we just wanted to test whether the impact of heat stress on black gram is more severe or not compared with mungbeans.

Table 1. Thirteen selected mungbean genotypes and one black gram genotype and their characteristics.

Genotype	Days to Flowering	Days to Maturity	Seed Size	Weight of 50 Seeds (g)
Jade-AU	42	70	Large	3.5
Crystal	38	72	Large	3.7
Celera-II AU	44	74	Small–Medium	2.8
Berken	40	69	Medium–Large	3.2
Satin II	42	71	Medium	3.0
AGG 324363/AGG325961	44	74	Medium	2.8
MOONG/AGG325960	41	72	Small	1.2
M08019/AGG325977	41	78	Large	4.0
CHIH-CO/AGG325966	42	70	Large	3.5
M10403/AG325964	40	73	Small	1.7
MAUS12-053/AGG325976	42	70	Medium	2.9
M11238/AGG325973	38	70	Medium	2.5
M12036	42	72	Medium–Large	3.2
Onyx-Au (black gram)	38	75	Small	2.5

2.2. Glasshouse Experiment

2.2.1. Experimental Details

Plants were grown in ANOVApot® (Anova Solutions, www.anovapot.com.au, accessed 30 June 2022; Green Genius, Brisbane, Australia): each pot (20 cm high and 20 cm in diameter) contained ~2.3 kg of a mixture of 70% composted bark mixed with 30% of cocoa peat and was fertilized with 2 g/L of osmocote and 1 g/L of dolomite. Plants were grown in two naturally lit, temperature-controlled glasshouses at the University of Queensland, St. Lucia, Australia (27°23′ S, 153°06′ E). The pots were kept on capillary mats to avoid water stress effecting the experiment and avoid the leaching of nutrients due to overwatering. Five seeds treated with a fungicide (Thiram) for soil-borne diseases and then inoculated with Rhizobia were sown at a 3 cm depth in each pot. The emerged seedlings were gradually thinned to one healthy seedling per pot. The experiment was laid out in a completely randomized design with 4 replications. The day and night temperatures in both rooms were kept at 30 °C vs. 22 °C at the start of the experiment.

At the start of the appearance of the first floral bud in the first pot, the temperature in one room was increased to day/night temperatures of 40 °C/22 °C, and this room was converted into the high-temperature treatment room (HT room). The other room, with day and night temperatures of 30 °C/22 °C, was treated as the optimum-temperature room (OT room). The plants in the OT room were monitored every day, and as soon as the first bud appeared on any plant, it was transferred to the HT room. The high-temperature treatment was maintained for 6–7 h (10 a.m.–5 p.m.) in the 12 h photoperiod. The high-temperature treatment was imposed at the onset of the first flower bud and maintained for 15 days during and around flowering. Thereafter, the plants were transferred back to the OT room until maturity. The seeds were sown in late October, and the plants were harvested at physiological maturity when 80% of the pods turned brown in colour in late December.

2.2.2. Measurements

Pollen germination/viability: Pollen germination was measured using an Impedance Flow Cytometry (IFC) Ampha Z32 instrument (Amphasys AG, Root, Switzerland). IFC technology has been demonstrated to be a fast, reliable, and label-free technology for studying pollen quantity and viability. IFC determines the electrical properties of cells by using a microfluid chip, varying the frequency (MHz) to measure the cell size, membrane integrity, and cytoplasmic conductivity [35]. Three random fresh flowers per plant, which opened on the same day, were collected early in the morning. The flowers were transferred to the laboratory in an esky to avoid temperature fluctuations during the transportation of the flowers to the lab. Whole anthers from all three flowers were gently removed with forceps and placed in Eppendorf tubes containing 1 mL of AF5 buffer (suitable for

mungbeans), using AF buffers designed for each pollen type and species. To extract the pollen out of the anther, the Eppendorf tube was scratched over the rack up to 4–5 times. The contents from the Eppendorf tube, including the pollen and buffer, were strained directly into microcentrifuge tubes to remove particles that were larger than the actual pollen grains in order to prevent clogging of the microfluid chip. Another 1 mL of buffer was added to the same Eppendorf tube to rinse off any pollen sticking to the tube, and then again the Eppendorf tube was scratched on the stand and the contents were strained into a microcentrifuge tube. The microcentrifuge was gently shaken to equally distribute the pollen throughout the suspension. The pollen suspension was then pumped through a microfluidic chip. As dead and viable cells behave differently in an electric field, they could be detected and distinguished using the instrument.

Before starting the sample preparation, a mungbean chip E00001 was inserted into Ampha Z32 (Amphasys AG, Root, Switzerland). The E chip had a channel size of 240 µm. AmphaChips are precisely fabricated microfluidic and microelectronic devices supplied with microelectrodes. This chip measures changes in the electrical resistance of fluid medium when particles or cells pass through the applied electric field. Before measuring each sample, the chip was rinsed twice. The sample tubing was inserted into the microcentrifuge tube and then the measurement of the pollen viability was started. Once the measurement was completed, a gating was created to compare viable and dead pollen cells. The same method was applied to all of the samples.

Data on phenology (days from sowing to emergence, appearance of first flower, and maturity) were recorded for all plants. At physiological maturity, all HT and OT plants were harvested. The plant height and number of branches were measured. After measuring the plant height, the leaves, pods (both mature and immature), and any remaining flowers were removed from the stem. The number of pods, leaves, and flowers were counted. Leaf area was determined by using the LI-3100 leaf area meter (LI-COR Bioscience, Lincoln, NE, USA). Pod size was measured by randomly selecting 10 pods and then using a ruler to measure the length. The leaves and stem of each plant were kept separately in brown paper bags and then dried at 70 °C for more than 48 h, and the dry weight was measured. All dried pods were threshed by hand to separate the seeds from the pods. The seed weight per plant was measured and the total seed number per plant was counted using a seed counter. The number of unviable (deformed or shrunken) seeds, which were only present in the high-temperature treatment, were separated and counted manually, whereas viable seeds were counted with the seed counter. Seed yield stress susceptibility (SSI) and tolerance (STI) indices were calculated as suggested in [36,37]):

$$SSI = 1 - (Y_{HT}/Y_{OT})/1 - (MeanY_{HT}/MeanY_{OT}); STI = (Y_{HT} \times Y_{OT})/(MeanY_{OT})^2$$

where Y indicates the seed yield; Y_{HT} indicates the seed yield at high temperatures; Y_{OT} indicates the seed yield at the optimum temperature.

2.3. Field Experiment

Four contrasting mungbean genotypes were selected from the glasshouse study: these included two commercial lines (Jade and Crystal) and two elite lines (M11238 and M12306). They were planted in the field in blocks using standard agronomy and maintenance practices for mungbean crops in a commercial field. Genotype rows were randomized within each block. There were eight blocks, each 2.5 m × 2.5 m: four blocks for the heat-stress treatment and four parallel blocks for the ambient-temperature treatment. There were four replications in each block. Blocks were separated by 1 m wide border rows (border rows were sown using a machine). The seeds in each treatment block were hand-sown on 16 January in rows (20 cm apart); emergence occurred on 28 January and the plants were thinned on the same day to maintain a 10 cm distance between the plants in a row. At the beginning of flowering on 9–11 March, ten randomly selected plants were tagged in each replication for two measurements: the first just after the heat-stress treatment around the time of flowering for plant morphological measurements, and the second at the end

of physiological maturity for pod and grain yield measurements. Heat-stress treatment for around 20 days during flowering was imposed using specifically designed heat tents (Figure 1).

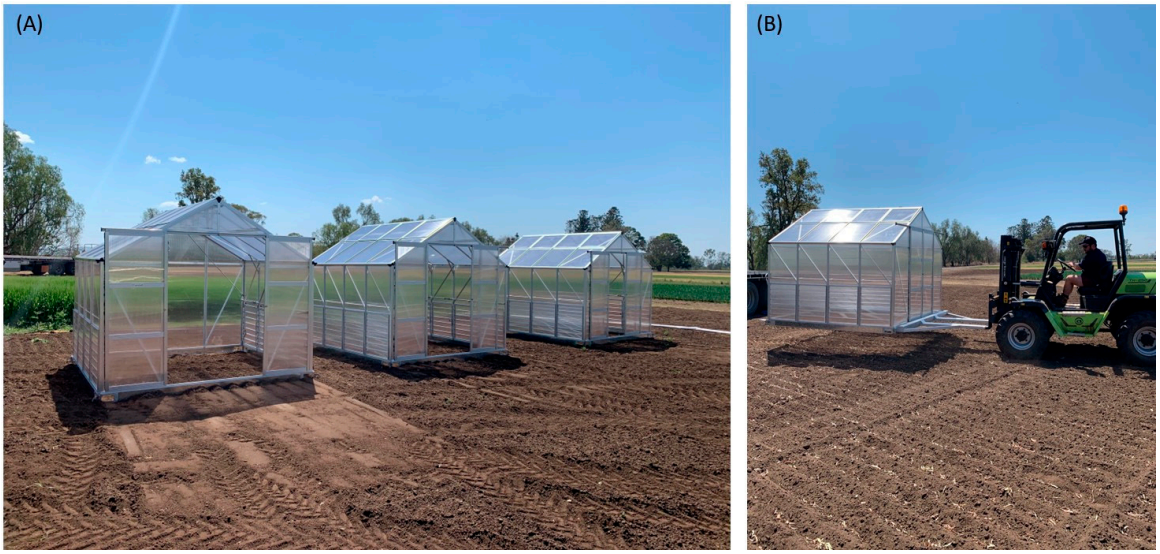

Figure 1. (**A**) Specially made heat tents; (**B**) a forklift was used to put the heat tents over the crops.

The 'heat tent' design was a 2.5 × 2.5 m steel-framed polycarbonate sheeted chamber, which was engineered to automatically open and close hydraulically operated vents using pre-programmed parameters. The vents, located at the top of the tent and along the bottom of two sides of the tent, were programmed to maintain a 10 °C temperature differential between the inside temperature and the outside (ambient) temperature using built-in temperature sensors. If the temperature inside reached >40 °C, the vents would open, allowing passive venting. At night, each tent was completely open to avoid imposing any treatment at night. The temperatures recorded on the built-in sensors were downloaded using a Bluetooth connection. To place and remove the tents in the field, a forklift was required (Figure 1B). The heat tents were removed on 31 March and morphological measurements (leaf area, plant height, leaf dry wt., and stem dry wt.) were taken on 5 tagged plants per treatment per rep (total: 80 plants per treatment). Physiologically matured pods were harvested on 14 April for 5 tagged plants in each block (total: 80 plants per treatment). Pod measurements included the number of pods and the length of each pod; the seeds were hand-thrashed per plant and the seed number and seed weight per plant were measured. It should be noted that the mungbean plants in the field experiment established and grew very well until the imposed heat-stress treatment (Figure 2A–E). However, towards the end of the imposed heat-stress treatment, insect infestation started to occur and became more severe towards the plants' physiological maturity. In particular, plants exposed to the heat treatment under the heat tent became more infested with insects compared with the plants outside the tents. The post-stress damage to the plants, once inside the tents, was severe (Figure 2F).

2.4. Statistical Analyses

An analysis of variance (ANOVA) was performed on the plant parameters using XLSTAT 2021.1 statistical software [38]. This software performs ANOVAs based on the same conceptual framework as linear regression. It standardises the residuals as a function of model prediction, so that residuals can be distributed randomly around the *x*-axis. A Shapiro–Wilk test is then performed on the residuals. XLSTAT allows for the correction of heteroscedasticity and autocorrelation, where homoscedasticity and independence of the error terms are the key hypotheses in linear regression and ANOVAs, where it is assumed that the variances of the error terms are independent and identically and normally

distributed. Fisher's LSD (least-significant difference) test was performed for multiple comparisons of the plant parameter means.

Figure 2. Various stages of crop growth: (**A**) early development; (**B**) beginning of flowering, when the heat-stress treatment was imposed by placing the crops under heat tents; (**C**,**D**) growth inside the heat tents; (**E**) growth outside the tents; and (**F**) infestation of insects towards the end of the heat-stress treatment. Infestation became more severe towards the plants' physiological maturity (plants and pods inside the tents were more infested than the outside plants at ambient temperatures).

3. Results

3.1. Glasshouse Experiment

The glasshouse experiment was conducted in a naturally lit glasshouse, and the diurnal temperature regimes are illustrated in Figure 3. The target optimum temperature (OT) and high temperature (HT) were 30 °C/22 °C and 40 °C/22 °C, respectively. For the OT, a minimum temperature of 22 °C was maintained throughout the 12 h dark period. Around 1 h after the onset of the light (5–6 a.m.), the temperature was increased until the pre-set maximum temperature of 30 °C was reached, which was maintained until 5 p.m., then decreased to 22 °C. For the HT treatment, which was imposed at the appearance of the first bud for 15 days (for each plant), the temperature was raised to a pre-set maximum of 40 °C for 6–7 h, around 10 a.m.; then, from around 5–6 p.m., it decreased to 22 °C (Figure 3).

An analysis of variance table showing the significance of the main effects (genotypes and heat treatments) and their interactions on the measured plant parameters is presented in Table 2. Both the high-temperature treatment (T) and genotypic (G) differences were highly significant ($p < 0.001$) for most parameters. However, the majority of parameters also showed significant interactions ($p < 0.05$–$p < 0.001$) between T and G, indicating that the effect of T varied with the genotype (Table 2). Supplementary Table S1 also presents detailed ANOVA results for the interaction effects, and multiple comparisons for the treatment means and significance of various plant parameters.

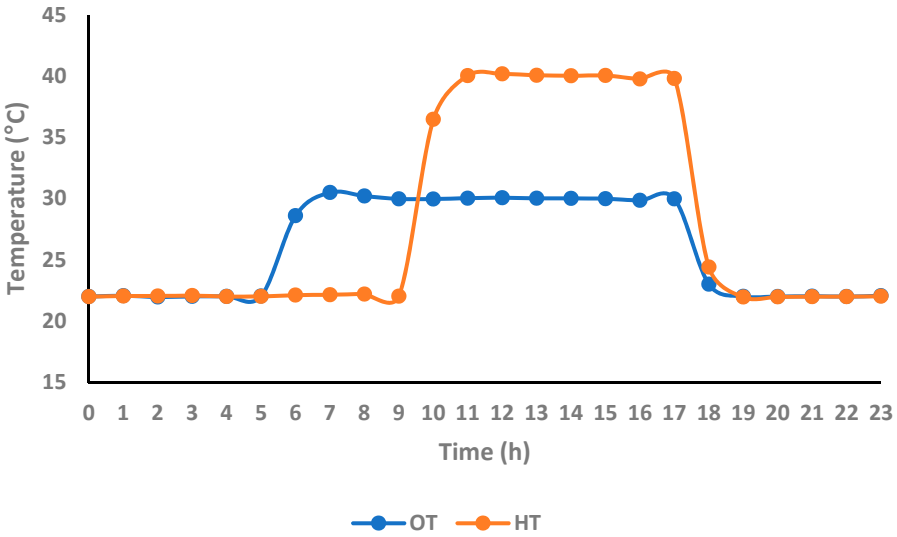

Figure 3. A typical diurnal pattern of the day and night temperatures for the optimum (OT) and high-temperature (HT) treatments in the controlled glasshouse facility.

Table 2. Mean values of plant growth parameters under high-temperature (HT) and optimum-temperature (OT) treatments, and the probability of significance (ANOVA) for the main effects of treatment temperature (T) and genotype (G) and their interactions (G × T). *, <0.05; **, <0.01; ***, <0.001; ns, not significant.

Plant Growth Parameter	Plant Traits	HT	OT	Pr. (Temp.)	Pr. (Genotype)	Pr. (G × T)
Shoot morphology	Plant height (cm)	73.5	67.3	**	***	***
	Number of nodes	15.1	9.6	***	ns	ns
	Secondary branching	42.6	29.0	***	***	*
	Total leaf number	80.8	75.6	*	***	*
	Leaf area (cm^2)	3335.7	3590.6	*	***	***
	Stem dry wt. (g)	16.1	14.1	***	***	***
	Leaf dry wt. (g)	13.8	14.6	ns	***	ns
	Total shoot wt. (g)	29.3	28.6	ns	***	**
	Days to first flower	37.3	34.9	***	***	ns
Phenology	Days to 50% flower	41.0	39.1	**	ns	ns
	Pollen viability (%)	29.6	67.2	***	ns	ns
Pods	Total number of pods	61.3	34.7	***	***	0.069
	Pod size/length (cm)	7.3	9.0	***	***	***
	Unfertilized pods	5.8	0.1	***	***	***
Seeds	Total seed number/plant	431.1	340.5	***	***	***
	Unviable seeds/plant	28.4	1.0	***	ns	ns
	Number of seeds per pod	7.1	10.5	***	***	**
	Viable seed wt. (g/seed)	0.061	0.065	**	***	ns
	Total seed wt. (g/plant)	22.4	19.7	***	***	**

3.1.1. Shoot Morphology

Exposure to HT during the reproductive growth stage resulted in increased plant height, on average, across the genotypes (Table 2). However, there were contrasting effects on plant height, with significant interactions between T and G ($p < 0.001$) (Table 2). At OT, plant height was similar for all genotypes, at around 70 cm; however, exposure to HT resulted in a significant increase in plant height for M12036 (above 100 cm), while the other genotypes did not show any effect on the plant height (Supplementary Table S1).

The number of nodes, secondary branching, and the total leaf number also increased with the HT (Table 2).

Leaf area and total leaf number: On average, OT plants had a greater leaf area than HT plants, indicating that most genotypes decreased their leaf area in response

to HT (Figure 4A). However, the leaf area increased significantly for M12036 and CHIH-CO/AGG325966 with HT compared to with OT, whereas Berken, M10403/AGG325964, and M11238/AGG325973 showed significant decreases in the leaf area with HT ($p < 0.001$), leading to an interaction between T and G (Supplementary Table S1). Similarly, the total leaf number of leaves also showed an interaction between T and G, where Celera II had a greater number of leaves with HT compared with that observed under OT (Supplementary Table S1). Most other genotypes also showed a tendency towards an increased number of leaves with HT (Supplementary Table S1).

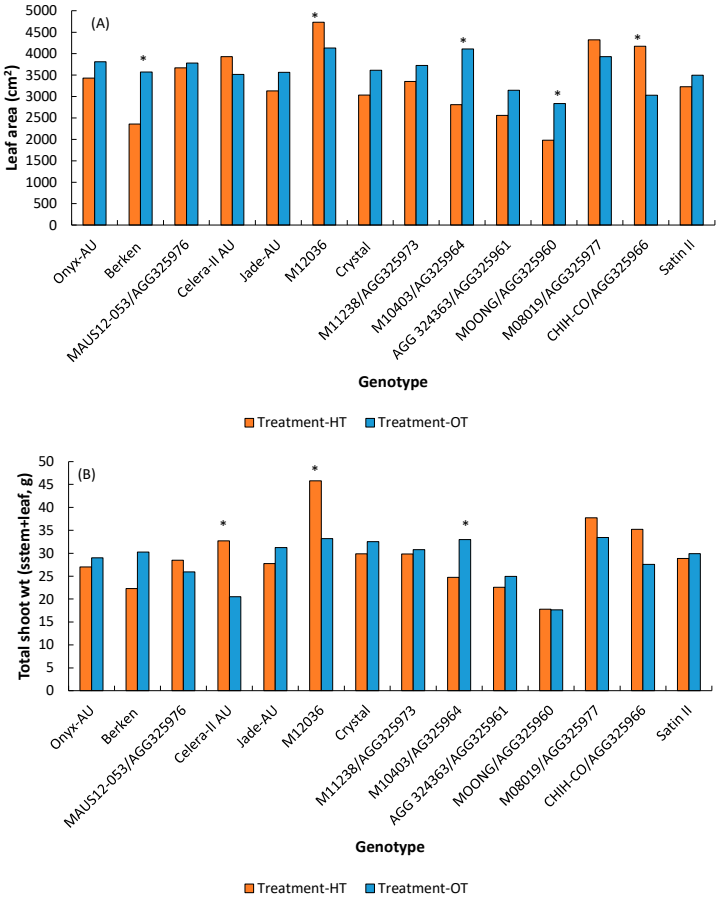

Figure 4. Interactions between the main effects of treatment temperature and genotype on (**A**) the leaf area and (**B**) the shoot weight. Genotypes with asterisk (*) signs indicate significant differences ($p < 0.05$) between the optimum (OT) and high-temperature (HT) treatments.

Shoot weight: There was an interaction between T and G ($p < 0.01$) where M12036 (46 g) followed by Celera II (33 g) responded to HT by increasing their shoot weight compared with that observed under OT (Figure 4B). The shoot weight tended to increase for four genotypes, seven genotypes showed no response, and two genotypes showed decreases in their shoot weight with HT (Berken and M10403/AGG325964). These effects on the shoot weight (stem + leaf) were broadly similar to those on plant height.

3.1.2. Phenology/Flowering

Flowering: Fifty-percent flowering occurred at 37 days and 39 days for the OT and HT groups, respectively. The number of flowers was recorded on three different dates: 10 December, 13 December, and 20 December. OT plants had the highest number of flowers (5.3) on 10 December, then this decreased gradually to 3.4 on 13 December and 0.15 on 20 December. The HT group showed an opposite trend of an increasing number of flowers, from 1.5 to 2.5 to 5.8, from 10 December to 13 December to 20 December.

Pollen viability percentage: HT decreased the pollen viability from 67% to 30% across all genotypes ($p < 0.001$) (Table 2). There were no differences in the pollen viability among the genotypes tested, nor was there any interaction between T and G. The pollen viability ranged from 49 to 78% at OT, and 15 to 39% at HT. Berken had the lowest viability of 15% at HT among all genotypes, whereas at OT, it had 68% viability, showing a severe reduction of around 80% from HT to OT. Other commercial genotypes showed reductions of around 50%.

3.1.3. Pods/Seeds

The number of pods increased with HT for all genotypes. At OT, the number of pods varied between 20 and 76, whereas at HT, it varied between 46 and 96 (Figure 5A). However, the effect of HT varied with the genotype ($p < 0.043$), where the greatest increase (200%) occurred for M12036, from 20 pods to 60 pods, and the lowest increase occurred for MOONG/AGG325960 (77–92, or 20%). Among the commercial genotypes, Crystal had the greatest increase, from 21 to 47 pods (120% increase), followed by Celera II (45 to 86 pods, or 91%); Onyx-Au (black gram) had the lowest increase (50 to 67 pods, or 32%).

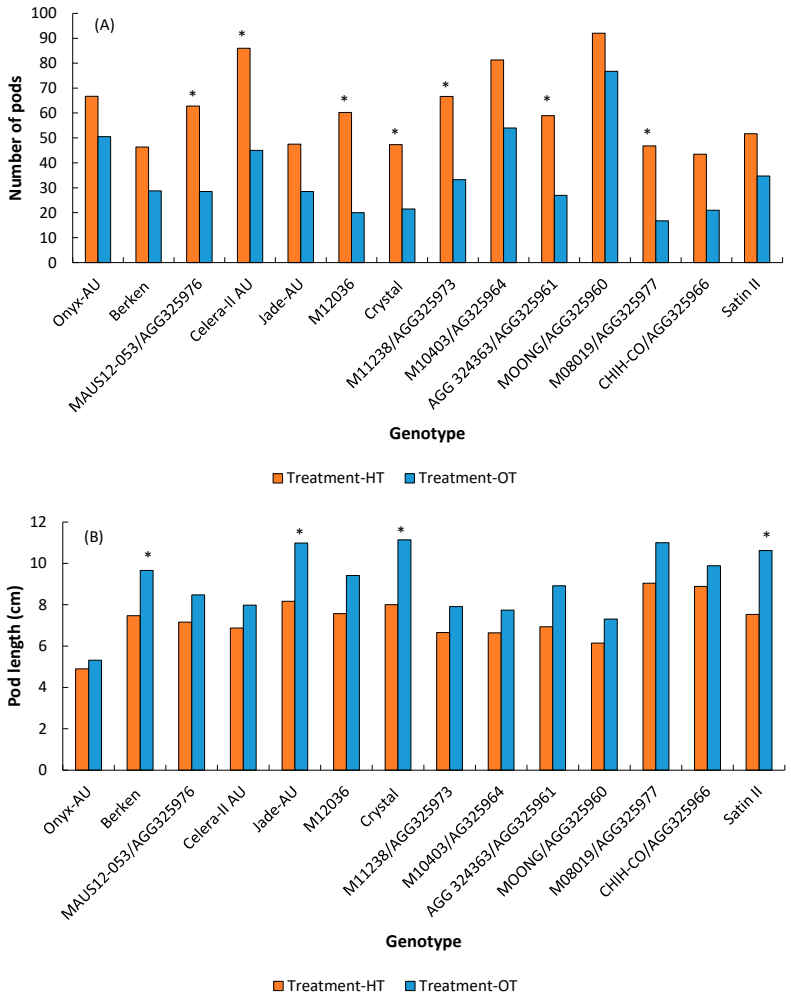

Figure 5. Interactions between the main effects of treatment temperature and genotype on (**A**) the number of pods and (**B**) the pod length. Genotypes with asterisk (*) signs indicate significant differences ($p < 0.05$) between the optimum (OT) and high-temperature (HT) treatments.

The pod length, on average and across genotypes, decreased from 9 cm to 7.3 cm with HT (Figure 5B). The effect of HT varied with the genotype ($p < 0.001$): the greatest decrease occurred for Satin II (10.6 to 7.5, or 29%), and the lowest decrease occurred for Onyx-AU (black gram) (5.3–4.9, or 8%). The pod length at OT for all commercial mungbean genotypes

ranged between 10 cm (Berken) and 11 cm (Crystal, Jade, Satin), whereas the black gram cultivar Onyx had the lowest pod length, at 5.3 cm. At HT, the pod length reduced to 7.5 cm for Berken and Satin II, and 8.0 cm for Jade and Crystal. Interestingly, genotypes M08019/AGG325977 and CHIH-CO/AGG325966 also had their pod lengths reduced from around 10.0–11.0 cm at OT to 9.0 cm at HT: these genotypes showed relatively longer pod lengths at HT compared with the commercial genotypes.

Total seed number: The total seed number was influenced by a highly significant interaction between T and G ($p < 0.001$) (Figure 6A). In comparison to the OT results, most genotypes tended to increase their total seed number with HT, but this effect was most pronounced for M11238/AGG325973, which showed a 99% increase (243 to 483 seeds), followed by M12036 with a 79% increase (233 to 417 seeds). Among the commercial genotypes, Celera II showed a 76% increase (406 to 716 seeds). Crystal had the lowest number of seeds at OT (217), which increased to 317 (about 46%) with HT: this increase was just on the borderline of showing a significant difference between OT and HT. MOONG/AGG325960 had the highest total seed number at OT (735), which insignificantly decreased with HT (to 685 seeds; a 7% decrease).

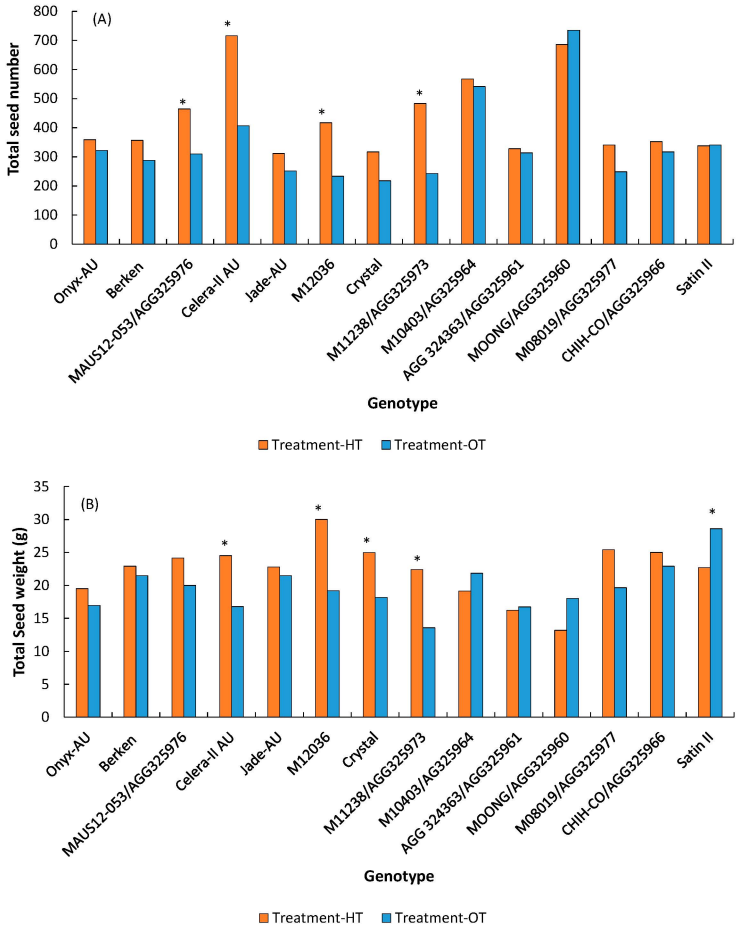

Figure 6. Interactions between the main effects of treatment temperature and genotype on (**A**) the total number of seeds, including viable and unviable, and (**B**) the total seed weight. Genotypes with asterisk (*) signs indicate significant differences ($p < 0.05$) between the optimum (OT) and high-temperature (HT) treatments.

Viability of seeds: HT resulted in only 28 seeds being unviable (7%), from a total seed number of 403, compared with just 1 unviable seed for the OT treatment, with a total seed number of 341 (Table 2).

Individual seed weight/seed size: HT significantly decreased the individual weight of viable seeds from 0.065 g to 0.061 ($p < 0.001$) (Table 2). Among the commercial genotypes,

Crystal and Jade had the largest individual seed weight/size (0.084 g), followed by Berken (0.073 g) and Celera II (0.039 g), whereas Satin II had the lowest (0.023 g). Other genotypes, namely M08019/AGG325977 and M12036, also had similar seed sizes (0.082–0.084 g) to those observed for the commercial genotypes Crystal and Jade.

Total seed weight: On average, HT increased the total seed weight compared with OT, but the effect varied with the genotype (Table 3, Figure 6B). HT significantly increased the seed weights for M12036, M11238/AGG325973, Celera II, and Crystal, whereas Satin II showed a significant decrease in seed weight with HT. The total seed weight was highest for Satin II at OT (29 g), which decreased to 23 g with HT (21% decrease). In contrast, M12036 showed a 56% increase in seed weight with HT in comparison to OT (30 g vs. 19 g). Celera and Crystal also recorded around 45% increases in their seed weights with HT (around 25–26 g vs. 17–18 g).

Table 3. Grain yield per plant under optimum-temperature (OT) and high-temperature (HT) treatments, stress tolerance index (STI), and stress susceptibility index (SSI), or compensatory growth index (CGI), for the genotypes used in this study. Grain yields under HT marked with asterisks (*) significantly ($p < 0.05$)differ from those under OT.

Genotype	OT	HT	STI	SSI/CGI
Satin II	28.6	22.7 *	1.69	−1.50
CHIH-CO/AGG325966	22.9	25.0	1.49	0.65
M10403/AG325964	21.9	19.1	1.09	−0.90
Berken	21.5	22.9	1.28	0.49
Jade-AU	21.5	22.8	1.27	0.45
MAUS12-053/AGG325976	20.0	24.2	1.26	1.52
M08019/AGG325977	19.7	25.4	1.30	2.12
M12036	19.2	30.0 *	1.50	4.09
Crystal	18.2	25.0 *	1.18	2.73
MOONG/AGG325960	18.0	13.2	0.62	−1.94
Onyx-AU	17.0	19.5	0.86	1.08
Celera-II AU	16.8	24.5 *	1.07	3.34
AGG 324363/AGG325961	16.8	16.2	0.71	−0.23
M11238/AGG325973	13.6	22.4 *	0.79	4.71

Ranking of genotypes for seed yield and tolerance index: At OT, the total seed yield per plant for Satin II was the highest (28.6 g) and M11238/AGG325973 (13.6 g) was the lowest-yielding genotype (Table 3). The ranking among the commercial cultivars was as follows: Satin II > Jade-AU = Berken > Crystal = Celera-II AU. However, the seed yield increased significantly for Crystal and Celera-II AU and decreased for Satin II with HT compared with the OT results. Berken and Jade-AU showed insignificant increases with the HT treatment. The stress tolerance index (STI) was the highest for Satin II (1.69), and Onyx-AU (black gram) had the lowest (0.86). The stress susceptibility index (SSI), which is designed to show the relative susceptibility (degree of reduction in yield) under stress conditions compared with non-stress conditions, could be instead used to show the post-stress compensatory growth index (CSI), as most genotypes exceeded the seed yield under heat stress, except Satin II, MOONG/AGG325960, and AGG 324363/AGG325961. The genotype most responsive to heat stress for compensatory growth (seed yield) (SSI/CGI) was M11238/AGG325973 (4.71), followed by M12036 (4.09), whereas among the commercial genotypes, the most responsive were Celera-II AU (3.34) and Crystal (2.73) (Table 3).

3.2. Field Experiment

Post-heat-stress plant growth measurements: In the field experiment, as expected, the specifically designed heat tents were able to increase the high temperature by around >10 °C compared to the ambient temperature outside the tent (Figure 7). Imposing heat stress for 20 days around flowering influenced the plant growth depending upon the genotypic responses, similar to the glasshouse experiment, which showed that out of

13 mungbean genotypes, a few genotypes tended to increase, a few tended to decrease, and others showed no changes in the plant growth parameters (leaf area, plant height, and shoot weight) with heat stress.

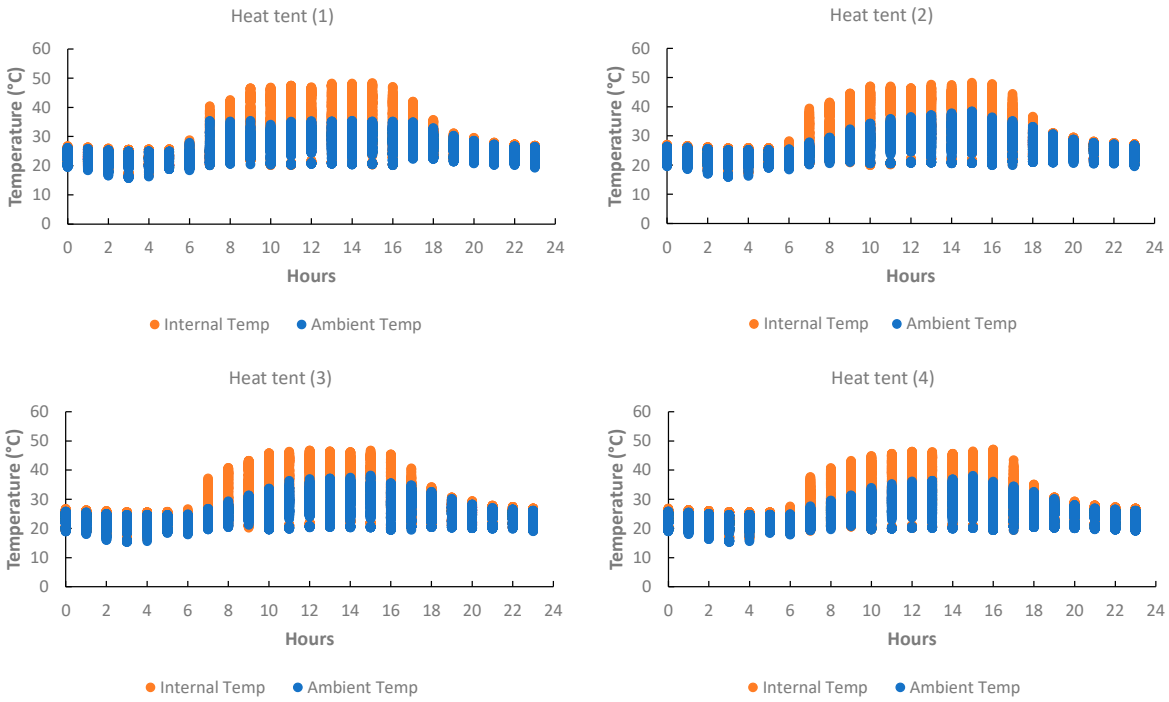

Figure 7. Daily variation in temperatures inside the tent (Internal Temp) and outside the tent (Ambient Temp) for the four heat tents used for imposing the high-temperature stress.

Only four selected genotypes from the glasshouse study were used (Jade-AU, Crystal, M12036, and M11238) in the field experiment. In comparison to the glasshouse experiment, the responses to high-temperature stress were similar for Crystal and M12036, showing increased shoot growth in both studies, and Jade-AU and M11238 showed a non-significant response to heat stress for shoot growth in both studies, but M11238 had a greater total seed yield in the glasshouse study (Table 2; Supplementary Tables S1 and S2).

Measurements at physiological maturity: As highlighted before, plants inside the tents became infested with insects towards the end of the high-temperature treatment, when the heat tents were removed and plant growth measurements were taken (Figure 2F). The data on shoot growth collected at the end of the heat treatment were not affected by insect damage. However, according to post-heat-stress measurements, at physiological maturity, insect damage on the pods and foliage became more severe on the heat-treated plants compared with the plants outside the tents. This might be due to a build-up of humidity inside the tent, which could have been conducive to the insect infestation. Therefore, the total pod number and total seed yield data per plant may not be reliable (Supplementary Table S2), except the pod length, seeds per pod, and individual seed weight data, which would be more reliable as they were collected from undamaged pods. Similar to the glasshouse experiment, the number of seeds per pod was significantly reduced, whereas there was no effect on the pod length and individual seed weight with the main treatment of high-temperature stress (Supplementary Table S2).

In this study, interestingly, the total seed weight was highly correlated to the plant height, leaf area, number of nodes, and leaf weight under high temperatures, whereas no relationship was noticed for these parameters under optimum-temperature conditions (Supplementary Table S3).

4. Discussion

This study aimed to understand the genotypic differences in tolerance to high-temperature stress (HT) and post-stress recovery among Australian commercial mungbean cultivars and a range of selected parents from the NAM population. The experiments were designed to determine the effects of HT on pollen viability, seed set, and yield when plants were exposed to 40 °C (HT) during the reproductive growth stage. Significant genotypic variability occurred in response to the high temperatures for post-stress overall plant growth, with some genotypes increasing while others decreased their plant growth and grain yield in both the field and glasshouse studies.

4.1. Temperature Threshold for Pollen Viability and Grain Yield in Mungbean Plants

The optimum temperature range for most grain legume crops (warm- or cool-season crops) is 10 °C to 36 °C; however, cool-season grain legumes are more sensitive to heat stress than warm-season varieties [39,40]. Nair et al. [41] reported that the optimum temperature for the growth and development of mungbeans is from 28 °C to 30 °C, with seed development continuing within the range of 33 °C to 35 °C. Kumar et al. [10] highlighted a higher temperature threshold for mungbeans in India, from 35 °C to 45 °C, leading to potential reductions in yields. Their study on mungbean plants experiencing temperatures exceeding 35 °C/25 °C, especially exceeding 40 °C, led to chlorosis, reduced vegetative and reproductive growth, and the abscission of buds, flowers, and pods. High-temperature stress significantly reduced pollen viability, grain yield, and yield parameters for tolerant and susceptible mungbean genotypes [14]. For instance, pollen viability for the susceptible mungbean genotype SML 668 reduced from 91.2% at the 30/20 °C optimum temperature to 73.4%, 51.3%, and 34.2% with higher temperatures of 40/30 °C, 43/30 °C, and 45/32 °C, respectively [14].

4.2. Impaired Pollen Viability Had No Impact on Grain Yield in This Study

Pollen viability significantly decreased in this study as well, from around 70% to 30%, when the daytime temperature increased from 30 °C to 40 °C for a period of two weeks around flowering. On average, across the genotypes, high-temperature stress in this study also severely decreased some grain-yield-related parameters such as the pod size and number of seeds per pod, and increased the number of unfertilized pods and unviable seeds, as reported in other studies. In contrast to other studies, under well-watered glasshouse conditions, this study found that the grain yield per plant increased significantly for some genotypes subjected to high-temperature stress compared with the plants growing at the optimum temperature, although pollen viability was severely reduced to 30%. For some genotypes, plants exposed to high-temperature stress significantly increased their plant height, number of nodes, secondary branching, total leaf number, stem dry weight, number of pods, pod weight, total seed number, and seed weight (Table 2). Other genotypes also showed a tendency towards increases in the grain yield per plant and yield-related parameters with the imposed high-temperature stress, but the effects were statistically insignificant (Figure 6B).

4.3. Post-Heat-Stress Compensatory Growth Response in Mungbean Plants

The post-heat-stress tendency of increasing grain yield and related parameters demonstrates a compensatory vegetative and reproductive growth habit in the mungbean plants. The primary reason for this compensatory growth could be the relatively more indeterminate growth habits of some mungbean genotypes compared with other mungbean genotypes and most mainstream grain legumes. Vadez et al. [21] highlighted that the indeterminate growth pattern of most legumes provides plasticity to environmental stresses by allowing the development of additional flowers and then seeds under favourable growing conditions. However, cultivars of mainstream grain legumes are largely bred/selected for a more determinate growth habit to decrease plant biomass and optimize the allocation between vegetative and reproductive growth [42]. Determinant growth also helps with

synchronous pod maturity, which is desirable for mechanical harvesting ([43] and references within). It also means that the earliest ripe pods are not exposed to weather damage while later pods ripen. Therefore, post-heat-stress compensatory growth has not been seen or reported for most mainstream grain legume crops, as the current cultivars have a more determinate growth habit. Nonetheless, in a few limited studies on mungbeans, compensatory growth to mitigate the impact of heat stress on grain yield, as observed in this study, was also not seen or reported.

The reasons for post-heat-stress compensatory growth not being observed in mungbean plants could be excessive and/or prolonged heat stress, or a combination of heat stress, low humidity, and limiting water conditions during the post-stress period in the reported studies. For example, most heat-stress studies in mungbeans have imposed heat stress of 45 °C or more [14,16,31,44,45]. The optimum temperature for the growth and development of mungbeans is from 28 °C to 30 °C [41]; therefore, 15 °C above the normal temperature, that is, 45 °C, could be excessive heat stress. Further, mungbean plants subjected to late-sowing strategies to impose prolonged heat stress (45 °C or more) from flowering to maturity [14,31,45] would not have a chance to compensate during the post-heat-stress period. Additionally, late-sown plants may also be exposed to low humidity and/or limiting moisture conditions towards pod filling and maturity as a result of terminal drought, mostly experienced by late-sown crops. High temperatures, low humidity, and/or limiting water conditions can cause the stomates to shut in order to maintain the relative leaf water content or increase the stomatal conductance and transpiration for cooling the leaves, but both of these physiological processes or tolerance mechanisms in response to heat stress will affect the water uptake and pod development. Kaur et al. [14] reported that the mungbean plants in their study were not moisture-limited because they had a similar leaf relative water content, whereas Kumar et al. [10] reported that the relative leaf water content decreased significantly at 40 °C/30 °C (10% over the control) and at 45 °C/35 °C (12% over the control) for young mungbean seedlings in a growth cabinet study. Sharma et al. [31] acknowledged that late-sown mungbean plants showed drought symptoms from time to time, though they were watered frequently. It should be noted that under field conditions, high-temperature stress is frequently associated with reduced water availability [46,47]. Nevertheless, imposing high temperatures through late planting would have a confounding effect, as mungbean plants show a substantial response to the sowing date, which influences the rate of development during both the sowing-to-flowering and flowering-to-maturity phases associated with the day lengths and temperatures of the growing seasons [48]. Part of the reason why the flowering-to-maturity phase was extended by long days/warm temperatures in the study of Lawn [49] was that those conditions favour indeterminate growth.

4.4. Evidence for Post-Heat-Stress Compensatory Growth in Other Studies

One growth chamber study showed evidence of post-stress compensation, where the grain yields of a couple of genotypes exceeded with high-temperature stress compared to those observed under an optimum-temperature treatment [44]. For example, the grain yield of the susceptible genotype G27-AUSTRCF 324136 doubled from 0.9 g/plant to 1.8 g/plant, and the grain yield of the commercial cultivar Crystal increased slightly from 1.1 g to 1.2 g/plant with a 45 °C temperature, compared with the control [44]. It should be noted that the seed yield per plant was drastically low in their study compared with the seed yield per plant in our study. For example, Crystal in our study yielded more than 15 g/plant at the optimum temperature, compared with 1.1 g/plant in their study. This may be due to the smaller pot size used in their study. Nevertheless, similar evidence of compensatory growth was also observed in the year 2014 for late-sown mungbean crops in India, where imposed heat stress during flowering (max. temperature varying between 44 °C and 47 °C) resulted in increased pod number, seed weight, and grain yield compared with normal-sown plants [50]). Further, Ha [51] showed that plant height and the number of seeds per pod increased with increasing temperatures (ambient + 3 °C) and then decreased

with further increases in temperature (ambient + 5 °C), whereas the number of branches, node number, and number of pods steadily increased with the increasing temperature, for mungbean genotypes. This evidence further supports that some mungbean genotypes with relatively more indeterminate growth habits are capable of mitigating the impact of heat stress through post-stress compensatory growth, provided that the imposed heat stress is not excessive and/or prolonged, and growing conditions are not impacted by low humidity and limiting moisture conditions. Evidence of post-stress recovery to some degree after short periods of temperature stress by adjusting yield components was also reported for chickpeas [33,34]. It should be noted that the post-stress recovery and compensatory growth mechanisms are different from the inherited tolerance and susceptibility of genotypes to heat stress. For example, Basu et al. [52] reported that seedlings of a tolerant mungbean genotype recovered well at 30 °C after a heat shock at 37–52 °C, whereas seedlings of a susceptible genotype failed to recover. The tolerant genotype was better adapted to heat stress and showed normal growth and fertile pollen even at 43 °C, whereas the susceptible genotype showed complete pollen sterility at 43 °C [52]. In our study, pollen viability reduced equally with the imposed heat stress during early flowering, but some genotypes showed greater post-stress compensatory vegetative growth and pod production. That is, they did not have an inherited tolerance to heat stress, but rather they had some sort of physiological mechanism for enhanced compensatory growth. This needs to be further investigated.

4.5. Performance of Genotypes

In the glasshouse experiment, among the commercial mungbean genotypes, Satin II had the greatest seed yield at OT, which significantly reduced at HT, but was still comparable to those of M12036, Crystal, Celera II AU, and other genotypes, which showed significant increases in grain yield with HT over OT. Satin II also had the greatest stress tolerance index and one of the lowest stress susceptibility indexes among the genotypes. On average, across the heat-stress treatments, the seed yields of Satin II and M12036 were considerably higher than those of the other genotypes, but the seed size of Satin-II (similar to Berken) was significantly smaller than that of M12036, which had a relatively larger seed size, comparable to those of Crystal and Jade-AU. In the field experiment, again like the glasshouse results, M12036 and Crystal outperformed Jade-AU and M11238, indicating persistence and reliability in the yield performance of these genotypes. Based on the seed yield per plant and seed size, M12036 appears to outperform most genotypes, followed by Crystal.

5. Conclusions

The present study highlights the post-stress compensatory growth response to high-temperature stress, imposed at flowering. As expected, heat stress severely reduced the pollen viability, but genotypes with relatively more indeterminate growth habits mitigated the impact of heat stress on their pollen viability by increasing their plant height and associated vegetative growth parameters (branching, node number, etc.). These genotypes also rapidly produced more flowers and pods during post-stress compensatory growth. The post-stress rates of flower production and podding exceeded those observed in the pre-stress period and resulted in the maturing of additional pods along with the pods of non-stressed plants around the same time. There have not been many studies on mungbeans with respect to genotypic variability in response to heat stress. However, a limited number of studies have also indirectly provided convincing evidence of greater compensatory growth responses post stress, particularly in some genotypes, including commercial ones, that effectively mitigated the impact of heat stress. These genotypes were able to increase the total seed yield by increasing the number of pods, with minimal impacts on the number of viable seeds and the seed size. The physiological mechanisms associated with the post-stress rapid growth and maturity of these genotypes in response to heat stress warrant further investigations.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/crops4030020/s1, Table S1. Glasshouse experiment results. Mean values of interaction effects (temperature x genotype) on plant growth parameters. HT, high temperature and OT, optimum temperature. Letters to indicate significant differences between mean values of treatments; Table S2. Field experiment results. Mean values of plant growth parameters effected by main treatments temperature and genotype and their interactions, measured after imposed high temperature and at the physiological maturity. Letters to indicate significant differences between mean values of treatments; Table S3. Correlation (Pearson) matrix between selected plant parameters at optimum temperature (OT) and high temperature (HT). Values in bold are different from 0 with a significance level alpha=0.05.

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