Impact of post-flowering heatwaves on individual grain weight and quality in wheat

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Abstract
Wheat is highly susceptible to heatwaves, especially during reproductive and grain-filling periods. Multi-environment trials were conducted over three years at three locations with different sowing dates to investigate the impact of heatwaves on individual grain weight, and grain protein content in 22 wheat genotypes. Genotypes were grown using a novel photoperiod-extension method (PEM) where single stems of synchronised phenology were tagged and hand-harvested at maturity. Adjacent to PEM trials, genotypes were cultivated in conventional yield plots and machine-harvested at maturity. Significant effects of sowing times and genotypes were observed for individual grain weight and protein content ($p<0.001$). Individual grain weight and grain protein content were also strongly correlated with the number of post-flowering hot days. The clear trade-off observed between grain weight and grain protein content underscored that adverse heat-stress effects resulting in reduced grain size were associated with increased protein content. The findings from this study will assist improvement of heat response in crop models, and selection of genotypes with better adaptation to warmer environments.

Keywords
Heat stress, grain weight, grain protein content, photoperiod extension method, crop adaptation.

Introduction
Wheat can be severely impacted by heat stress during the post-flowering stage, particularly in regions where high temperatures are prevalent. Late-season heat can significantly reduce grain yield and quality by decreasing individual grain weight (IGW) and altering grain protein content (GPC), particularly the fractions of gliadins and glutenins (Prasad et al. 2011; Labuschagne et al. 2021; Ababaei and Chenu, 2020). Considering the recent rise in average temperatures and the expected escalation in the frequency of heatwaves (Collins and Chenu, 2021), enhancing heat tolerance of wheat through genetic approaches appears as a promising avenue to increase yield and yield stability. In previous studies, methods such as late sowing dates, field heat chambers, or controlled conditions have been used to evaluate heat tolerance of various genotypes (e.g. Telfer et al. 2021; Thistlethwaite et al. 2020). However, field-based methods often result in genotypes experiencing heat stress at different developmental stages. To address this challenge, we developed the photoperiod-extension method (PEM), which extends the photoperiod using artificial lights creating a developmental gradient in each row of plants, with plants close to the light flowering earlier than those further away (Ullah et al. 2023). This method allows a focus on plants flowering at the same time irrespective of the genotype maturity type, so that heat tolerance of genotypes can be assessed under natural field conditions in plants at matched developmental stages.

In this study, 22 wheat genotypes were grown with the PEM at different sowing dates to explore the genotypic variability of effects from post-flowering heatwaves on grain size and quality. Results were compared to impacts observed in adjacent trials where genotypes were sown at same dates in traditional field plots.

Material and Methods
Irrigated field trials were conducted in the years 2018, 2019 and 2020 at three locations in southern Queensland, Australia. Twenty-two genotypes of spring wheat (*Triticum aestivum* L.) were tested in both (i) conventional plots sown at standard and late sowing dates, and (ii) in trials using the newly developed photoperiod extension method (PEM) also with standard and late sowing dates (Figure 1; Ullah et al. 2023

and 2024). Each year, the trials were established in a randomized complete block design with four replicates per genotype. Crop management practices included non-limiting fertilizer application as well as control of weeds, diseases, and pests at all trials.
Conventional plots were harvested with a combine harvester and IGW of each plot was estimated from 2 or 3 sub-samples of 100 grains. For each replicate in the PEM, approximately 20 spikes that flowered on the same day for all genotypes were tagged and manually harvested at maturity. The IGW was then estimated from these spikes. Grain protein content (GPC) was estimated from near-infrared scans using a Fourier Transform Near-Infrared Spectroscopy (FT-NIR) instrument (Bruker, Germany). Linear regression, and ANOVA analyses were performed using R (R Core Team 2018).

Results and Discussion
Individual grain weight (IGW) and grain protein content (GPC) varied significantly across trials depending on the number of post-flowering hot days that occurred in those trials. A clear correlation was found between IGW and the number of post-flowering hot day (>30ºC) in harvested spikes from PEM trials ($p = 0.0002$, $R^2 = 0.91$), with a decrease in IGW by 1.6 mg for each extra post-flowering hot day (Figure 2). For instance, 22 post-flowering hot days in the late sowing (‘s2’) of trial TOS19 resulted in a 60% reduction in IGW compared to trials with no or low heat stress (0-5 post-flowering hot days). By contrast, no significant correlation ($p = 0.11$) was observed between IGW and the number of post-flowering hot day (>30ºC) in conventional plots; and the reduction in IGW in the plot harvest of TOS19 sowing s2 was only 12.5% in comparison to average results from trials with no or low post-flowering heat stress (0-5 post-flowering hot days). Previous studies also reported declines in IGW with exposure to elevated temperatures during grain filling stage (Asseng et al. 2011; Liu et al. 2016; Sehgal et al. 2018). The ‘dilution’ of heatwave effects in plots compared to those in the PEM is likely due to genotype phenology differences during heat events in the conventional plot trials.

Figure 1. The photoperiod-extension method (PEM; Ullah et al. 2023) with LED lights in the middle for the trial was employed to develop a gradient of phenology with plants nearest to the lights, at the right of figure, flowered earlier than those further away. The study focused on spikes from different genotypes that flowered at the same time, i.e. spikes from plants growing at different distance from the light depending on the maturity type of the genotype.

In association with reduced IGW, high occurrence of hot days after flowering resulted in increased GPC (Figure 2). This aligns with findings reporting that heat stress typically leads to higher GPC due to reduced carbohydrate accumulation (Liu et al. 2016; Fernie et al. 2022). GPC increased significantly ($p= 0.003$) with the number of post-flowering hot days (>30ºC) in spikes from the PEM, at a rate of 0.32% more GPC for each extra post-flowering hot day. In contrast, in conventional plots, the GPC increase with post-flowering hot days was not statistically significant ($p = 0.18$). Hence for both IGW and GPC, response to post-flowering heatwaves were clearer and more pronounced when considering organ-level harvest in PEM trials than canopy-level harvest in conventional plots, likely due to genotype phenology differences during heat events in the conventional plot trials.

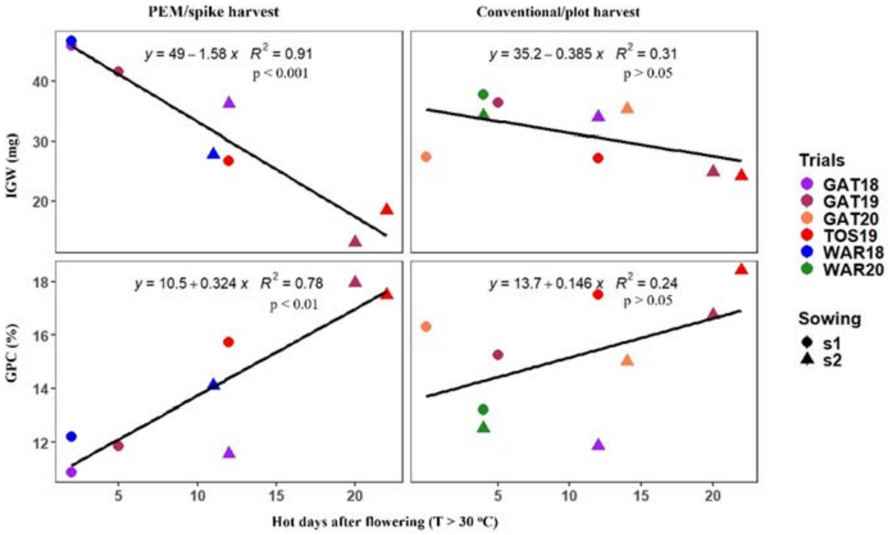

Figure 2. Individual grain weight (IGW) and grain protein content (GPC) of wheat exposed to post-flowering hot days (temperature > 30°C) for both (i) conventional plots that were machine harvests and (ii) the photoperiod-extension method (PEM) trials where harvested spikes were marked at matched flowering Each point represents the average value from 22 genotypes for each of the studied environments. Trials are indicated by different colours, and sowing times by different symbols (circle, conventional sowing ('s1'); triangle, late sowing ('s2')).

Compared to machine-harvested conventional plots, the harvest of spikes that had synchronized flowering in PEM trials also allowed a better discrimination between genotypes for heat-induced responses in both IGW and GPC (data not shown). In PEM spike harvests, substantial genotypic variability was observed for IGW and GPC responses to the number of post-flowering hot days (Figure 3). IGW sensitivity to hot environment ('slope') ranged from 1.89 to -1.29 mg hot-day^{-1} among studied genotypes. To put in context, for a crop experiencing 10 post-flowering hot days, this corresponds to an IGW reduction of ~6 mg more in the most sensitive compared to the less sensitive genotypes (i.e. 600 kg ha^{-1} for a crop with 100.10^{6} grain ha^{-1}). Berkut had the greatest sensitivity (-1.89 mg hot-day^{-1}) to heat stress, reflecting important decrease in its IGW in environment with post-flowering heatwaves. On the other hand, Sokoll/FRTL (-1.28 mg hot-day^{-1}) exhibited the most stable IGW with relatively low IGW decrease in response to post-flowering heatwaves.

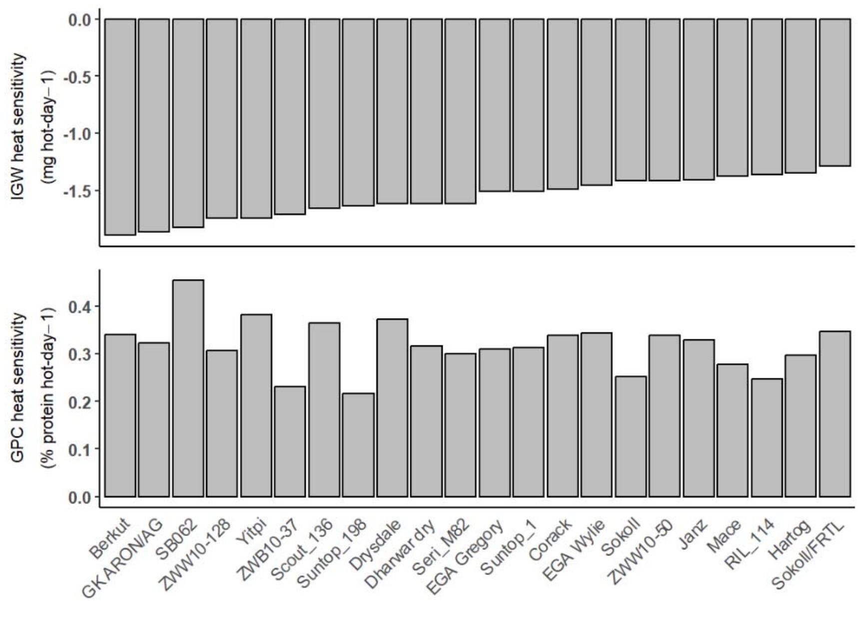

Figure 3. Sensitivity of IGW (mg hot-day^{-1}) and GPC (% protein hot-day^{-1}) in response to the number of hot days (temperature > 30°C) occurring after flowering in PEM trials. Genotypes are ordered according to increasing sensitivity of IGW to heat stress.

Post-flowering sensitivity of GPC (i.e. slope of GPC vs. hot days) varied by more than 2-fold, from 0.21 to 0.45 % protein hot-day^{-1}, among studied genotypes (Figure 3). Suntop_198 and ZWB10-37 exhibited the smallest increase in GPC in response to post-flowering heat stress suggesting a more stable response to late heatwaves. By contrast, line SB062 (0.45 % protein hot-day^{-1}) exhibited the greatest GPC increase observed in response to post-flowering hot days. Earlier studies have also reported genotypic differences in protein accumulation under heat stress (Labuschagne et al. 2021; Zheng et al. 2009).

Conclusion

The findings emphasize the importance of selecting and breeding wheat genotypes that exhibit both industry-acceptable IGW under non-limiting conditions, and a stable performance under heat-stress conditions. This conclusion is reinforced by the fact that the results of this study concern irrigated conditions and thus are likely to underestimate heat impacts in production environments, where heatwaves typically occur during drought periods. Identifying genotypes that maintain good IGW and grain quality under both non-limiting and stressed conditions, i.e. with low sensibility to heat stress is crucial for developing heat-tolerant wheat varieties capable of maintaining both yield and quality in the face of climate change.

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