A field-based technique for screening heat tolerance of wheat lines differing in maturity at matched developmental phases

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Abstract
Field-based screening of heat tolerant wheat germplasm is complicated by confounding effects of phenological differences between genotypes at the timing of natural heat events. Here, we developed and tested during two years at three locations and at different sowing dates, a novel technique, which allows field screening of diverse wheat genotypes at matched developmental stages. Wheat genotypes were planted in single rows and artificial light was provided at the end of the rows extending the photoperiod. This supplemental light induced a gradient of flowering time along the rows for each genotype, so that genotypes could be tagged at a closely matched developmental phase. The method was found to facilitate comparison of all tested genotypes at a matched developmental stage when heat events occured. The novel technique resulted in robust genotype rankings for heat-tolerance that were relatively stable across environments subjected to similar heat stress. The method has potential to improve the efficiency of heat-tolerance field screening, and to breed for germplasm with heat tolerance.

Keywords
Phenotyping, heat stress, genotype x environment interaction, crop improvement.

Introduction
With the recent rate of climate change, a significant increase in the frequency of extreme temperatures was recorded over the last decades (Ababaei and Chenu 2020) and further increases are projected by the end of this century (Field et al. 2012). Wheat crops are highly sensitive to high temperature and the degree of damage depends on which developmental stage is exposed to heat. Reproductive and grain filling stages in wheat crop are reported to be highly sensitive to heat and a mild increase in temperature during these stages can significantly reduce grain yield (Stone and Nicolas 1998). In Australia, heat-stress events primarily occur during the grain-filling period, thus impacting individual grain weight more than grain numbers (Ababaei and Chenu 2020). Over recent decades, the frequency of hot days (Tmax > 26°C) during the grain-filling period of wheat crops has significantly increased across Australian wheatbelt (Ababaei and Chenu 2020). Developing heat tolerant wheat genotypes appears urgent for sustaining wheat grain yields under current and future hot environments. Conventionally, wheat genotypes are screened for heat tolerance by staggering sowing dates in the field (Passioura 2006). Given that the degree of impact of heat is highly dependent on the developmental-phase of the crop, ranking of wheat genotypes for heat tolerance can often be confounded by variation in developmental stage during a natural heat event. A suitable technique that could phenotype for high temperature stress at closely matched developmental stages of wheat genotypes in the field will reduce genotype x environment interactions and assist in the selection of heat tolerant genotypes. In this study, we developed an artificial photoperiod-extension method, which allows screening wheat genotypes with different maturity types at a common developmental stage during natural heat events.

Methods
Field experiments were conducted during 2018 and 2019 at three locations across southern Queensland, namely Gatton (GAT), Warwick (WAR) and Tosari (TOS) near Tummaville. In each location, 32 wheat genotypes with contrasting phenology were planted in a randomised block design with two times of sowing (‘s1’, July sowing; ‘s2’, late August sowing) and four replicates. Each

genotype was hand planted in a 5 m single row with 30 cm row spacing and a target population density of 130 plants m^{-2}. LED lamps were set up at one end of each row, extending the day length to 20 h close to the lights (Figure 1). The intensity of light diminishes in proportion to the square of the distance from the lights along the test row. This induced a gradient of flowering times within each row. The crops were partially (Tosari) or fully (elsewhere) irrigated and cultivated under non-limiting nutritional conditions. Standard crop management practices were used for controlling weeds, pests and diseases. For each trial (site x year x sowing date), at least 20 spikes of each genotype in each block were tagged at anthesis (at different distances from the light depending on the genotype). These spikes were manually harvested at maturity and processed to determine grain yield components. To act as a comparison to the new photoperiod-extension screening method, the performance of same wheat genotypes was assessed in 'conventional' field plots adjacent to most of the photoperiod-extension trials. The plots were managed in the same way (e.g. same sowing dates) but harvested using a small plot machine harvester at maturity.
Data were analysed using R (R Core Team, 2018). Statistical differences were tested with student's t-tests at a 5% level.

Figure 1. Experimental set up to extend the photoperiod and allow screening of plants at closely matched developmental stages during naturally occurring heat shock events. Lights were installed in the middle of the field, with rows blocked for different sowing dates. Plants closer to the supplemental light flowered earlier than the plants away from light.

Results

In conventional field-based screening for heat tolerance of spring wheat, sowing dates are manipulated in a way that the late sown crop is likely to receive a higher degree of heat stress. However, wheat genotypes typically flower at different times, and are likely to experience a naturally-occurring heat event at different developmental stages. Interpreting the results can be difficult as genotype performance may be due to either heat tolerance per se and/or differences in phenology relative to the critical period for heat sensitivity (i.e. some may possible escape from the heat stress during the most sensitive developmental stages).
We developed and tested a novel screening method, which allows the phenotyping of wheat genotypes with varying phenology at similar developmental stages. In our study, individual grain size was strongly correlated with the total number of hot days (maximum daily temperature > 30°C) during grain filling period (i.e. 0 to 500°Cd after flowering). The crops that receiving 0-5 hot days experienced no significant change in grain weight but further increase in the number of hot days significantly reduced the grain weight of the tested wheat genotypes. For instance, compared with five hot days during grain filling at the Gatton 2019 trial (GAT19), the crops receiving 9 and 22 post-flowering hot days (maximum daily temperature >30°C) had a 14 and 61% reduction in grain weight (average across 32 genotypes), respectively.

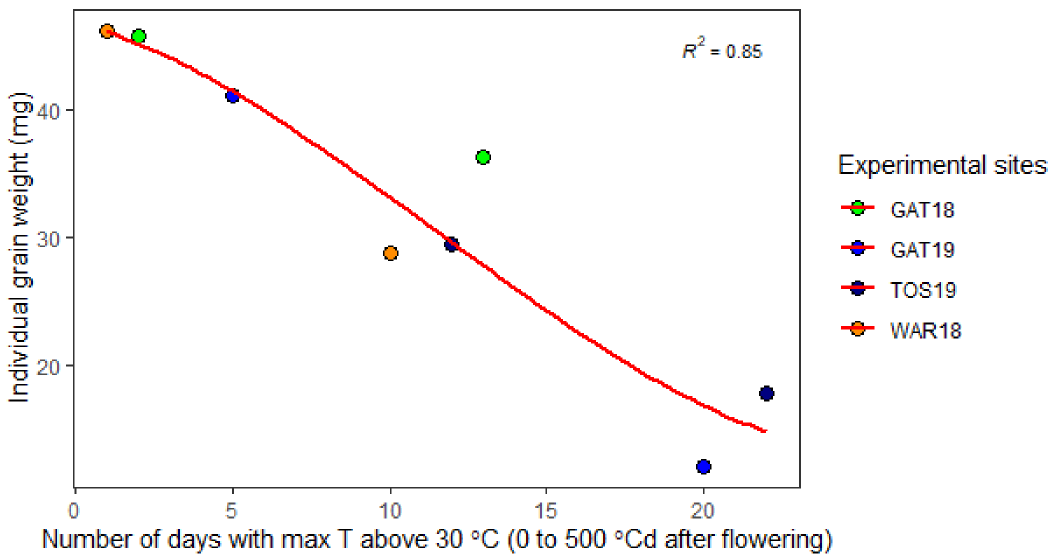

Figure 2. Relationship between individual grain weight and the number of post-flowering hot days (maximum temperature > 30°C) at the Gatton, Tosari and Warwick trials in 2018-19. Each point corresponds to the trial mean of 32 wheat genotypes compared at a matched development stage.

In the conventional method, based on comparison between different sowing dates without photoperiod extension, genotype ranking for grain weight varied widely across environments, both between sowings and sites (Fig. 3A). A maximum correlation of 0.48 was found between first sowings at Gatton and Tosari in 2019 (GAT19s1 and TOS19s1). For the second sowings, which were more prone to heat stress, the maximum correlation was only 0.10 between trials sown at Gatton and Tosari in 2019 (GAT19s2 and TOS19s2). In contrast, with our new photoperiod extension method, genotypes were ranked more reliably for heat tolerance (Fig. 3B). Importantly, genotype rankings were moderate to strong in environments experiencing similar heat stress. The correlations were (i) 0.89 for environments with 1-2 hot days, (ii) 0.54 for environments with 5-10 hot days, (iii) 0.8 for environments with 10-13 hot days, and (iv) 0.46 for environments with 20-22 hot days. This suggests that our method can more reliably rank wheat genotypes for heat tolerance under natural environments compared with the conventionally used screening technique.

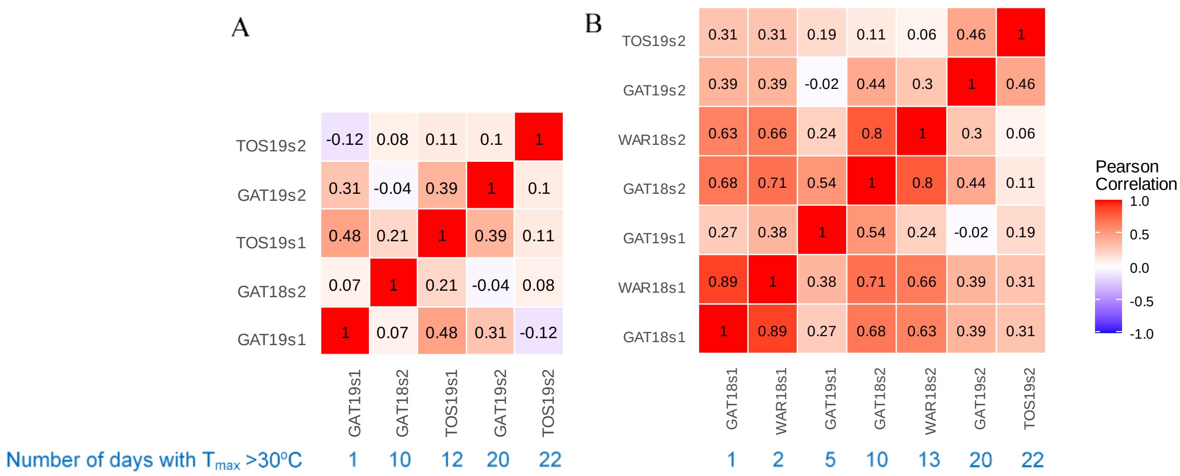

Figure 3. Correlations for individual grain weight of all studied genotypes between (A) irrigated plot trials at multiple sites and sowing dates that were affected by different heat-shocks events without photoperiod extension (i.e. commonly-used field screening method not accounting for differences in developmental stages), and (B) field trials with the new screening photoperiod extension method with supplemental light to allow selection of plants at a closely matched developmental stage. Individual grain

weight was estimated from measurements at the plot level in (A) and at the spike level in (B). Below the heat maps, in blue, are indicated the number of days with a maximum temperature above 30ºC during the grain-filling period for each trial. Trials are in ascending order from left to right for the number of days with maximum temperature above 30ºC during grain fill.

Conclusion

We developed and tested a new field-based method, which allows comparison of all studied genotypes at closely matched developmental stages when a heat event occurred. The method provided robust heat tolerance ranks for the genotypes tested in similar heat-stress environments. This method promises to improve the efficiency of heat tolerance field screening, particularly when comparing genotypes of different maturity types.

Acknowledgments

The research was made possible thanks to the support of The University of Queensland and the Queensland government, Department of Agriculture and Fisheries. Najeeb Ullah was supported by a Queensland Government, Advance Queensland Fellowship.

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