

RESEARCH PAPER

Wheat photosystem II heat tolerance responds dynamically to short- and long-term warming

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Abstract

Wheat photosynthetic heat tolerance can be characterized using minimal chlorophyll fluorescence to quantify the critical temperature (T_{crit}) above which incipient damage to the photosynthetic machinery occurs. We investigated intraspecies variation and plasticity of wheat T_{crit} under elevated temperature in field and controlled-environment experiments, and assessed whether intraspecies variation mirrored interspecific patterns of global heat tolerance. In the field, wheat T_{crit} varied diurnally—declining from noon through to sunrise—and increased with phenological development. Under controlled conditions, heat stress (36 °C) drove a rapid (within 2 h) rise in T_{crit} that peaked after 3–4 d. The peak in T_{crit} indicated an upper limit to PSII heat tolerance. A global dataset [comprising 183 *Triticum* and wild wheat (*Aegilops*) species] generated from the current study and a systematic literature review showed that wheat leaf T_{crit} varied by up to 20 °C (roughly two-thirds of reported global plant interspecies variation). However, unlike global patterns of interspecies T_{crit} variation that have been linked to latitude of genotype origin, intraspecific variation in wheat T_{crit} was unrelated to that. Overall, the observed genotypic variation and plasticity of wheat T_{crit} suggest that this trait could be useful in high-throughput phenotyping of wheat photosynthetic heat tolerance.

Keywords: Acclimation, chlorophyll fluorescence, heat stress, phenotypic plasticity, photosynthesis, photosystem II, thermotolerance, *Triticum* species.

Introduction

As the climate changes, global mean land surface temperature has continued to rise, alongside more frequent, longer, and more intense heatwaves (Perkins-Kirkpatrick and Lewis, 2020). This is particularly concerning for the prospect of improving crop yields, as heat stress is associated with significant declines in the yield of widely cultivated crops, including wheat (Asseng *et al.*, 2015; Tack *et al.*, 2015; Hochman *et al.*, 2017; Liu *et al.*, 2019; Ortiz-Bobea *et al.*, 2019). Photosynthesis is a primary determinant of wheat yield, and it is particularly sensitive to heat stress (Berry and Bjorkman, 1980; Way and Yamori, 2014). Improving the heat tolerance of photosynthesis could future-proof wheat yield in a warming world (Cossani and Reynolds, 2012; Scafaro and Atkin, 2016; Iqbal *et al.*, 2017). To realize improvements in wheat photosynthetic heat tolerance, it is paramount that we first understand and quantify patterns of wheat photosynthetic heat tolerance so that we can then successfully exploit them.

Decreased leaf photosynthetic rate under high temperature is partially linked to disruption of the chloroplast electron transport chain, of which the thylakoid membrane-embedded PSII is considered the most sensitive component (Sharkey, 2005; Brestic *et al.*, 2012). Heat-induced reactive oxygen species and lipid peroxidation both cause cleavage of the reaction centre-binding D1 protein of PSII (Yamashita *et al.*, 2008), inhibiting electron flow and thus the production of ATP. For decades PSII damage has been measured with Chl *a* fluorescence metrics, including the critical temperature of F_0 (henceforth T_{crit}) (Schreiber *et al.*, 1975; Schreiber and Berry, 1977; Hüve *et al.*, 2011; Geange *et al.*, 2021). T_{crit} is the critical temperature above which minimal Chl *a* fluorescence (F_0) rises rapidly, indicating incipient damage to PSII (Schreiber and Berry, 1977; Melcarek and Brown, 1979; Neuner and Pramsohler, 2006; Slot *et al.*, 2019). T_{crit} is associated with increased thylakoid membrane fluidity, disruption of the light-harvesting antennae (Raison *et al.*, 1982; Figueroa *et al.*, 2003), dissociation of chloroplast membrane-bound proteins (Berry and Bjorkman, 1980), and loss of chloroplast thermostability (Armond *et al.*, 1978). As a standardized metric, T_{crit} has been used to examine global patterns of heat tolerance, quantify phenotypic plasticity in response to warming, and assess vulnerability to climate change across plant species (O'Sullivan *et al.*, 2017; Zhu *et al.*, 2018; Lancaster and Humphreys, 2020; Geange *et al.*, 2021). While the number of publications examining plant T_{crit} is growing (Ferguson *et al.*, 2020; Arnold *et al.*, 2021; Slot *et al.*, 2021), most studies focused on woody, non-crop species, and characterization of intraspecific variation in T_{crit} of crop species has been limited (see Ferguson *et al.*, 2020 for a recent exception). Wheat, as the most widely cultivated crop (with >220 Mha cultivated worldwide) with a diverse range of genotypes originating from across the globe, is an ideal crop species for examining intraspecific variation and acclimation of T_{crit} . In addition, although wheat is a temperate crop, there is increasing

evidence of warming in many wheat-producing regions, including China, the USA, and Australia, resulting in either stalled or reduced wheat yield (Hochman *et al.*, 2017; Zhao *et al.*, 2017). Understanding the response of T_{crit} to warming and the magnitude of intraspecific variation in T_{crit} could thus provide opportunities for improving photosynthetic heat tolerance and yield resilience in wheat and other crops.

Quantification of intraspecific variation in physiological traits of crops commonly encounters bottlenecks at the phenotyping stage. However, high-throughput phenotyping techniques are being developed, including a robotic system offering a 10-fold increase in the measurement speed of dark respiration (Scafaro *et al.*, 2017; Coast *et al.*, 2019, 2021), and the proximal remote sensing of leaf hyperspectral reflectance signatures for rapidly assaying photosynthetic characteristics and dark respiration (Silva-Pérez *et al.*, 2018; Coast *et al.*, 2019; Fu *et al.*, 2019). Though chlorophyll fluorescence techniques are well established for assessing photosynthetic heat tolerance, they are typically cumbersome. This limits their incorporation in breeding programmes that screen hundreds of genotypes for heat tolerance. However, recently, Arnold *et al.* (2021) described a high-throughput chlorophyll fluorescence screening technique for a diversity of wild species. Previous studies of photosynthetic thermal tolerance also largely assumed that T_{crit} is diurnally and phenologically constant. However, these assumptions may be flawed. Substantial changes in metabolic capacity and demand for photosynthetic products vary diurnally, with phenological development and in response to fluctuations in temperature (Steer, 1973; Rashid *et al.*, 2020). Thus, it seems reasonable that T_{crit} may demonstrate similar variation. However, these assumptions remain untested.

The extent to which plants physiologically adjust to warming is important in determining productivity and survival (Scheiner, 1993; Leung *et al.*, 2020). Acclimation of photosynthetic electron transport to elevated temperature is evidenced by an increase in T_{crit} . Zhu *et al.* (2018) reported acclimation at a rate of a 0.34 °C increase in T_{crit} for every 1 °C increase in average temperature over the growing season for a range of Australian species. Acclimation of T_{crit} may also increase plant thermal safety margins, thus protecting against damage to PSII under future heat stress. Thermal safety margins are estimated as the difference between the upper limit of leaf function (e.g. T_{crit}) and the maximum growth temperature experienced in an environment (Sastry and Barua, 2017), and they provide a useful representation of a species' potential vulnerability to global warming (Sunday *et al.*, 2014). A reduction in this margin indicates increasing vulnerability to heat stress, while an increase in this margin indicates better capacity to tolerate the effects of climate warming (Hoffmann *et al.*, 2013). Thermal safety margins of 10–15 °C have been reported for many plant species (Weng and Lai, 2005; O'Sullivan *et al.*, 2017; Perez and Feeley, 2020), with some as high as 12–31 °C (Leon-Garcia and Lasso, 2019) when leaf temperature, rather than air temperature, was used. However, many plant species have low thermal safety

margins (e.g. ≤ 5 °C; Sastry and Barua, 2017). Unfortunately, reports quantifying the acclimation capacity and thermal safety margins of food crops are scarce. Reports on acclimation of T_{crit} to warming have been in response to a sustained increase in long-term growth temperature. Similar descriptions of T_{crit} acclimation to short-term heat stress (e.g. heatwaves) are not well documented. Considering that heatwaves are predicted to become more frequent and intense (Perkins-Kirkpatrick and Lewis, 2020), it is pertinent that we understand if and how T_{crit} responds to heatwaves. Whether acclimation of T_{crit} to heatwaves has an upper threshold (i.e. a ceiling temperature) is currently unknown.

Previous uses of T_{crit} to assess global patterns of heat tolerance have been underpinned by ecological theories established in terrestrial ectotherms and endotherms (Addo-Bediako *et al.*, 2000; Deutsch *et al.*, 2008; Sunday *et al.*, 2011; Araújo *et al.*, 2013). One such theory is that organism physiology correlates closely with large-scale geographical patterns in the thermal environment where populations of an individual species evolved (Gabriel and Lynch, 1992). Indeed, greater photosynthetic heat tolerance of non-crop plants originating from hotter, equatorial environments has been reported for numerous species (O'Sullivan *et al.*, 2017; Drake *et al.*, 2018; Zhu *et al.*, 2018; Lancaster and Humphreys, 2020). It remains unknown whether such global patterns of interspecies variation hold for intraspecific comparisons, for example in a widely cultivated crop such as wheat, with genotypes originating from across the globe.

In this study, we employed a high-throughput system to describe intraspecific variation and high temperature acclimation of T_{crit} in wheat. Our objectives were to: (i) examine whether leaf T_{crit} varies diurnally and with phenology; (ii) determine the thermal safety margins and assess vulnerability of wheat to high temperatures in the Australian grain belt; and (iii) to assess if there is an upper threshold for leaf T_{crit} exposed to a sustained heat shock. To achieve these objectives, we conducted three field studies and one controlled-environment experiment. In addition, we conducted a systematic literature review of wheat T_{crit} and used the global data we generated to investigate if intraspecific variation in wheat leaf T_{crit} is related to the latitude of genotype (as a proxy for climate of origin) of wheat genotypes or species.

Materials and methods

Field experiments: assessing diel and phenological variation in wheat T_{crit} and estimating thermal safety margins of Australian wheat

Germplasm

A set of 20–24 wheat genotypes (Supplementary Table S1) were used in three field experiments conducted in Australia across 3 years. Twenty of

these genotypes were used by Coast *et al.* (2021) to assess acclimation of wheat photosynthesis and respiration to warming in two of the fields. The genotypes included: commercial Australian cultivars; heat-tolerant materials developed by the centres of the Consultative Group on International Agricultural Research (CGIAR) in Mexico and Morocco and tested in warm areas globally; materials derived from targeted crosses between adapted hexaploid cultivars and heat-tolerant Mexican hexaploid landraces; tetraploid emmer wheat (*T. dicoccon* Schrank ex Schübl.), Indian cultivars; and synthetic wheat derived by crossing *Aegilops tauschii* with modern tetraploid durum wheat. All genotypes evaluated were hexaploid and chosen for their contrasting heat tolerance under high temperature conditions in Sudan (Gezira; 14.9°N, 33°E), Australia (Narrabri, NSW; 30.27°S, 149.81°E), and Mexico (Ciudad Obregón; 27.5°N, 109.90°W).

Experimental design and husbandry

The first 2 years of field experiments were undertaken in regional Victoria (2017, Dingwall; and 2018, Barraport West), and the third was in regional New South Wales (2019, Narrabri). A detailed description of the experimental designs for the 2017 and 2018 experiments is given in Coast *et al.* (2021). Briefly, a diverse panel of genotypes were sown on three dates each in 2017 (20 genotypes) and 2018 (24 genotypes) to expose crops to different growth temperatures at a common developmental stage. The first time of sowing (TOS) for both experiments was within the locally recommended periods for sowing (early May). Subsequent sowing times were 1 month apart in June and July. Experiments were sown in three adjacent strips, one for each TOS. Each strip consisted of four replicate blocks. The 2019 field experiment was similar in all aspects to the 2018 field experiment, except for the following: (i) only two sowing times were incorporated in the design; and (ii) the sowing times were ~2 months apart (17 May 2019 and 15 July 2019). Of the 24 genotypes sown in 2018, only 20, which were common to the 2017 and 2019 experiments, were assessed for T_{crit} . All three field experiments were managed following standard agronomic practices for the region by the Birchip Cropping Group (www.bcg.org.au) in regional Victoria, and staff of the IA Watson Grains Research Centre at The University of Sydney, and Australian Grain Technologies, in Narrabri. A summary of the field experiments is presented in Table 1.

Diel measurements of wheat T_{crit}

Six of the 20 genotypes in the 2017 field experiment at Dingwall, Victoria were used to investigate diel variation. The six genotypes were two commercial cultivars (Mace and Suntop) and four breeding lines (with reference numbers 143, 2254, 2267, and 2316). These were chosen because they are representative of the diversity of the set of 20 genotypes (Coast *et al.*, 2021). To determine if T_{crit} varied diurnally, one flag leaf from each of four replicate plants was harvested at anthesis [Zadok growth stage (GS) 60–69; Zadoks *et al.*, 1974] from plants of TOS 3 at four consecutive time points occurring every 6 h over an 18 h period (solar noon, sunset, midnight, and sunrise).

Phenological measurements of wheat T_{crit}

A subset of four genotypes from the 20 in the 2018 field experiment at Barraport West, Victoria was used to assess phenological variation in T_{crit} . The four genotypes were the breeding lines 2062, 2150, 2254, and 2267, the latter two of which were also used for diel measurements as described in the previous paragraph. Plants at heading (Zadok GS50–59), anthesis (Zadok GS60–69), and grain filling (Zadok GS70–79) were respectively chosen from fields of the three sowing times. One flag leaf was harvested from the tallest tiller of each replicate plant (minimum eight replicates) at the different phenological stages at 10.00 h on the same day and used to determine T_{crit} .

Table 1. Information on field experiments in Australia

Experiment location and year	Year	Genotypes studied ^a	Mean daily maximum temperature at anthesis (°C)	Radiation ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$) ^b
Dingwall, Victoria	2017	20	21.4–31.6	1394–1934
Barraport West, Victoria	2018	20	22.8–33.4	1706–2331
Narrabri, New South Wales	2019	24	22.6–34.1	1823–1950
Experiment objective	TOS ^c	Genotypes studied	Brief description of method	
Diurnal variation in T_{crit}				
Dingwall, Victoria	3	6	Flag leaf T_{crit} determined at anthesis at four consecutive time points occurring every 6 h over an 18 h period (i.e. solar noon, sunset, midnight, and sunrise).	
Phenological variation in T_{crit}				
Barraport West, Victoria	1–3	4	Flag leaf T_{crit} determined at heading, anthesis, and grain filling on the same day at 10.00 h from all three time of sowing plots.	
Rate of acclimation of T_{crit} ^d and calculation of thermal safety margins				
Dingwall, Victoria	1–3	20	Times of sowing varied so that plants sown later experienced warmer growth environments at a common developmental stage. Flag leaf T_{crit} determined at anthesis. Thermal safety margins were estimated as the difference between genotype mean flag leaf T_{crit} at anthesis and the maximum recorded air temperature at Dingwall/Barraport West (40 °C) or Narrabri (40.8 °C) in October (typical month of peak wheat anthesis).	
Barraport West, Victoria	1–3	20		
Narrabri, New South Wales	1–2	24		

^a Twenty genotypes were common to all experiments. The designation of all genotypes used in this study is provided in [Supplementary Table S1](#).

^b Mean maximum photosynthetically active radiation measured with Licor 6400XTs light sensors.

^c Time of sowing, where the first time of sowing was within the locally recommended sowing window, with subsequent times of sowing separated in 1 month intervals at Victoria, or 2 months at New South Wales.

^d An additional T_{crit} high temperature acclimation study was conducted under controlled environments with two of the 24 genotypes.

Estimation of thermal safety margin of Australian wheat

All 20 genotypes in the 2017 and 2018 field experiments in Dingwall and Barraport West respectively, as well as all 24 genotypes in the 2019 field experiment in Narrabri were used to estimate thermal safety margins. Thermal safety margins were estimated as the difference between individual genotype T_{crit} and the maximum recorded air temperature at either Dingwall or Narrabri in October. Similar definitions of thermal safety margins as the difference between the measured temperature at which a species experiences irreversible physiological damage and the maximum measured temperature of the species' habitat have been used in studies of animal ectotherms and plants (Deutsch *et al.*, 2008; Sunday *et al.*, 2014; O'Sullivan *et al.*, 2017; Sastry and Barua, 2017). We considered Barraport West and Dingwall together for their historical weather records as they are in close proximity to one another in the Mallee district of Victoria, Australia. Weather data for Dingwall and Barraport West were obtained from the Australian Bureau of Meteorology covering the period 1910–2020. We used 40 °C and 40.8 °C (the maximum recorded air temperature for October) in Dingwall/Barraport West and Narrabri, respectively, to quantify thermal safety margins under current climatic scenarios. October was chosen as the upper threshold of exposure of field plants at anthesis to heat because all the later sown plants in this study were at anthesis in October. For future climatic conditions, we added 2.6 °C and 5 °C to the current maximum temperature, with the 2.6 °C addition representing the top end of the intermediate emission Representative Concentration Pathway (RCP) 4.5 IPCC scenario predicted for Eastern Australia by 2090 (1.3–2.6 °C), and the 5 °C addition similarly representing the top end of the high emission RCP 8.5 IPCC scenario predicted for Eastern Australia by 2090 (2.8–5 °C; [Climate Change Australia, 2021](#)). Across all times of sowing in the three field experiments, flag leaves were harvested at anthesis (Zadok GS60–69) at a standardized time of 09.00–10.00 h to determine T_{crit} and estimate thermal safety margins.

Controlled-environment experiment: speed of acclimation and upper limit of leaf T_{crit} during heat shock

A controlled-environment experiment was conducted to determine the speed and threshold of the response of T_{crit} to a sudden heat shock. Two wheat genotypes, 29 and 2267 ([Supplementary Table S1](#)), which contrasted in T_{crit} under common conditions were used to assess the speed and potential threshold of the response of wheat leaf T_{crit} to sudden heat shock. This experiment was conducted at the Controlled Environment Facilities of the Australian National University (ANU), Canberra, Australia.

Plant husbandry

Seeds were germinated on saturated paper towels in covered plastic containers under darkness for 1 week. Germinated seedlings were planted in 1.05 litre pots (130 mm diameter) filled with potting mix (80% composted bark, 10% sharp sand, 10% coir) with 4g l⁻¹ fertilizer (Osmocote Exact Mini fertiliser, ICL, Tel Aviv, Israel) mixed through.

Temperature treatment

Potted plants were grown in glasshouses in which a 24/18 °C day/night temperature regime with a 12 h photo-thermal period was maintained until tillering. At tillering, when all plants had a fully extended third leaf (Zadok GS22–29; [Zadoks *et al.*, 1974](#)), plants were moved into growth cabinets (TPG-2400-TH, Thermoline Scientific, Wetherill Park, NSW, Australia) for temperature treatment. One of two temperature conditions were imposed: a day/night regime of 24/18 °C, or a heat shock with day/night temperatures of 36/24 °C. White fluorescent tubes provided a 12 h photoperiod of photosynthetically active radiation of 720–750 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at plant height. Leaf discs were sampled from fully extended third leaves from main tillers and used to determine T_{crit} after 2, 4, 24, 48,

72, and 120 h in the growth cabinets. Four replicate plants were used for T_{crit} measurement at each sampling time and for each temperature condition. Plant husbandry followed standard practice at the ANU Controlled Environment Facilities.

Meta-study (field experiments, glasshouse studies, and a systematic literature review) of wheat T_{crit} relationship with origins of genotypes

To explore how our results compare with previous studies that have assessed wheat leaf T_{crit} , and whether genotypes from hot habitats exhibit higher T_{crit} , we undertook a systematic review of the published literature and compiled data from >30 years (1988–2020) of wheat leaf T_{crit} studies. A database was generated using information from a recently published systematic review on global plant thermal tolerance (Geange *et al.*, 2021) and additional literature search. These published data were combined with data from the three field experiments described above. The multiple times of sowings in each of the three Australian field experiments provided us with eight thermal environments for obtaining wheat leaf T_{crit} from a total of 24 wheat genotypes. We also included unpublished wheat leaf T_{crit} data from nine other experiments conducted in controlled-environment facilities at the Australian National University. Overall, our global dataset included 3223 leaf T_{crit} samples from 183 wheat genotypes of various species (*Triticum aestivum* L., *T. turgidum* L., ssp. durum Desf., *T. turgidum* L., ssp. diococcoides Thell.), and wild wheat (*Aegilops* species).

Determination of leaf T_{crit}

One leaf per plant was harvested and stored in plastic bags alongside a saturated paper towel and were left to dark adapt for a minimum of 20 min while being transported back to the laboratory. There was ~60–90 min between harvest and measurement time, which was maintained consistently across all experiments. Previous trial experiments with the fluorometer demonstrated that change in wheat T_{crit} post-harvest is minimal (< 0.5 °C) within this time frame. Water (90 µl) was placed in each well of a 48-well Peltier heating block in order to ensure that leaf samples remained hydrated throughout the assay. A single 6 mm diameter leaf disc was excised from the middle of each harvested dark-adapted leaf and placed within each well of the heating block. Once discs were all loaded into the heating block, a glass plate was used to enclose the wells to prevent leaf pieces from drying out during the assay. The block was then placed directly beneath the lens of an imaging fluorometer (FluorCam 800MF, Photon Systems Instruments, Brno, Czech Republic) and programmed to heat from 20 °C to 65 °C at a rate of 1 °C min⁻¹. Prior independent tests using copper–constantan thermocouples [42 SWG (standard wire gauge), 64 µm diameter] referenced against a PT-100 platinum resistance thermometer confirmed that changes in programmed block temperature were strongly correlated with leaf temperature in all wells. The fluorometer recorded F_0 throughout the heating period (approximately one record per minute). Following the conclusion of the temperature ramp, fluorescence data were processed and used to estimate T_{crit} , which was calculated according to the method described by Schreiber and Berry (1977), using the R package 'segmented' (Mugge, 2008). Briefly, the package identifies the breakpoint in data containing a broken-line relationship by estimating linear and generalised linear models. This breakpoint in the F_0 curve was taken as T_{crit} .

Statistical analysis

Statistical analyses were carried out within the R statistical environment (v. 3.4.4; R Core Team, 2018) with R Studio. For analysis of the field data on diurnal and phenological variation in T_{crit} , we employed linear mixed models in R using the packages lmerTest (Kuznetsova *et al.*, 2017) and

emmeans (Lenth, 2020). Genotype was a fixed term in all models, while other fixed terms were time of day (for analysis of diel variation), developmental stage (for analysis of phenological variation), and time of sowing (for analysis of growth temperature variation), with replicate included as a random term in all models. The ceiling threshold of T_{crit} under heat stress (of 36 °C), in the controlled-environment experiment, was determined by fitting a non-linear regression to the T_{crit} by time relationship. Then using the coefficients of the fitted regressions, we estimated the time at which the fitted T_{crit} was highest, and this was taken as the time to peak acclimation. To test the relationships between T_{crit} and growth environment temperature, we only used data from the three field experiments in Australia, for which we had reliable data. The 24 genotypes studied under field conditions in Australia were grouped based on the region of origin of their pedigree (Aleppo, Syria; Gezira, Sudan; Narrabri, Australia; Obregón, Mexico; Pune, India; and Roseworthy, Australia) and the relationship was examined using linear or bivariate regressions. Our global dataset (see Table 2 for sources) was used to ascertain the link between wheat leaf T_{crit} and climate of origin by regressing mean genotype T_{crit} with genotype latitude (as a proxy for climate) of origin.

Results

Diel and phenological variation in T_{crit}

Flag leaf chlorophyll fluorescence measurements of 20–24 wheat genotypes (grown at one of three experimental sites in the Australian wheat belt) were used to assess variation in T_{crit} across time, phenology, and genotype origin. There was a significant genotype by time of day interaction for T_{crit} ($P=0.042$; Supplementary Table S2), highlighting the heterogeneity in this diel variation of T_{crit} among our genotypes. In all except genotype 2316, T_{crit} tended to be highest at solar noon before then declining through sunset, midnight, and sunrise (Fig. 1A). The slope of these trends was only significant for genotype 2267, with T_{crit} declining by 3.1 °C from solar noon to sunrise. In contrast, genotype 143 exhibited the narrowest diel range in T_{crit} , with difference of 1.1 °C between solar noon and sunrise. Irrespective of genotype, T_{crit} at solar noon was significantly higher than at sunrise ($P<0.001$ for time of day). T_{crit} also showed a significant genotype by phenology interaction, and highly significant differences for the main effects of genotype as well as phenology (Supplementary Table S2). The interaction effect was largely due to the increasing trend in T_{crit} as plants developed from heading to anthesis and grain filling for genotypes 2267, 2254, and 2062, but not for 2150 (Fig. 1B). T_{crit} of genotype 2150 rose slightly between heading and anthesis then declined significantly at grain filling relative to anthesis. Genotype 2254 showed the largest increase in T_{crit} between heading and anthesis, rising 1.8 °C from 44.4 °C to 46.2 °C.

Thermal safety margins of Australian wheat

At Dingwall, only the main effect of TOS was significant (Supplementary Table S3). In comparison with TOS 1, T_{crit} of TOS2 tended to be higher and TOS 3 tended to be lower (Fig. 2A). At Barraport, T_{crit} varied amongst the genotypes over a

Table 2. Source of data used for assessment of global variation in leaf photosynthetic heat tolerance (T_{crit})

Study ^a	Origin	Species	Mean T_{crit} (n)
This study	Asia	<i>Triticum aestivum</i> L.	45.1 (8)
	Africa	<i>T. aestivum</i> L.	44.6 (1)
	Australia	<i>T. aestivum</i> L. and <i>T. dicoccum</i> Schrank	44.7 (9)
	North America	<i>T. aestivum</i> L.	45.0 (6)
Average			44.8 (24)
Havaux et al. (1988)	Africa	<i>T. turgidum</i> L., ssp. <i>durum</i> Desf.	49.7 (9)
	Europe	<i>T. turgidum</i> L., ssp. <i>durum</i> Desf.	48.1 (19)
	North America	<i>T. turgidum</i> L., ssp. <i>durum</i> Desf.	51.8 (1)
	South America	<i>T. turgidum</i> L., ssp. <i>durum</i> Desf.	49.0 (2)
Average			48.7 (31)
Végh et al. (2018)	Europe	<i>T. aestivum</i> L.	41.8 (5)
	Africa	<i>T. turgidum</i> L., ssp. <i>durum</i> Desf.	37.0 (1)
Rekika et al. (1997)	North America	<i>T. turgidum</i> L., ssp. <i>durum</i> Desf.	35.0 (1)
	Europe	<i>T. turgidum</i> L., ssp. <i>durum</i> Desf.	37.2 (3)
		<i>T. turgidum</i> L., ssp. <i>diococcoides</i> Thell.	38.0 (1)
	Europe	<i>Aegilops species</i>	38.2 (5)
	(wild wheat)		
Average			37.5 (11)
Data from nine studies (see Supplementary Table S5)	Asia	<i>T. aestivum</i> L.	43.8 (21)
	Africa	<i>T. aestivum</i> L.	45.2 (1)
	Australia	<i>T. aestivum</i> L. and <i>T. dicoccum</i> Schrank	44.5 (79)
	North America		43.9 (32)
Average			44.2 (133)

^a The fluorescence temperature response curves used in these studies were similar (ramp rate of 1–1.5 °C min⁻¹, in the 20–65 °C range). Values in bold are study averages and those in parentheses indicate the number of genotypes/species used.

range of ~2 °C and the effect of TOS on T_{crit} depended on the genotype ($P < 0.01$ for genotype by TOS interaction). T_{crit} increased more in some genotypes (e.g. 1132, 143, and Trojan) under later sowing than others (e.g. 2267 and 29), but also did not change significantly in some (e.g. 1898 and 1943; Fig. 2B). At Narrabri, only the main effects were significant—genotypes varied in their T_{crit} , and TOS 3 T_{crit} was higher than TOS 1 (Fig. 2C; Supplementary Table S3). Across TOS, the genotypes with the lowest and highest mean T_{crit} were 1704 (45.3 °C) and 143 (46.7 °C), respectively. Across the 24 genotypes, T_{crit} increased by 0.5 °C from TOS 1 (at 45.7 °C) to TOS 3 (at 46.2 °C). An ANOVA run on a linear mixed effects model of the entire field dataset revealed field site to be the largest source of variation in T_{crit} of all our independent variables (df = 2, F -value = 190.9, $P < 0.001$). The overall mean T_{crit} at each of the field sites was 45.1 °C at Barraport West, 44.1 °C at Dingwall, and 45.9 °C at Narrabri.

Thermal safety margins were calculated for all field-grown genotypes by quantifying the difference between T_{crit} and the maximum air temperature recorded during October. All genotypes demonstrated a higher T_{crit} than the historical maximum October air temperatures recorded at each field site (Fig. 2, dash-dot line). Thermal safety margins in the TOS 1 fields ranged from 3.2 °C to 4.8 °C in Dingwall (Fig. 2A), from 2.8 °C to 5.3 °C in Barraport West (Fig. 2B) and from 3.8 °C to

5.8 °C in Narrabri (Fig. 2C). For the later sown crops (i.e. TOS 2 and 3) which experienced warmer growth temperatures, thermal safety margins increased relative to TOS 1 in Dingwall (3.7–5.6 °C for TOS 2), in Barraport West (4.6–6.8 °C for TOS 2, and 4.3–8.1 °C for TOS 3), and in Narrabri (4.5–6.1 °C). The exception to this pattern was TOS 3 at Dingwall where the lower end of the thermal safety margin range declined, resulting in a range of 2.1–4.8 °C. At both Narrabri and Barraport West, mean T_{crit} of all genotypes was above the +2.6 °C mark associated with the RCP 4.5 intermediate emission scenario (Fig. 2B, C, dashed line). Most genotypes were also largely clear of the RCP 4.5 mark at Dingwall, except the T_{crit} of genotypes 2255 and 2328 sown at TOS 3 which were below this threshold. The +5 °C warming mark associated with the high emission RCP 8.5 scenario was equal to or above the T_{crit} of many genotypes at all three field sites, though there was some variation across the locations. At Narrabri, half of the genotypes were below the RCP 8.5 threshold when sown at TOS 1, while this fell to a quarter of genotypes when sown at TOS 3 (Fig. 2C). At TOS 1 in Barraport West, 17 genotypes fell below the RCP 8.5 threshold, with only one and three genotypes below this mark for TOS 2 and 3, respectively (Fig. 2B). At Dingwall, T_{crit} of all genotypes sown at TOS 1 and TOS 3 was below the RCP 8.5 threshold, while 14 genotypes at TOS 2 were below this mark (Fig. 2A).

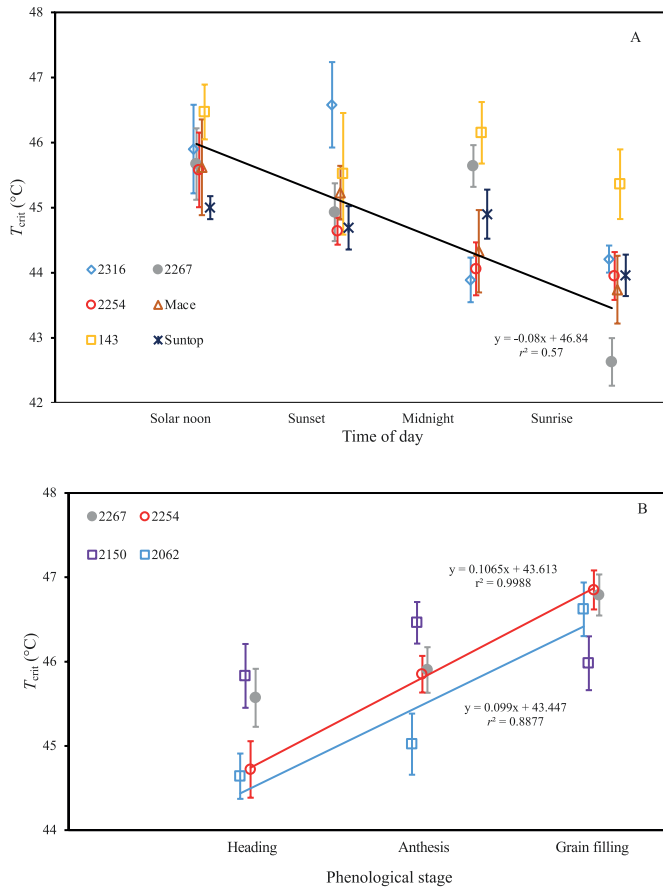


Fig. 1. Variation in flag leaf T_{crit} (°C) of wheat genotypes over the course of an 18 h period (A), and across three phenological stages (B). Solid lines indicate significant linear trends ($P < 0.05$) for all genotypes pooled across time (A) and for genotypes 2254 and 2062 across phenology (B). Plants were grown at field sites in Dingwall, Victoria in 2017 (A), and in Barraport West, Victoria in 2018 (B). Points represent the mean \pm SE, $n=4$ for (A) and $n=8-18$ for (B).

Genotype origin does not predict variation or acclimation in T_{crit}

The 24 genotypes grown across the three field sites were grouped by the regions from which they originated (Supplementary Table S1; Aleppo, Syria; Gezira, Sudan; Narrabri, Australia; Obregón, Mexico; Pune, India; and Roseworthy, Australia) in order to determine if this explained any of the observed variation in T_{crit} . Genotype origin had a significant effect on T_{crit} at both Barraport West and Narrabri (Supplementary Table S4). At Barraport West, genotypes that originated in Narrabri had the highest T_{crit} and Sudan the lowest (Fig. 3B). At Narrabri, the genotype that originated from Syria had the highest T_{crit} , whereas those from Roseworthy had the lowest. In contrast, genotype origin had no significant effect on T_{crit} at Dingwall. TOS had a significant effect on T_{crit} at all three sites irrespective of origin. In Dingwall T_{crit} was lower for TOS 3 relative to TOS 1 and 2, while in Barraport West T_{crit} was lower for

TOS 1 than for TOS 2 and 3 (Fig. 3). At the Narrabri site, T_{crit} increased from TOS 1 to TOS 3 for all origin groupings (Fig. 3C). No interaction between time of sowing and genotype origin was observed at any field site (Supplementary Table S4).

Response of T_{crit} to short-term exposure to high temperature and upper limit of T_{crit} plasticity

To characterize the short-term response of T_{crit} to high temperature, two genotypes were grown under controlled conditions and measured while at the tillering stage of development. In both genotypes, T_{crit} increased significantly following 2 h of heat shock (Fig. 4). As the heat shock progressed, T_{crit} increased following a curvilinear pattern which peaked after 3.4 d for genotype 2267 and after 4.2 d for genotype 54. Although the time to reach peak T_{crit} during the heat shock differed for the two genotypes, their maximum T_{crit} values were similar, being 43.8 °C for genotype 29 and 43.6 °C for genotype 2267 (Fig. 4). T_{crit} for both genotypes remained largely constant over the 120 h period for those plants that were maintained at the control day/night temperature regime of 24/12 °C.

Global variation in wheat T_{crit}

We combined data from our experiments with previously published data (covering genotypes grown across field and controlled-environment experiments) to examine the degree of variation in T_{crit} in wheat genotypes on a global scale based on the latitude of origin as a proxy for climate of origin of their pedigree (Fig. 5). We found three studies (Havaux *et al.*, 1988; Rekika *et al.*, 1997; Véghe *et al.*, 2018) that reported wheat leaf T_{crit} using similar fluorescence temperature response curves (with ramp rates of 1–1.5 °C min⁻¹ between 20 °C and 65 °C) to estimate T_{crit} . Our final data collation comprised 183 wheat species/varieties (comprising *T. aestivum* L., *T. turgidum* L., ssp. *durum* Desf., *T. turgidum* L., ssp. *diococcoides* Thell., and wild wheat *Aegilops* species) originating from all continents except Antarctica (Table 2). Globally, wheat leaf T_{crit} varied by up to 20 °C (35–55 °C) and there were more data for studies under warm conditions for genotypes originating from the lower latitudes than from high latitudes (Fig. 5). The larger variation in T_{crit} for genotypes originating from the higher latitudes coincided with the cooler growth conditions. Overall, there was less variation in T_{crit} under the warm conditions. We found no relationship between mean wheat leaf T_{crit} and the absolute latitude of genotype climate of origin (Fig. 5).

Discussion

In this study, we used a high-throughput technique to record minimal Chl *a* fluorescence and quantified the critical temperature (T_{crit}) of PSII damage—a measure of leaf photosynthetic heat tolerance—for wheat genotypes grown in

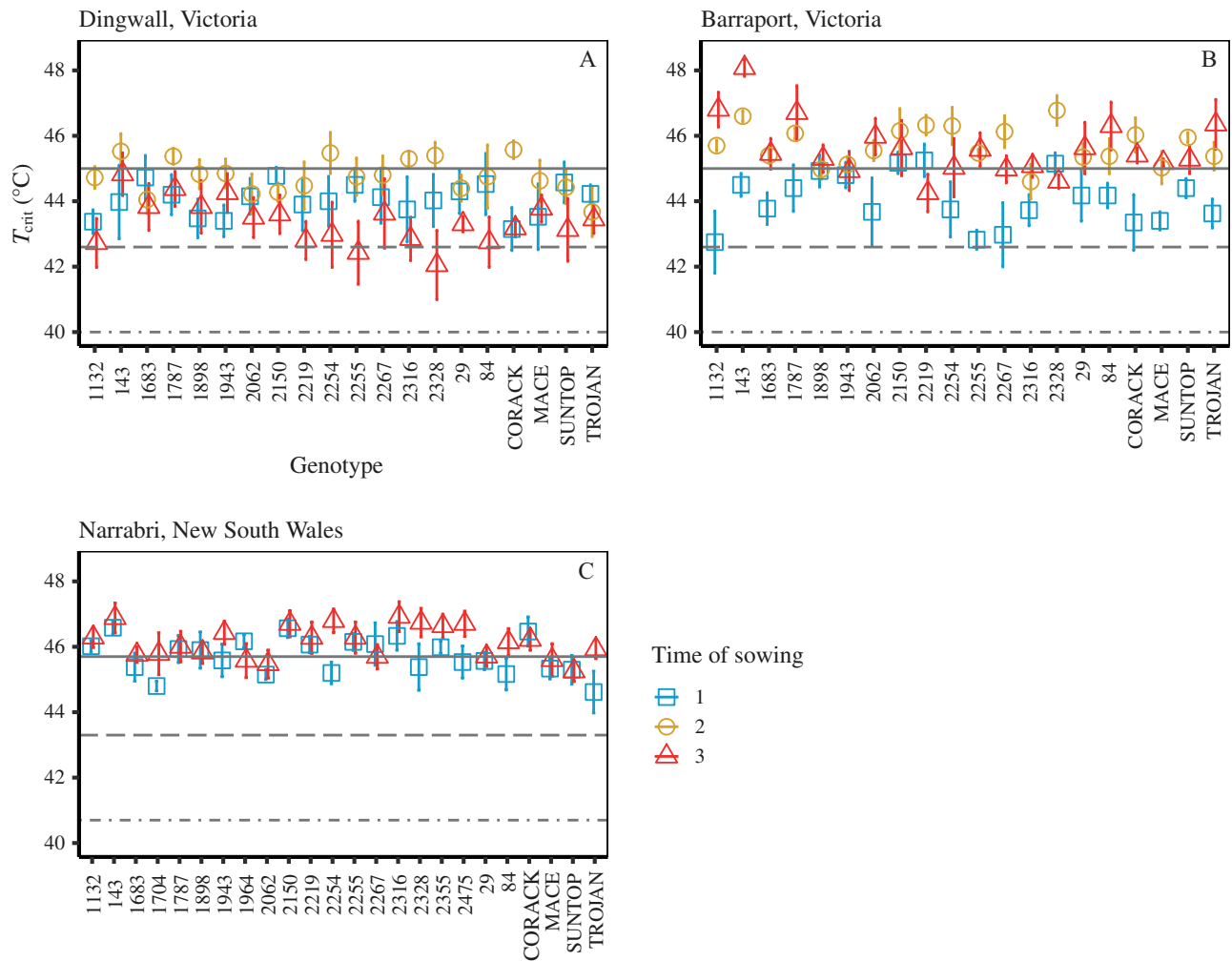


Fig. 2. Phenotypic plasticity of leaf T_{crit} and thermal safety margins of 20–24 wheat genotypes. The genotypes were sown at either the locally recommended time of year (time of sowing 1; blue squares); 1 month after the recommended time (time of sowing 2; yellow circles); or 2 months after the recommended time (time of sowing 3; red triangles) at three Australian field sites. Delayed times of sowing were used to impose warmer average growth temperatures for plants sown at times of sowing 2 and 3. The field sites were Dingwall (A) and Barrarport West (B) Victoria, and Narrabri, New South Wales (C). Twenty genotypes were sown at Dingwall in 2017 and Barrarport West in 2018, and the same 20 plus an additional four genotypes were sown at Narrabri in 2019. The dash-dot lines mark the hottest recorded maximum temperature during the typical anthesis month (October) at each field site (40.7 °C for Narrabri, and 40 °C for Dingwall, data from the Australian Bureau of Meteorology; due to the close proximity of Dingwall and Barrarport West we used the same climate records for these sites), while the dashed line and the solid line mark the RCP 4.5 IPCC and RCP 8.5 IPCC emission scenarios (+2.6 °C and +5 °C), respectively. The difference between the observed T_{crit} and these current and future maximum temperatures is termed the thermal safety margin. Here we assume that leaf temperature is equal to air temperature. Points represent the mean \pm SE, minimum $n=4$.

multiple field experiments, as well as a controlled-environment experiment. The field experiments demonstrated the extent of variation in T_{crit} over the course of a single day, as well as across several crucial stages of phenological development. They also showed that the region of origin of wheat genotypes was unrelated to T_{crit} in three representative Australian wheat-growing regions, and that sowing time (and thus, growth temperature) was responsible for significant variation in T_{crit} . Delayed sowing (i.e. elevated growth temperature) was generally associated with increases in T_{crit} , resulting in higher thermal safety margins at both field sites.

When two genotypes were subjected to a sudden heat shock in a controlled environment, we observed a slight difference between genotypes in the speed with which T_{crit} increased. However, both genotypes exhibited a similar peak T_{crit} value during this heat shock. Finally, when combining these data with previously published wheat T_{crit} data, as well as unpublished data from other experiments conducted in controlled-environment facilities at The Australian National University, we found that the absolute latitude of pedigrees of wheat genotypes were not significantly linked with variation in T_{crit} for either cool- or warm-grown plants.

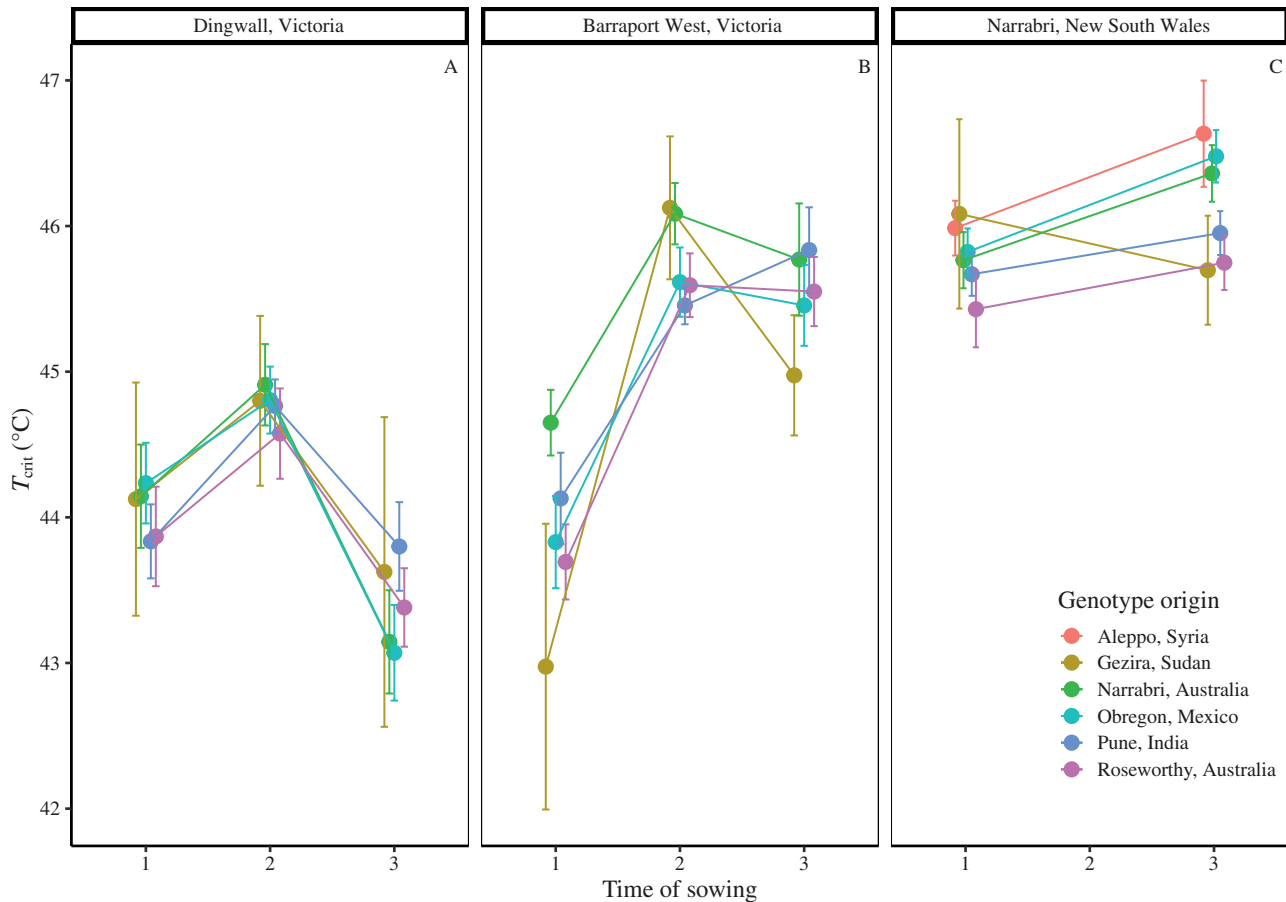


Fig. 3. Phenotypic response of wheat flag leaf PSII heat tolerance (T_{crit}) to time of sowing at three Australian field sites: (A) Dingwall, Victoria; (B) Barraport West, Victoria; and (C) Narrabri, New South Wales. Genotypes are grouped according to the six locations of the breeding programmes where they were developed. Twenty genotypes were grown at Dingwall in 2017 and at Barraport West in 2018, while the same 20 plus an additional four genotypes were grown in Narrabri in 2019. In order to generate increasingly warmer growth temperature regimes, plants were sown at one of three times of sowing: time of sowing 1 (TOS 1) was the locally recommended time of sowing, while TOS 2 and TOS 3 were 1 and 2 months after TOS 1, respectively. Points represent the mean \pm SE, minimum $n=4$.

Temporal fluctuations in wheat T_{crit} may be linked to changes in leaf sugar content

Wheat T_{crit} varied significantly over the course of a single day, declining by an average of 1.7 °C over the 18 h from solar noon to sunrise (Fig. 1A). This pattern resembles the extent of change in T_{crit} in a temperate tree species reported by Hüve *et al.* (2006); specifically, a linear increase over 14 h, from a low point at 05.00 h to a peak at 19.00 h. Taken together, these findings suggest that T_{crit} generally increases to a peak during the late afternoon before declining to a minimum between midnight and dawn. Hüve *et al.* (2006) linked this diel variation in T_{crit} with daily variation in leaf sugar content, and demonstrated that T_{crit} increased when leaves were fed sugar solutions. Further work is needed to determine if the diel variation in T_{crit} that we observed in wheat was also influenced by corresponding variation in leaf sugar content.

It is also interesting to compare the extent of variation in T_{crit} that was observed over the course of a single day with the

extent of variation that was observed across phenology. In the 18 h between solar noon and sunrise, T_{crit} declined by 1.7 °C (Fig. 1A), a fluctuation that was similar in size to the 1.5 °C rise in T_{crit} that we observed from heading to anthesis (Fig. 1B). This comparison highlights the high level of plasticity in T_{crit} , and that variation in T_{crit} is clearly responsive to factors on both an hours-long time scale (i.e. diurnal fluctuations in leaf sugar content) and a longer term weeks-long scale (as evidenced by changes in T_{crit} from heading to anthesis and grain filling). Anthesis is widely considered the stage of phenology at which wheat is most vulnerable to high temperature (Ferris *et al.*, 1998; Thistlethwaite *et al.*, 2020), with this vulnerability largely due to a reduction of sink strength to import and utilize assimilates within the reproductive organs, rather than of assimilate supply from leaf photosynthesis *per se* (Li *et al.*, 2012; Ruan *et al.*, 2012). While this increase could reflect an ongoing rise in heat tolerance coinciding with seasonal warming, there was no significant difference in T_{crit} between plants undergoing anthesis versus those at the grain-filling stage, and so

anthesis may be the phenological stage at which T_{crit} reaches its peak.

Drivers of variation in wheat T_{crit}

The field site at which plants were grown was the most significant source of variation in T_{crit} ; the overall average T_{crit} at

Narrabri was 1.8 °C higher than recorded at Dingwall and 0.8 °C higher than at Barraport West. In addition to environment, genotype had a significant effect on T_{crit} at the Barraport West and Narrabri sites. These results suggest that environment, genotype, and most probably the genotype by environment interaction (GxE) may play large roles in determining wheat flag leaf T_{crit} . Breeding for genotypes with greater photosynthetic heat tolerance (i.e. higher T_{crit}) may be challenging if variation in T_{crit} is also influenced by GxE effects. GxE effects have been reported for other abiotic stress tolerance traits including lodging tolerance in spring wheat (Dreccer *et al.*, 2020), and drought tolerance in maize (Dias *et al.*, 2018).

Genotypes maintain moderate photosynthetic thermal safety margins

We observed variation in the thermal safety margins of wheat genotypes, predominantly associated with differences between field sites and the effect of sowing time at these sites. The thermal safety margin was 2.1 °C when averaged across all genotypes (Fig. 2). Thermal safety margins in three representative Australian wheat-growing regions were at least 2–4 °C for all genotypes. T_{crit} was always several degrees greater than the hottest recorded air temperature during the typical month of anthesis at each site (Narrabri, 40.8 °C; Dingwall/Barraport West 40 °C; denoted by the dot-dash lines in Fig. 2). Under the IPCC’s RCP 4.5 intermediate emission scenario for Eastern Australia by 2090, most genotypes would maintain a positive, yet reduced, thermal safety margin in the studied growing regions. However, under the high emission

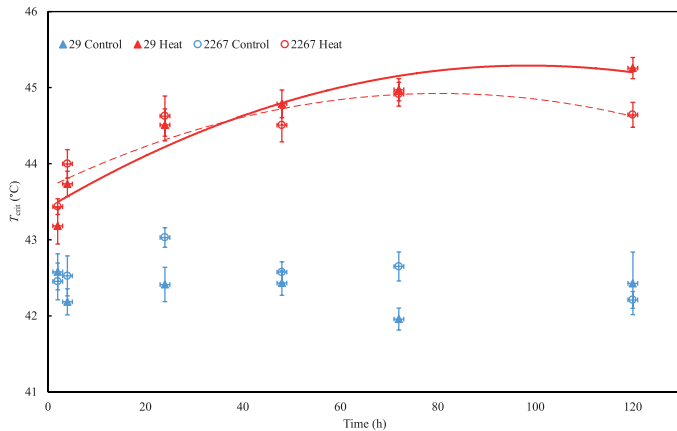


Fig. 4. Leaf T_{crit} (°C) of two wheat genotypes, 29 (triangles and solid lines) and 2267 (circles and dashed lines), exposed to 24 °C (control; blue shapes and lines) or 36 °C (heat; red shapes and lines) for varying durations (2, 4, 24, 48, 72, or 120 h) in a growth cabinet. Leaf samples for T_{crit} were from the third fully extended leaves on the main stem. Equations for the curvilinear relationships between T_{crit} at 36 °C (T_{crit}^{36} ; °C) and time (t ; h) under heat for genotype 29 is $T_{crit}^{36}=43.42 + 0.038t-0.00019t^2$ and for genotype 2267 is $T_{crit}^{36}=43.69 + 0.031t-0.00019t^2$. Points represent the mean \pm SE, $n=4$.

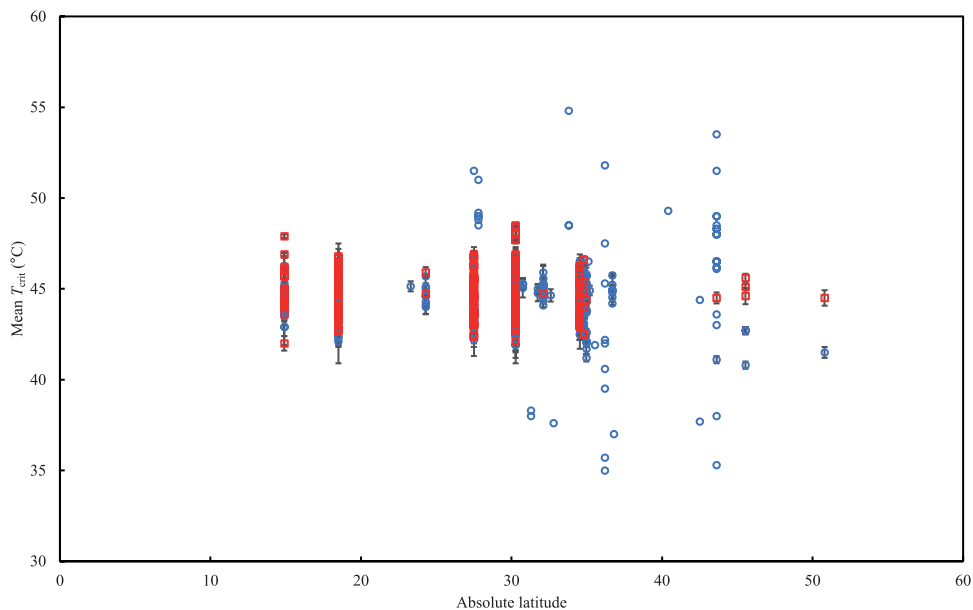


Fig. 5. Relationship between T_{crit} and the absolute latitude of the climate of origin for wheat genotypes when grown under cool (blue circles) and warm (red squares) conditions. For both conditions, $P>0.1$. Data obtained from 183 wheat genotypes (3223 measurements of leaf T_{crit} overall) from experiments in Australia (this study) and the published literature (Havaux *et al.*, 1988; Rekika *et al.*, 1997; Véghe *et al.*, 2018). Data points represent the mean T_{crit} (\pm SE where visible) for each genotype.

RCP 8.5 scenario, the thermal safety margins of most genotypes grown at the Dingwall site and a few genotypes at the Barraport West site would be exceeded (Fig. 2A, B). For genotypes grown at the Narrabri site, thermal safety margins under the RCP 8.5 scenario would be drastically reduced and, in some cases, disappear (Fig. 2C). According to our T_{crit} observations, only genotypes originating from Obregón and Aleppo would retain positive thermal safety margins under the RCP 8.5 scenario when sown at either optimal or delayed sowing times. The rise in T_{crit} with delayed sowing (and thus increased growth temperature) that we observed in the majority of genotypes indicates a widespread capacity for the thermal acclimation of wheat flag leaf T_{crit} . This suggests that thermal safety margins for wheat photosynthetic heat tolerance could yet increase in response to warming under future climate scenarios. However, given that we also observed an apparent limit to the acclimation of T_{crit} following sudden heat shock (Fig. 4), it is possible that daytime maximum temperatures could approach this physiological thermal limit of wheat PSII if the most severe global warming predictions are borne out. A hard limit to the high temperature acclimation of T_{crit} could indicate a physiological limitation of PSII, or a temperature that represents the absolute maximum tolerance. Given that the considerable thermal plasticity of PSII electron transport has been closely linked with improving photosynthetic heat tolerance more generally (Yamasaki *et al.*, 2002), the prospect of air temperatures approaching the physiological threshold of PSII high temperature acclimation is concerning.

Thus far, in assessing thermal safety margins, we have assumed parity between air and leaf temperatures; however, wheat leaf/canopy temperature can differ substantially from air temperature. Balota *et al.* (2007) reported canopy temperatures ranging from 3 °C below noon air temperatures to 10 °C above noon air temperatures in dryland wheat, and from 3 °C below noon air temperatures to 5.7 °C above noon air temperatures in irrigated wheat. Similarly, canopy temperatures of Australian wheat have also been recorded exceeding afternoon air temperature by 0.3–2.3 °C (Rathey *et al.*, 2011) and 3–5 °C (Rebetzke *et al.*, 2013). These examples, along with other previous instances (Rashid *et al.*, 1999; Thapa *et al.*, 2018), highlight the significant genotypic variation in canopy cooling and thus the potential for achieving gains in performance under high temperature by exploiting this variation. While greater levels of canopy cooling could increase thermal safety margins by limiting leaf temperature, achieving gains in wheat T_{crit} could also provide an avenue to maintaining positive thermal safety margins by increasing the threshold to PSII damage. Enhancing thermal safety margins by increasing T_{crit} could be particularly important in water-limited environments considering that heatwaves are frequently accompanied by drought, which increases stomatal closure and limits transpirational cooling, resulting in increased leaf temperature (Aspinwall *et al.*, 2019).

Thermal environment of growth site may be more influential than genotype origin in determining variation in wheat flag leaf T_{crit}

Considering the potential benefits to wheat heat tolerance and performance under high temperature that could arise from achieving increases in T_{crit} , as well as the extent of variation that we observed in T_{crit} among 24 genotypes at three field sites, it would be beneficial to identify characteristics that predict high T_{crit} in wheat genotypes. Thus, we analysed whether the distinct regions from which our genotypes originated could reliably predict variation in T_{crit} . Previous studies of (mostly) woody, non-crop species found that T_{crit} was correlated with climate of origin (O'Sullivan *et al.*, 2017; Zhu *et al.*, 2018). In a similar vein, we found evidence of genotype region of origin significantly affecting T_{crit} at two of our field sites (Fig. 3A, C). One consistency at both of these sites was that genotypes originating from Roseworthy, Australia generally exhibited the lowest or second lowest mean T_{crit} values. In contrast, the genotype from Aleppo exhibited the highest T_{crit} at the Narrabri site (Fig. 3C), while at the Barraport West site the genotypes originating from Narrabri had the highest mean T_{crit} across all sowing times (Fig. 3A). However, it seems unlikely that the effect of genotype region of origin is the result of differences in temperature at these locations: for instance, the average daily maximum April temperature in Aleppo, Syria is 23 °C (NOAA), while the average daily October maximum in Roseworthy, Australia is 23.8 °C (Australian Bureau of Meteorology). Therefore, the differences associated with genotype origin in the current study are likely to be related to a more complex combination of environmental differences between locations (e.g. rainfall, temperature, soil quality, and agricultural practices). Differences in the aims and methods of breeding programmes at various locations could also explain variation in T_{crit} associated with genotype origin. We also note that our experiments did not include genotypes that were developed in cooler environments, such as wheat-growing regions in Europe or Northern America, and so further work may be required to capture the full extent of global variation in wheat T_{crit} .

T_{crit} increases within hours of heat shock, and peaks after 3–4 d

We observed widespread evidence of wheat T_{crit} plasticity following exposure to high temperature, including elevated growth temperature in the field (via delayed sowing, Figs 2, 3) and sudden heat shock under controlled conditions (Fig. 4). We also saw clear genotypic variation in the plasticity of T_{crit} across these experiments. In some genotypes, T_{crit} rose by upwards of 4 °C when sowing time was delayed by 2 months (Fig. 2B), while in others T_{crit} showed no change or even declined by up to 1.2 °C from TOS 1 to TOS 3 (Fig. 2A). Similarly, following a heat shock imposed under controlled conditions,

we observed a difference between two genotypes in the speed at which T_{crit} increased despite the two genotypes eventually reaching a similar peak T_{crit} (Fig. 4). Genotypic variation is thus evident not only in wheat flag leaf T_{crit} under common non-stressful temperatures, but also in the extent of T_{crit} plasticity in response to sudden heat shock. Increases in T_{crit} with warming have been reported previously (O'Sullivan *et al.*, 2017; Zhu *et al.*, 2018), and these are considered examples of high temperature acclimation. That we observed similar patterns in wheat T_{crit} , as well as genotypic variation in this acclimation, suggests that the capacity to increase PSII heat tolerance could be a trait worth targeting for the development of wheat genotypes with greater heat tolerance. However, further work is needed to first investigate whether such acclimation is associated with enhanced performance under high temperature in the field.

One aspect of the current study that may aid such future efforts is the development of high-throughput minimal Chl *a* fluorescence assays that can be used for large-scale screening of wheat PSII heat tolerance. When combined with other burgeoning high-throughput techniques for measuring photosynthetic characteristics (Sharma *et al.*, 2012; Silva-Pérez *et al.*, 2018; Fu *et al.*, 2019; McAusland *et al.*, 2019; Arnold *et al.*, 2021), it is becoming increasingly achievable to efficiently measure a range of traits that provide insight into the photosynthetic thermal tolerance of entire plots in crop breeding trials.

Genotypic variation in wheat leaf T_{crit} is not consistent with latitudinal trends in general plant heat tolerance

In contrast to previous results that observed a decrease in PSII heat tolerance (measured as T_{crit}) as latitude moved further from the equator (O'Sullivan *et al.*, 2017; Lancaster and Humphreys, 2020), we found no relationship between wheat leaf T_{crit} and the latitude of genotype climate of origin, irrespective of thermal acclimation (Fig. 5). This discrepancy could be related to differences between cultivated and wild species: the studies of O'Sullivan *et al.* (2017) and Lancaster and Humphreys (2020) demonstrated a relationship between heat tolerance and latitude based almost entirely on records of different wild species. In contrast, our study focuses solely on one domesticated species. Wheat is known as a crop with a particularly narrow genetic background (Tanksley and McCouch, 1997), but we observed a large range of T_{crit} in wheat here (up to 20 °C) which compares with the ~30 °C global range reported across 218 plant species spanning seven biomes reported by O'Sullivan *et al.* (2017). This large range of wheat leaf T_{crit} can be exploited to improve heat tolerance in modern crop varieties, as has been done recently in successful efforts to improve wheat drought tolerance (Reynolds *et al.*, 2015). Still, wheat is cultivated in a wide range of ecological and climatic conditions, covering >220 Mha, including areas where it is exposed to high temperature stress. As such, we predicted that the rise in T_{crit} that we observed with elevated growth

temperature in our experimental dataset (Figs 1–4) would also be apparent in the meta-analysis. However, there was no evidence of any thermal acclimation response of T_{crit} in this larger dataset. This could partly be due to the diversity of experimental methods used to generate the data in Fig. 5, as well as variation in the duration and intensity of elevated growth temperature treatments. Given that the plant thermal tolerance field uses a large and diverse range of experimental designs and assays (Geange *et al.*, 2021), the results of our systematic review of wheat T_{crit} could be further evidence of a need to better standardize the approaches used to measure and describe photosynthetic heat tolerance.

Conclusion

Wheat leaf T_{crit} varied dynamically with changes in growth conditions, notably increasing in response to short- and long-term high temperatures, and exhibiting an upper ceiling in acclimating to heatwaves. There was also evidence of developmental, diel, and genotypic variation in T_{crit} , as well as a strong genotype-by-environment interaction. Interestingly, global wheat leaf T_{crit} which spanned up to 20 °C was unrelated to genotype climate of origin and latitude, unlike reported associations with global interspecies variation in leaf T_{crit} of 171 plant species (~30 °C). However, the observed genotypic variation and plasticity of wheat T_{crit} , combined with the recent development of a high-throughput technique for measuring T_{crit} (Arnold *et al.* 2021), indicate that this trait would be useful for high-throughput screening, understanding photosynthetic heat tolerance, and the development of heat-tolerant wheat.

Supplementary data

The following supplementary data are available at [JXB online](#).

Table S1. Wheat pedigree information for genotypes grown in the three field experiments and one controlled-environment experiment described in this study.

Table S2. ANOVA of factors influencing wheat T_{crit} at two Australian field sites.

Table S3. ANOVA of effect of time of sowing and genotype on wheat T_{crit} at three Australian field sites.

Table S4. ANOVA of effect of time of sowing and genotype origin on wheat T_{crit} at three Australian field sites.

Table S5. Mean wheat leaf T_{crit} from nine studies conducted under controlled-environment and field conditions in Australia.

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Author contributions

OKA, HB, and Y-LR: funding acquisition; RT: development of the seed materials; BCP, JH, OC, HB, and OKA: designing the experiments; BCP, JH, and OC: data collection; BCP, JH, and OC: data analysis; BCP and OC: writing with contributions from all authors.

Conflict of interest

The authors declare no conflict of interest.

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Data availability

Data supporting the findings of this study are available from the corresponding author upon request.

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