

1 **Title:** Wheat photosystem II heat tolerance responds dynamically to short and long-term  
2 warming

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27 **Running title:** Wheat photosystem II heat tolerance

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## 45 Abstract

46

47 Heat-induced inhibition of photosynthesis is a key factor in declining wheat performance and  
48 yield. Variation in wheat heat tolerance can be characterised using the critical temperature  
49 ( $T_{crit}$ ) above which incipient damage to the photosynthetic machinery occurs. We investigated  
50 intraspecies variation and plasticity of wheat  $T_{crit}$  under elevated temperature in field and  
51 controlled environment experiments. We also assessed whether intraspecies variation in  
52 wheat  $T_{crit}$  mirrors patterns of global interspecies variation in heat tolerance reported for  
53 mostly wild, woody plants. In the field, wheat  $T_{crit}$  varied through the course of a day,  
54 peaking at noon and lowest at sunrise, and increased as plants developed from heading to  
55 anthesis and grain filling. Under controlled temperature conditions, heat stress (36°C) was  
56 associated with a rapid rise in wheat  $T_{crit}$  (i.e. within two hours of heat stress) that peaked  
57 after 3–4 days. These peaks in  $T_{crit}$  indicate a physiological limitation to photosystem II heat  
58 tolerance. Analysis of a global dataset (comprising 183 *Triticum* and wild wheat (*Aegilops*)  
59 species) generated from the current study and a systematic literature review showed that  
60 wheat leaf  $T_{crit}$  varied by up to 20°C (about two-thirds of reported global plant interspecies  
61 variation). However, unlike global patterns of interspecies  $T_{crit}$  variation which has been  
62 linked to latitude of genotype origin, intraspecific variation in wheat  $T_{crit}$  was unrelated to  
63 that. Yet, the observed genotypic variation and plasticity of wheat  $T_{crit}$  suggests that this trait  
64 could be a useful tool for high-throughput phenotyping of wheat photosynthetic heat  
65 tolerance.

66

## 67 Introduction

68

69 As the climate changes, global mean land-surface temperature has continued to rise,  
70 alongside more frequent, longer, and more intense heatwaves (Perkins-Kirkpatrick and  
71 Lewis, 2020). This is particularly concerning for the prospect of improving crop yields, as  
72 heat stress is associated with significant declines in the yield of widely-cultivated crops,  
73 including wheat (Asseng *et al.*, 2015; Tack *et al.*, 2015; Hochman *et al.*, 2017; Liu *et al.*,  
74 2019; Ortiz-Bobea *et al.*, 2019). Photosynthesis is a primary determinant of wheat yield and it  
75 is particularly sensitive to heat stress (Berry and Bjorkman, 1980; Way and Yamori, 2014).  
76 Improving the heat tolerance of photosynthesis could future-proof wheat yield in a warming  
77 world (Cossani and Reynolds, 2012; Scafaro and Atkin, 2016; Iqbal *et al.*, 2017). To realise  
78 improvements in wheat photosynthetic heat tolerance, it is paramount that we first understand  
79 and quantify patterns of wheat photosynthetic heat tolerance so that we can then successfully  
80 exploit them.

81 Decreased leaf photosynthetic rate under high temperature is partially linked to  
82 disruption of the chloroplast electron transport chain, of which the thylakoid membrane-  
83 embedded photosystem II (PSII) considered the most sensitive component (Sharkey, 2005;  
84 Brestic *et al.*, 2012). Heat-induced reactive oxygen species and lipid peroxidation both cause  
85 cleavage of the reaction centre-binding D1 protein of PSII (Yamashita *et al.*, 2008), inhibiting  
86 electron flow and thus the production of ATP. For decades PSII damage has been measured  
87 with chlorophyll *a* fluorescence metrics, including  $T_{crit}$  of  $F_0$  (Schreiber *et al.*, 1975; Schreiber  
88 and Berry, 1977; Hüve *et al.*, 2011; Geange *et al.*, 2021).  $T_{crit}$  of  $F_0$  (henceforth  $T_{crit}$ ) is the  
89 critical temperature above which minimal chlorophyll *a* fluorescence ( $F_0$ ) rises rapidly,  
90 indicating incipient damage to PSII (Schreiber and Berry, 1977; Melcarek and Brown, 1979;  
91 Neuner and Pramsohler, 2006; Slot *et al.*, 2019).  $T_{crit}$  is associated with increased thylakoid  
92 membrane fluidity, disruption of the light-harvesting antennae (Raison *et al.*, 1982; Figueroa  
93 *et al.*, 2003), dissociation of chloroplast membrane-bound proteins (Berry and Bjorkman,  
94 1980), and loss of chloroplast thermostability (Armond *et al.*, 1978). As a standardized

95 metric,  $T_{crit}$  has been used to examine global patterns of heat tolerance, quantify phenotypic  
96 plasticity in response to warming, and assess vulnerability to climate change across plant  
97 species (O'Sullivan *et al.*, 2017; Zhu *et al.*, 2018; Lancaster and Humphreys, 2020; Geange *et al.*,  
98 *et al.*, 2021). While the number of publications examining plant  $T_{crit}$  is growing (Ferguson *et al.*,  
99 2020; Arnold *et al.*, 2021; Slot *et al.*, 2021), most studies focused on woody, non-crop  
100 species, and characterisation of intraspecific variation in  $T_{crit}$  of crop species has been limited  
101 (see Ferguson *et al.*, 2020 for a recent exception). Wheat, as the most widely-cultivated crop  
102 (with over 220 million ha cultivated worldwide) with a diverse range of genotypes  
103 originating from across the globe, is an ideal crop species for examining intraspecific  
104 variation and acclimation of  $T_{crit}$ . In addition, although wheat is a temperate crop, there is  
105 increasing evidence of warming in many wheat-producing regions, including China, the USA  
106 and Australia, resulting in either stalled or reduced wheat yield (Hochman *et al.*, 2017; Zhao  
107 *et al.*, 2017). Understanding the response of  $T_{crit}$  to warming and the magnitude of  
108 intraspecific variation in  $T_{crit}$  could thus provide opportunities for improving photosynthetic  
109 heat tolerance and yield resilience in wheat and other crops.

110 Quantification of intraspecific variation in physiological traits of crops commonly  
111 encounters bottlenecks at the phenotyping stage. However, high-throughput phenotyping  
112 techniques are being developed, including a robotic system offering a ten-fold increase in the  
113 measurement speed of dark respiration (Scafaro *et al.*, 2017; Coast *et al.*, 2019, 2021), and  
114 the proximal remote sensing of leaf hyperspectral reflectance signatures for rapidly assaying  
115 photosynthetic characteristics and dark respiration (Silva-Pérez *et al.*, 2018; Coast *et al.*,  
116 2019; Fu *et al.*, 2019). Though chlorophyll fluorescence techniques are well-established for  
117 assessing photosynthetic heat tolerance, they are typically cumbersome. This limits their  
118 incorporation in breeding programmes that screen hundreds of genotypes for heat tolerance.  
119 But recently, Arnold *et al.* (2021) described a high-throughput chlorophyll fluorescence  
120 screening technique for a diversity of wild species. Previous studies of photosynthetic  
121 thermal tolerance also largely assumed that  $T_{crit}$  is diurnally and phenologically constant.  
122 However, these assumptions may be flawed. Substantial changes in metabolic capacity and  
123 demand for photosynthetic products vary diurnally, with phenological development and in  
124 response to fluctuations in temperature (Steer, 1973; Rashid *et al.*, 2020). Thus, it seems  
125 reasonable that  $T_{crit}$  may demonstrate similar variation. However, these assumptions remain  
126 untested.

127 The extent to which plants physiologically adjust to warming is important in  
128 determining productivity and survival (Scheiner, 1993; Leung *et al.*, 2020). Acclimation of  
129 photosynthetic electron transport to elevated temperature is evidenced by an increase in  $T_{crit}$ .  
130 Zhu *et al.* (2018) reported acclimation at a rate of 0.34°C increase in  $T_{crit}$  for every 1°C  
131 increase in average temperature over the growing season for a range of native Australian  
132 species. Acclimation of  $T_{crit}$  may also increase plant thermal safety margins, thus protecting  
133 against damage to PSII under future heat stress. Thermal safety margins are estimated as the  
134 difference between the upper limit of leaf function (e.g.  $T_{crit}$ ) and the maximum growth  
135 temperature experienced in an environment (Sastry and Barua, 2017), and they provide a  
136 useful representation of a species' potential vulnerability to global warming (Sunday *et al.*,  
137 2014). A reduction in this margin indicates increasing vulnerability to heat stress, while an  
138 increase in this margin indicates better capacity to tolerate the effects of climate warming  
139 (Hoffmann *et al.*, 2013). Thermal safety margins of 10–15°C have been reported for many  
140 plant species (Weng and Lai, 2005; O'Sullivan *et al.*, 2017; Perez and Feeley, 2020), with  
141 some as high as 12–31°C (Leon-Garcia and Lasso, 2019) when leaf temperature, rather than  
142 air temperature, was used. However, many plant species have low thermal safety margins  
143 (e.g.  $\leq 5^\circ\text{C}$ ; Sastry and Barua, 2017). Unfortunately, reports quantifying the acclimation  
144 capacity and thermal safety margins of food crops are scarce. Reports on acclimation of  $T_{crit}$

145 to warming have been in response to a sustained increase in long-term growth temperature.  
146 Similar descriptions of  $T_{crit}$  acclimation to short-term heat stress (e.g. heatwaves) are not well  
147 documented. Considering heatwaves are predicted to become more frequent and intense  
148 (Perkins-Kirkpatrick and Lewis, 2020), it is pertinent that we understand if and how  $T_{crit}$   
149 responds to heatwaves. Whether acclimation of  $T_{crit}$  to heatwaves has an upper threshold (i.e.  
150 a ceiling temperature) is currently unknown.

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

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

172

## 173 **Materials and Methods**

174

175 *Field experiments: Assessing diel and phenological variation in wheat  $T_{crit}$  and estimating*  
176 *thermal safety margins of Australian wheat*

177

178 *Germplasm:* A set of 20–24 wheat genotypes (Table S1) were used in three field experiments  
179 conducted in Australia across three years. Twenty of these genotypes were used in Coast *et*  
180 *al.* (2021) to assess acclimation of wheat photosynthesis and respiration to warming in two of  
181 the fields. The genotypes included: commercial Australian cultivars; heat tolerant materials  
182 developed by the centres of the Consultative Group on International Agricultural Research  
183 (CGIAR) in Mexico and Morocco and tested in warm areas globally; materials derived from  
184 targeted crosses between adapted hexaploid cultivars and heat tolerant Mexican hexaploid  
185 landraces, tetraploid emmer wheat (*T. dicoccon* Schrank ex Schübl.), Indian cultivars and  
186 synthetic wheat derived by crossing *Aegilops tauchii* with modern tetraploid durum wheat.  
187 All genotypes evaluated were hexaploid and chosen for their contrasting heat tolerance under  
188 high temperature conditions in Sudan (Gezira; 14.9°N 33°E), Australia (Narrabri, NSW;  
189 30.27°S, 149.81°E) and Mexico (Ciudad Obregón; 27.5°N, 109.90°W).

190 *Experimental design and husbandry:* The first two years of field experiments were  
191 undertaken in regional Victoria (2017, Dingwall; and 2018, Barraport West), and the third  
192 was in regional New South Wales (2019, Narrabri). A detailed description of the  
193 experimental designs for the 2017 and 2018 experiments are reported in Coast *et al.* (2021).  
194 Briefly, a diverse panel of genotypes were sown on three dates each in 2017 (20 genotypes)



195 and 2018 (24 genotypes) to expose crops to different growth temperatures at a common  
196 developmental stage. The first time of sowing (TOS) for both experiments were within the  
197 locally recommended periods for sowing (early May). Subsequent sowing times were one  
198 month apart in June and July. Experiments were sown in three adjacent strips, one for each  
199 TOS. Each strip consisted of four replicate blocks. The 2019 field experiment was similar in  
200 all aspects to the 2018 field experiment, except for the following: (i) only two times of  
201 sowing were incorporated in the design; and (ii) the sowing times were approximately two  
202 months apart (17 May 2019 and 15 July 2019). Of the 24 genotypes sown in 2018 only 20,  
203 which were common to the 2017 and 2019 experiments, were assessed for  $T_{crit}$ . All three field  
204 experiments were managed following standard agronomic practices for the region by the  
205 Birchip Cropping Group ([www.bcg.org.au](http://www.bcg.org.au)) in regional Victoria, and staff of the IA Watson  
206 Grains Research Centre at The University of Sydney and AGT, in Narrabri. A summary of  
207 the field experiments is presented in Table 1.

208 *Diel measurements of wheat  $T_{crit}$ :* Six of the 20 genotypes in the 2017 field  
209 experiment at Dingwall, Victoria were used to investigate diel variation. The six genotypes  
210 were two commercial cultivars (Mace and Suntop) and four breeding lines (with reference  
211 numbers 143, 2254, 2267, and 2316). These were chosen because they are representative of  
212 the diversity of the set of 20 genotypes (Coast *et al.*, 2021). To determine if  $T_{crit}$  varied  
213 diurnally, flag leaves were harvested at anthesis (Zadok GS60-69; Zadoks *et al.*, 1974) from  
214 plants of TOS3 at four consecutive time points occurring every six hours over an 18-hour  
215 period (solar noon, sunset, midnight, and sunrise).

216 *Phenological measurements of wheat  $T_{crit}$ :* A subset of four genotypes from the 20 in  
217 the 2018 field experiment at Barraport West, Victoria was used to assess phenological  
218 variation in  $T_{crit}$ . The four genotypes were the breeding lines 2062, 2150, 2254, and 2267, the  
219 latter two of which were also used for diel measurements as described in the previous  
220 paragraph. Plants at heading (Zadok GS50–59), anthesis (Zadok GS60–69), and grain filling  
221 (Zadok GS70–79) were respectively chosen from fields of the three times of sowing. Flag  
222 leaves were harvested from the tallest tillers of plants at the different phenological stages at  
223 10 am on the same day and used to determine  $T_{crit}$ .

224 *Estimation of thermal safety margin of Australian wheat:* All 20 genotypes in the  
225 2017 and 2018 field experiments in Dingwall and Barraport West respectively, as well as all  
226 24 genotypes in the 2019 field experiment in Narrabri were used to estimate thermal safety  
227 margins. Thermal safety margins were estimated as the difference between individual  
228 genotype  $T_{crit}$  and the maximum recorded air temperature at either Dingwall or Narrabri in  
229 October. Similar definitions of thermal safety margins as the difference between the  
230 measured temperature at which a species experiences irreversible physiological damage and  
231 the maximum measured temperature of the species' habitat have been used in studies of  
232 animal ectotherms and plants (Deutsch *et al.*, 2008; Sunday *et al.*, 2014; O'Sullivan *et al.*,  
233 2017; Sastry and Barua, 2017). We considered Barraport West and Dingwall together for  
234 their historical weather records as they are in close proximity to one another in the Mallee  
235 district of Victoria, Australia. Weather data for Dingwall and Barraport West were obtained  
236 from the Australian Bureau of Meteorology covering the period 1910–2020. We used 40°C  
237 and 40.8°C (the maximum recorded air temperature for October) in Dingwall/Barraport West  
238 and Narrabri, respectively, to quantify thermal safety margins under current climatic  
239 scenarios. October was chosen as the upper threshold of exposure of field plants at anthesis to  
240 heat because all the later sown plants in this study were at anthesis in October. For future  
241 climatic conditions we added 2.6°C and 5°C to the current maximum temperature, with the  
242 2.6°C addition representing the top end of the intermediate emission Representative  
243 Concentration Pathway (RCP) 4.5 IPCC scenario predicted for Eastern Australia by 2090  
244 (1.3–2.6°C), and the 5°C addition similarly representing the top end of the high emission

245 RCP 8.5 IPCC scenario predicted for Eastern Australia by 2090 (2.8–5°C; Climate Change  
246 Australia, 2021). Across all times of sowings in the three field experiments, flag leaves were  
247 harvested at anthesis (Zadok GS60-69) at a standardised time of 9–10 am to determine  $T_{crit}$   
248 and estimate thermal safety margins.

249

250 *Controlled environment experiment: speed of acclimation and upper limit of leaf  $T_{crit}$  during*  
251 *heat shock*

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

258 *Plant husbandry:* Seeds were germinated on saturated paper towel in covered plastic  
259 containers under darkness for one week. Germinated seedlings were planted in 1.05 L pots  
260 (130 mm diameter) filled with potting mix (80% composted bark, 10% sharp sand, 10% coir)  
261 with 4g L<sup>-1</sup> fertiliser (Osmocote Exact Mini fertiliser, ICL, Tel Aviv, Israel) mixed through.

262 *Temperature treatment:* Potted plants were grown in glasshouses in which a 24/18°C  
263 day/night temperature regime with a 12-hour photo-thermal period was maintained until  
264 tillering. At tillering, when all plants had a fully-extended third leaf (Zadok growth scale 22–  
265 29; Zadoks *et al.*, 1974), plants were moved into growth cabinets (TPG-2400-TH,  
266 Thermoline Scientific, Wetherill Park, NSW, Australia) for temperature treatment. One of  
267 two temperature conditions were imposed: a day/night regime of 24/18°C, or a heat shock  
268 with day/night temperatures of 36/24°C. White fluorescent tubes provided a 12 h photoperiod  
269 of photosynthetically active radiation of 720–750  $\mu\text{mol m}^{-2} \text{s}^{-1}$  at plant height. Leaf discs  
270 were sampled from fully extended third leaves from main tillers and used to determine  $T_{crit}$   
271 after 2, 4, 24, 48, 72, and 120 hours in the growth cabinets. Four replicate plants were used  
272 for  $T_{crit}$  measurement at each sampling time and for each temperature condition. Plant  
273 husbandry followed standard practice at the ANU Controlled Environment Facilities.

274

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

277

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

291

292 *Determination of leaf  $T_{crit}$*

293

294 Leaves were harvested and stored in plastic bags alongside a saturated paper towel and were  
295 left to dark adapt for a minimum of 20 minutes. Water (90  $\mu$ l) was placed in each well of a  
296 48-well Peltier heating block in order to ensure leaf samples remained hydrated throughout  
297 the assay. A single 6 mm diameter leaf disc was excised from the middle of each harvested  
298 dark-adapted leaf and placed within each well of the heating block. Once discs were all  
299 loaded into the heating block, a glass plate was used to enclose the wells to prevent leaf  
300 pieces from drying out during the assay. The block was then placed directly beneath the lens  
301 of an imaging fluorometer (*FluorCam 800MF*, Photon Systems Instruments, Brno, Czech  
302 Republic) and programmed to heat from 20 to 65°C at a rate of 1°C min<sup>-1</sup>. The fluorometer  
303 recorded  $F_0$  throughout the heating period (approximately one record per minute). Following  
304 the conclusion of the temperature ramp, fluorescence data was processed and used to estimate  
305  $T_{crit}$ , which was calculated by fitting linear regressions to both the flat and the steep parts of  
306 the  $F_0$  curves and then recording the temperature at which these two lines intersected.

307

### 308 *Statistical analysis*

309

310 Statistical analyses were carried out within the R statistical environment (v. 3.4.4; R Core  
311 Team, 2018) with R Studio. For analysis of the field data on diurnal and phenological  
312 variation in  $T_{crit}$  we employed linear mixed models in R using the packages lmerTest  
313 (Kuznetsova *et al.*, 2017) and emmeans (Lenth, 2020). In the models we treated genotype and  
314 time of day (for the diel variation) or developmental stage (for the phenological variation) as  
315 fixed terms and replicate as a random term. The ceiling threshold of  $T_{crit}$  under heat stress (of  
316 36°C), in the controlled environment experiment, was determined by fitting a non-linear  
317 regression to the  $T_{crit}$  by time relationship. Then using the coefficients of the fitted  
318 regressions, we estimated the time at which the fitted  $T_{crit}$  was highest and this was taken as  
319 the time to peak acclimation. To test the relationships between  $T_{crit}$  and growth environment  
320 temperature, we only used data from the three field experiments in Australia, for which we  
321 had reliable data. The 24 genotypes studied under field conditions in Australia were grouped  
322 based on the region of origin of their pedigree (Aleppo, Syria; Gezira, Sudan; Narrabri,  
323 Australia; Obregón, Mexico; Pune, India; and Roseworthy, Australia) and the relationship  
324 examined using linear or bivariate regressions. Our global dataset (see Table 2 for sources)  
325 was used to ascertain the link between wheat leaf  $T_{crit}$  and climate of origin by regressing  
326 mean genotype  $T_{crit}$  with genotype latitude (as a proxy for climate) of origin.

327

## 328 **Results**

329

### 330 *Diel and phenological variation in $T_{crit}$*

331 There was significant genotype by time of day interaction for  $T_{crit}$  ( $P=0.042$ ; Table S2),  
332 highlighting the heterogeneity in this diel variation of  $T_{crit}$  among our genotypes. In all but  
333 genotype 2316,  $T_{crit}$  tended to be highest at solar noon before then declining through sunset,  
334 midnight, and sunrise. The slope of these trends was only significant for genotype 2267, with  
335  $T_{crit}$  declining by 3.1°C from solar noon to sunrise. By contrast, genotypes 143 exhibited the  
336 narrowest diel range in  $T_{crit}$ , with difference of 1.1°C between solar noon and sunrise.  
337 Irrespective of genotype,  $T_{crit}$  at solar noon was significantly higher than at sunrise ( $P<0.001$   
338 for time of day).  $T_{crit}$  also showed significant genotype by phenology interaction, and highly  
339 significant differences for the main effects of genotype as well as phenology (Table S2). The  
340 interaction effect was largely due to the increasing trend in  $T_{crit}$  as plants developed from  
341 heading to anthesis and grain filling for genotypes 2267, 2254 and 2062 but not for 2150  
342 (Fig. 1B).  $T_{crit}$  of genotype 2150 rose slightly between heading and anthesis then declined

343 significantly at grain filling relative to anthesis. Genotype 2254 showed the largest increase in  
344  $T_{crit}$  between heading and anthesis, rising 1.8°C from 44.4°C to 46.2°C.

345

#### 346 *Thermal safety margins of Australian wheat*

347 At Dingwall, only the main effect of TOS was significant (Table S3). In comparison to TOS  
348 1,  $T_{crit}$  of TOS 2 tended to be higher and TOS 3 tended to be lower (Fig. 2A). At Barraport,  
349  $T_{crit}$  varied amongst the genotypes over a range of about 2°C and the effect of TOS on  $T_{crit}$   
350 depended on the genotype ( $P < 0.01$  for Genotype by TOS interaction).  $T_{crit}$  increased more in  
351 some genotypes (e.g. 1132, 143 and Trojan) under later sowing than others (e.g. 2267 and  
352 29), but also did not change significantly in some (e.g. 1898 and 1943; Fig. 2B). At Narrabri,  
353 only the main effects were significant, i.e. genotypes varied in their  $T_{crit}$  and TOS 3  $T_{crit}$  was  
354 higher than TOS 1 (Table S3, Fig. 2C). Across TOS, the genotypes with the lowest and  
355 highest mean  $T_{crit}$  were 1704 (45.3°C) and 143 (46.7°C) respectively. Across the 24  
356 genotypes,  $T_{crit}$  increased by 0.5°C from TOS 1 (at 45.7°C) to TOS 3 (at 46.2°C). An analysis  
357 of variance run on a linear mixed effects model of the entire field data set revealed field site  
358 to be the largest source of variation in  $T_{crit}$  of all our independent variables (d.f. = 2,  $F$  value =  
359 190.9,  $P < 0.001$ ). The overall mean  $T_{crit}$  at each of the field sites was 45.1°C at Barraport  
360 West, 44.1°C at Dingwall, and 45.9°C at Narrabri.

361 Thermal safety margins were calculated for all field-grown genotypes by quantifying  
362 the difference between  $T_{crit}$  and the maximum air temperature recorded during October. All  
363 genotypes demonstrated a higher  $T_{crit}$  than the historical maximum October air temperatures  
364 recorded at each field site (Fig. 2, dash-dot blue line). Thermal safety margins in the TOS 1  
365 fields ranged from 3.2–4.8°C in Dingwall (Fig. 2A), to 2.8–5.3°C in Barraport West (Fig. 2B)  
366 and from 3.8–5.8°C in Narrabri (Fig. 2C). For the later grown crops (i.e. TOS 2 and 3) which  
367 experienced warmer growth temperatures, thermal safety margins increased relative to TOS 1  
368 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  
369 for TOS 3) and in Narrabri (4.5–6.1°C). The exception to this pattern was TOS 3 at Dingwall  
370 where the lower end of the thermal safety margin range declined, resulting in a range of 2.1–  
371 4.8°C. At both Narrabri and Barraport West, mean  $T_{crit}$  of all genotypes was above the +2.6°C  
372 mark associated with the RCP 4.5 intermediate emission scenario (Fig. 2B & 2C, dashed  
373 yellow line). Most genotypes were also largely clear of the RCP 4.5 mark at Dingwall, except  
374 the  $T_{crit}$  of genotypes 2255 and 2328 sown at TOS 3 were below this threshold. The +5°C  
375 warming mark associated with the high emission RCP 8.5 scenario was equal to or above the  
376  $T_{crit}$  of many genotypes at all three field sites, though there was some variation across the  
377 locations. At Narrabri, half of the genotypes were below the RCP 8.5 threshold when sown at  
378 TOS 1, while this fell to a quarter of genotypes when sown at TOS 3 (Fig. 2C). At TOS 1 in  
379 Barraport West, 17 genotypes fell below the RCP 8.5 threshold, with only one and three  
380 genotypes below this mark for TOS 2 and 3, respectively (Fig. 2B). At Dingwall,  $T_{crit}$  of all  
381 genotypes sown at TOS 1 and TOS 3 was below the RCP 8.5 threshold, while 14 genotypes  
382 at TOS 2 were below this mark (Fig. 2A).

383

#### 384 *Genotype origin does not predict variation or acclimation in $T_{crit}$*

385 The 24 genotypes grown across the three field sites were grouped by the regions from which  
386 they originated (Table S1; Aleppo, Syria; Gezira, Sudan; Narrabri, Australia; Obregón,  
387 Mexico; Pune, India; and Roseworthy, Australia) in order to determine if this explained any  
388 of the observed variation in  $T_{crit}$ . Genotype origin had a significant effect on  $T_{crit}$  at both  
389 Barraport West and Narrabri (Table S4) At Barraport West, genotypes that originated in  
390 Narrabri had the highest  $T_{crit}$  and Sudan the lowest (Fig. 3B). At Narrabri, the genotype that  
391 originated from Syria had the highest  $T_{crit}$  whereas those from Roseworthy had the lowest. By  
392 contrast, genotype origin had no significant effect on  $T_{crit}$  at Dingwall. TOS had a significant



393 effect on  $T_{crit}$  at all three sites irrespective of origin.  $T_{crit}$  was lower than TOS 3 relative to  
394 TOS 1/TOS 2 in Dingwall, and was higher than TOS 1 in TOS 2/TOS 3 in Barraport West  
395 (Fig. 3). At the Narrabri site,  $T_{crit}$  increased from TOS 1 to TOS 3 for all origin groupings  
396 (Fig. 3C). No interaction between time of sowing and genotype origin was observed at any  
397 field site (Table S4).

398

399 *Response of  $T_{crit}$  to short-term exposure to high temperature and upper limit of  $T_{crit}$  plasticity*  
400 In the two genotypes studied,  $T_{crit}$  increased significantly following two hours of heat shock  
401 (Fig. 4). In both genotypes,  $T_{crit}$  increased during the heat shock following a curvilinear  
402 pattern which peaked after 3.4 days for genotype 2267 and 4.2 days for genotype 54.  
403 Although the time to reach peak  $T_{crit}$  during the heat shock differed for the two genotypes,  
404 their maximum  $T_{crit}$  values were similar, being 43.8°C for genotype 29 and 43.6°C for  
405 genotype 2267 (Fig. 4).  $T_{crit}$  for both genotypes remained largely constant over the 120-hour  
406 period for those plants that were maintained at the control day/night temperature regime of  
407 24/12°C.

408

409 *Global variation in wheat  $T_{crit}$*

410 We combined data from our experiments with previously published data (covering genotypes  
411 grown across field and controlled environment experiments) to examine the degree of  
412 variation in  $T_{crit}$  in wheat genotypes on a global scale based on the latitude of origin as a  
413 proxy for climate of origin of their pedigree (Fig. 5). We found three studies (Havaux *et al.*,  
414 1988; Rekika *et al.*, 1997; Végh *et al.*, 2018) that reported wheat leaf  $T_{crit}$  using similar  
415 fluorescence temperature response curves (with ramp rates of 1–1.5°C min<sup>-1</sup> between 20–  
416 65°C) to estimate  $T_{crit}$ . Our final data collation comprised 183 wheat species/varieties  
417 (comprising *T. aestivum* L., *T. turgidum* L., ssp. *durum* Desf., *T. turgidum* L., ssp.  
418 *diococcoides* Thell., and wild wheat – *Aegilops* species) originating from all continents  
419 except Antarctica (Table 2). Globally, wheat leaf  $T_{crit}$  varied by up to 20°C (35–55°C) and  
420 there were more data for studies under warm conditions for genotypes originating from the  
421 lower latitudes than high latitudes (Fig 5). The larger variation in  $T_{crit}$  for genotypes  
422 originating from the higher latitudes coincided with the cooler growth conditions. Overall,  
423 there was less variation in  $T_{crit}$  under the warm conditions. We found no relationship between  
424 wheat leaf  $T_{crit}$  and the absolute latitude of genotype climate of origin (Fig. 5).

425

## 426 Discussion

427

428 In this paper we used a high-throughput technique to record minimal chlorophyll *a*  
429 fluorescence and quantified the critical temperature ( $T_{crit}$ ) of photosystem II damage – a  
430 measure of leaf photosynthetic heat tolerance – for wheat genotypes grown in multiple field  
431 experiments, as well as a controlled environment experiment. The field experiments  
432 demonstrated the extent of variation in  $T_{crit}$  over the course of a single day, as well as across  
433 several crucial stages of phenological development. They also showed that the region of  
434 origin of wheat genotypes were unrelated to  $T_{crit}$  in three representative Australian wheat  
435 growing regions, and that sowing time (and thus, growth temperature) was responsible for  
436 significant variation in  $T_{crit}$ . Delayed sowing (i.e. elevated growth temperature) was generally  
437 associated with increases in  $T_{crit}$ , resulting in higher thermal safety margins at both field sites.  
438 When two genotypes were subjected to a sudden heat shock in a controlled environment, we  
439 observed a slight difference between genotypes in the speed with which  $T_{crit}$  increased.  
440 However, both genotypes exhibited a similar peak  $T_{crit}$  value during this heat shock. Finally,  
441 when combining these data with previously published wheat  $T_{crit}$  data, as well as unpublished  
442 data from other experiments conducted in controlled environment facilities at The Australian

443 National University, we found that the absolute latitude of pedigrees of wheat genotypes were  
444 not significantly linked with variation in  $T_{crit}$  for either cool or warm grown plants.

445

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

447 Wheat  $T_{crit}$  varied significantly over the course of a single day, declining by an average of  
448 1.7°C over the 18 hours from solar noon to sunrise (Fig. 1A). This pattern resembles the  
449 extent of change in  $T_{crit}$  in a temperate tree species reported by Hüve *et al.* (2006);  
450 specifically, a linear increase over 14 hours, from a low point at 5 am to a peak at 7 pm.  
451 Taken together, these findings suggest that  $T_{crit}$  generally increases to a peak during the late  
452 afternoon before declining to a minimum between midnight and dawn. Hüve *et al.* (2006)  
453 linked this diel variation in  $T_{crit}$  with daily variation in leaf sugar content, and demonstrated  
454 that  $T_{crit}$  increased when leaves were fed sugar solutions. Further work is needed to determine  
455 if the diel variation in  $T_{crit}$  that we observed in wheat was also influenced by corresponding  
456 variation in leaf sugar content.

457 It is also interesting to compare the extent of variation in  $T_{crit}$  that was observed over  
458 the course of a single day with the extent of variation that was observed across phenology. In  
459 the 18 hours between solar noon and sunrise  $T_{crit}$  declined by 1.7°C (Fig. 1A), a fluctuation  
460 that was similar in size to the 1.5°C rise in  $T_{crit}$  that we observed from heading to anthesis  
461 (Fig. 1B). This comparison highlights the high level of plasticity in  $T_{crit}$ , and that variation in  
462  $T_{crit}$  is clearly responsive to factors on both an hours-long timescale (i.e. diurnal fluctuations  
463 in leaf sugar content) and a longer-term weeks-long scale (as evidenced by changes in  $T_{crit}$   
464 from heading to anthesis and grain filling). Anthesis is widely considered the stage of  
465 phenology at which wheat is most vulnerable to high temperature (Ferris *et al.*, 1998;  
466 Thistlethwaite *et al.*, 2020), and so it was somewhat surprising to see  $T_{crit}$  rise as plants  
467 moved from heading to anthesis at the first glance. However, it is not surprising when  
468 considering the fact that anthesis vulnerability to heat stress is largely due to reduction of sink  
469 strength to import and utilize assimilates within the reproductive organs, rather than of  
470 assimilate supply from leaf photosynthesis per se (Li *et al.*, 2012; Ruan *et al.*, 2012). While  
471 this increase could reflect an ongoing rise in heat tolerance coinciding with seasonal  
472 warming, there was no significant difference in  $T_{crit}$  between plants undergoing anthesis  
473 versus those at the grain filling stage, and so anthesis may be the phenological stage at which  
474  $T_{crit}$  is at its peak.

475

#### 476 *Drivers of variation in wheat $T_{crit}$*

477 The field site at which plants were grown was the most significant source of variation in  $T_{crit}$ ;  
478 the overall average  $T_{crit}$  at Narrabri was 1.8°C higher than recorded at Dingwall and 0.8°C  
479 higher than at Barraport West. In addition to environment, genotype had significant effect on  
480  $T_{crit}$  at the Barraport West and Narrabri sites. These results suggest that environment,  
481 genotype and most likely the genotype-by-environment interactions (GEI) may play large  
482 roles in determining wheat flag leaf  $T_{crit}$ . Breeding for genotypes with greater photosynthetic  
483 heat tolerance (i.e. higher  $T_{crit}$ ) may be challenging if variation in  $T_{crit}$  is also influenced by  
484 GEI effects. GEI effects have been reported for other abiotic stress tolerance traits including  
485 lodging tolerance in spring wheat (Dreccer *et al.*, 2020), and drought tolerance in maize (Dias  
486 *et al.*, 2018).

487

#### 488 *Genotypes maintain moderate photosynthetic thermal safety margins*

489 We observed variation in the thermal safety margins of wheat genotypes, predominantly  
490 associated with differences between field sites and the effect of sowing time at these sites.  
491 The thermal safety margin was 2.1°C when averaged across all genotypes (Fig. 2). Thermal  
492 safety margins in three representative Australian wheat-growing regions were at least 2–4°C

493 for all genotypes.  $T_{crit}$  was always several degrees greater than the hottest recorded air  
494 temperature during the typical month of anthesis at each site (Narrabri, 40.8°C,  
495 Dingwall/Barraport West 40°C; denoted by the blue dot-dash lines in Fig. 2). Under the  
496 IPCC's RCP 4.5 intermediate emission scenario for Eastern Australia by 2090, most  
497 genotypes would maintain a positive, yet reduced, thermal safety margin in the studied  
498 growing regions. However, under the high emission RCP 8.5 scenario, the thermal safety  
499 margins of most genotypes grown at the Dingwall site and a few genotypes at the Barraport  
500 West site would be exceeded (Fig. 2A & 2B). For genotypes grown at the Narrabri site,  
501 thermal safety margins under the RCP 8.5 scenario would be drastically reduced and, in some  
502 cases, disappear (Fig. 2C). According to our  $T_{crit}$  observations, only genotypes originating  
503 from Obregón and Aleppo would retain positive thermal safety margins under the RCP 8.5  
504 scenario when sown at either optimal or delayed sowing times. The rise in  $T_{crit}$  with delayed  
505 sowing (and thus increased growth temperature) that we observed in the majority of  
506 genotypes indicates a widespread capacity for the thermal acclimation of wheat flag leaf  $T_{crit}$ .  
507 This suggests that thermal safety margins for wheat photosynthetic heat tolerance could yet  
508 increase in response to warming under future climate scenarios. However, given that we also  
509 observed an apparent limit to the acclimation of  $T_{crit}$  following sudden heat shock (Fig. 4), it  
510 is possible that daytime maximum temperatures could approach this physiological thermal  
511 limit of wheat PSII if the most severe global warming predictions are borne out. A hard limit  
512 to the high temperature acclimation of  $T_{crit}$  could indicate a physiological limitation of PSII,  
513 or a temperature that represents the absolute maximum tolerance. Given that the considerable  
514 thermal plasticity of PSII electron transport has been closely linked with improving  
515 photosynthetic heat tolerance more generally (Yamasaki *et al.*, 2002), the prospect of air  
516 temperatures approaching the physiological threshold of PSII high temperature acclimation is  
517 concerning.

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

535  
536 *Thermal environment of growth site may be more influential than genotype origin in*  
537 *determining variation in wheat flag leaf  $T_{crit}$*

538 Considering the potential benefits to wheat heat tolerance and performance under high  
539 temperature that could arise from achieving increases in  $T_{crit}$ , as well as the extent of variation  
540 that we observed in  $T_{crit}$  among 24 genotypes at three field sites, it would be beneficial to  
541 identify characteristics that predict high  $T_{crit}$  in wheat genotypes. Thus, we analysed whether  
542 the distinct regions from which our genotypes originated could reliably predict variation in

543  $T_{crit}$ . Previous studies of (mostly) woody, non-crop species found that  $T_{crit}$  was correlated with  
544 climate of origin (O'Sullivan *et al.*, 2017; Zhu *et al.*, 2018). In a similar vein, we found  
545 evidence of genotype region of origin significantly affecting  $T_{crit}$  at two of our field sites (Fig.  
546 3A & 3C). One consistency at both of these sites was that genotypes originating from  
547 Roseworthy, Australia generally exhibited the lowest or second-lowest mean  $T_{crit}$  values. By  
548 contrast, the genotype from Aleppo exhibited the highest  $T_{crit}$  at the Narrabri site (Fig. 3C),  
549 while at the Barraport West site the genotypes originating from Narrabri had the highest  
550 mean  $T_{crit}$  across all times of sowing (Fig. 3A). However, it seems unlikely that the effect of  
551 genotype region of origin is the result of differences in temperature at these locations: for  
552 instance, the average daily maximum April temperature in Aleppo, Syria is 23°C (NOAA),  
553 while the average daily October maximum in Roseworthy, Australia is 23.8°C (Australian  
554 Bureau of Meteorology). Therefore, the differences associated with genotype origin in the  
555 current study are likely related to a more complex combination of environmental differences  
556 between locations (e.g. rainfall, temperature, soil quality, agricultural practices). Differences  
557 in the aims and methods of breeding programs at various locations could also explain  
558 variation in  $T_{crit}$  associated with genotype origin. We also note that our experiments did not  
559 include genotypes that were developed in cooler environments, such as wheat growing  
560 regions in Europe or Northern America, and so further work may be required to capture the  
561 full extent of variation in  $T_{crit}$  that is associated with various wheat growing regions.

562  
563  *$T_{crit}$  increases within hours of heat shock, and peaks after 3–4 days*

564 We observed widespread evidence of wheat  $T_{crit}$  plasticity following exposure to high  
565 temperature, including elevated growth temperature in the field (via delayed sowing, Fig. 2 &  
566 3) and sudden heat shock under controlled conditions (Fig. 4). We also saw clear genotypic  
567 variation in the plasticity of  $T_{crit}$  across these experiments. In some genotypes  $T_{crit}$  rose by  
568 upwards of 4°C when sowing time was delayed by two months (Fig. 2B), while in others  $T_{crit}$   
569 showed no change or even declined by up to 1.2°C from TOS 1 to TOS 3 (Fig. 2A).  
570 Similarly, following a heat shock imposed under controlled conditions we observed a  
571 difference between two genotypes in the speed at which  $T_{crit}$  increased despite the two  
572 genotypes eventually reaching a similar peak  $T_{crit}$  (Fig. 4). Genotypic variation is thus evident  
573 not only in wheat flag leaf  $T_{crit}$  under common non-stressful temperatures, but also in the  
574 extent of  $T_{crit}$  plasticity in response to sudden heat shock. Increases in  $T_{crit}$  with warming have  
575 been reported previously (O'Sullivan *et al.*, 2017; Zhu *et al.*, 2018) and these are considered  
576 examples of high temperature acclimation. That we observed similar patterns in wheat  $T_{crit}$ , as  
577 well as genotypic variation in this acclimation, suggests that the capacity to increase PSII  
578 heat tolerance could be a trait worth targeting for the development of wheat genotypes with  
579 greater heat tolerance. However, further work is needed to first investigate whether such  
580 acclimation is associated with enhanced performance under high temperature in the field.

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

588  
589 *Genotypic variation in wheat leaf  $T_{crit}$  is not consistent with latitudinal trends in general*  
590 *plant heat tolerance*

591 Contrary to previous results that observed a decrease in PSII heat tolerance (measured as  $T_{crit}$ )  
592 as latitude moved further from the equator (O'Sullivan *et al.*, 2017; Lancaster and



593 Humphreys, 2020), we found that, irrespective of thermal acclimation, wheat leaf  $T_{crit}$  did not  
594 vary with the latitude of genotype climate of origin (Fig. 5). This discrepancy could be  
595 related to differences between cultivated and wild species: the O'Sullivan *et al.* (2017) and  
596 Lancaster and Humphreys (2020) studies demonstrated a relationship between heat tolerance  
597 and latitude based almost entirely on records of different wild species. By contrast, our study  
598 focuses solely on one domesticated species. Wheat is known as a crop with a particularly  
599 narrow genetic background (Tanksley and McCouch, 1997), but we observed a large range of  
600  $T_{crit}$  in wheat here (up to 20°C) which compares with the approximately 30°C global range  
601 reported across 218 plant species spanning seven biomes reported by O'Sullivan *et al.*  
602 (2017). This large range of wheat leaf  $T_{crit}$  can be exploited to improve heat tolerance in  
603 modern crop varieties, similar to recent successes in improving wheat drought tolerance using  
604 airborne remote sensing (Reynolds *et al.*, 2015). Still, wheat is cultivated in a wide range of  
605 ecological and climatic conditions, covering over 220 million hectares, including areas where  
606 it is exposed to high temperature stress. As such, we predicted that the rise in  $T_{crit}$  that we  
607 observed with elevated growth temperature in our experimental data set (Fig. 1–4) would also  
608 be apparent in the meta-analysis. However, there was no evidence of any thermal acclimation  
609 response of  $T_{crit}$  in this larger data set. This could partly be due to diversity of experimental  
610 methods used to generate the data in Figure 5, as well as variation in the duration and  
611 intensity of elevated growth temperature treatments. Given that the plant thermal tolerance  
612 field uses a large and diverse range of experimental designs and assays (Geange *et al.*, 2021),  
613 the results of our systematic review of wheat  $T_{crit}$  could be further evidence of a need to better  
614 standardise the approaches used to measure and describe photosynthetic heat tolerance.

615

### 616 *Conclusion*

617 Wheat leaf  $T_{crit}$  varied dynamically with changes in growth conditions, notably increasing in  
618 response to short and long-term high temperatures, and exhibiting an upper ceiling in  
619 acclimating to heatwaves. There was also evidence of developmental, diel and genotypic  
620 variation in  $T_{crit}$ . These results suggested a strong genotype-by-environment interaction  
621 effects on wheat leaf  $T_{crit}$  and potential links between  $T_{crit}$  and leaf sugar content.  
622 Interestingly, global wheat leaf  $T_{crit}$  which spanned up to 20°C was unrelated to genotype  
623 climate of origin and latitude, unlike reported associations with global interspecies variation  
624 in leaf  $T_{crit}$  of 171 plant species (*cf.* ~30°C). However, the observed genotypic variation and  
625 plasticity of wheat  $T_{crit}$ , combined with a recent developments of high throughput technique  
626 for measuring  $T_{crit}$  (Arnold et al 2021), indicate that this trait would be useful for high-  
627 throughput screening, understanding photosynthetic heat tolerance and development of heat  
628 tolerant wheat.

629

630

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648

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- 851



## 1 Tables and figure legends

2 **Table 1.** Information on field experiments in Australia

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

5 **Figure 1.** Variation in flag leaf  $T_{crit}$  (°C) of wheat genotypes over the course of an 18 h period  
6 (A), and across three phenological stages (B). Solid lines indicate significant linear trends.  
7 Plants were grown at field sites in Dingwall, Victoria in 2017 (A), and in Barraport West,  
8 Victoria in 2018 (B). Points represent mean  $\pm$  se,  $n = 4$  for (A) and  $n = 8-18$  for (B).

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

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

36 **Figure 4.** Leaf  $T_{crit}$  (°C) of two wheat genotypes – 29 (triangles and solid lines) and 2267  
37 (circles and dashed lines) exposed to 24°C (control; blue shapes and lines) or 36°C (heat; red  
38 shapes and lines) for varying durations (2, 4, 24, 48, 72, or 120 h) in a growth cabinet. Leaf  
39 samples for  $T_{crit}$  were from the third fully extended leaves on the main stem. Equations for the  
40 curvilinear relationships between  $T_{crit}$  at 36°C ( $T_{crit}^{36}$ ; °C) and time ( $t$ ; hour) under heat for  
41 genotype 29 is  $T_{crit}^{36} = 43.42 + 0.038t - 0.00019t^2$  and for genotype 2267 is  $T_{crit}^{36} = 43.69 +$   
42  $0.031t - 0.00019t^2$ . Points represent mean  $\pm$  se,  $n = 4$ .

43 **Figure 5.** Relationship between  $T_{crit}$  and the absolute latitude of the climate of origin for  
44 wheat genotypes when grown under cool (blue circles) and warm (red squares) conditions.

- 1 Data obtained from 183 wheat genotypes (3223 measurements of leaf  $T_{\text{crit}}$  overall) from
- 2 experiments in Australia (this study) and published literature (Havaux *et al.*, 1988; Rekika *et*
- 3 *al.*, 1997; Végh *et al.*, 2018). Data points represent mean  $T_{\text{crit}}$  ( $\pm$  SE where visible) for each
- 4 genotype.

1 **Table 1.** Information on field experiments in Australia

Experiment location and year	Year	Genotypes studied <sup>a</sup>	Mean daily maximum temperature at anthesis (°C)	Radiation ( $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) <sup>b</sup>
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 <sup>c</sup>	Genotypes studied	Brief description of method	
<i>Diurnal variation in <math>T_{crit}</math></i>				
Dingwall, Victoria	3	6	Flag leaf $T_{crit}$ determined at anthesis at four consecutive time points occurring every six hours over an 18-hour period (i.e. solar noon, sunset, midnight, and sunrise).	
<i>Phenological variation in <math>T_{crit}</math></i>				
Barraport West, Victoria	1–3	4	Flag leaf $T_{crit}$ determined at heading, anthesis, and grain filling on the same day at 10 am from all three time of sowing plots.	
<i>Rate of acclimation of <math>T_{crit}</math><sup>+d</sup> and calculation of thermal safety margins</i>				
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		

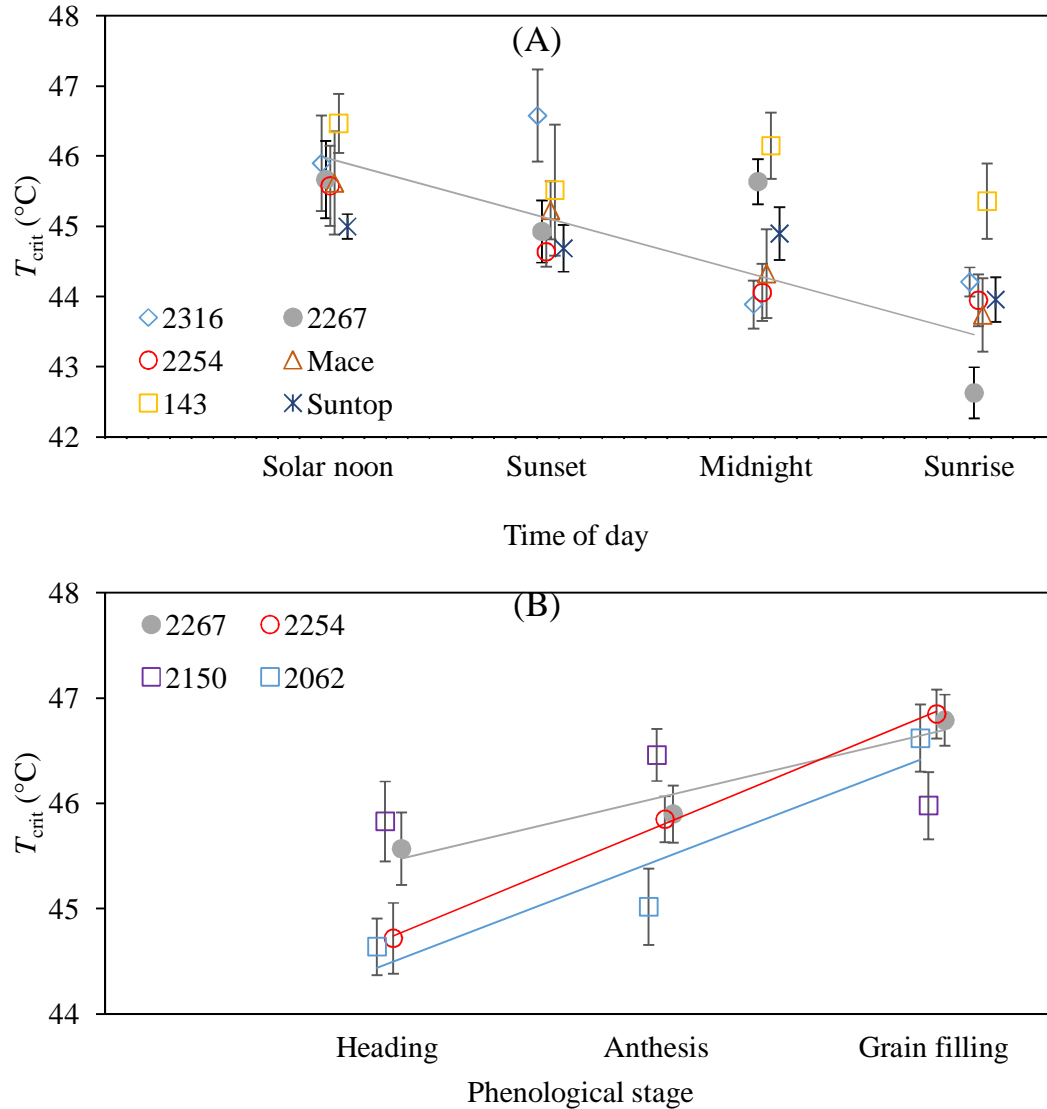
2 <sup>a</sup>Twenty genotypes were common to all experiments. The designation of all genotypes used in this study is provided in Supplementary Table S1;

3 <sup>b</sup>Mean maximum photosynthetically active radiation measured with Licor 6400XTs light sensors; <sup>c</sup>Time of sowing, where the first time of  
 4 sowing was within the locally recommended sowing window, with subsequent times of sowing separated in one month intervals at Victoria, or  
 5 two months at New South Wales; <sup>+d</sup>an additional  $T_{crit}$  high temperature acclimation study was conducted under controlled environments with two  
 6 of the 24 genotypes.

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

<b>Study<sup>1</sup></b>	<b>Origin</b>	<b>Species</b>	<b>Mean <math>T_{crit}</math> (n)</b>
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)
<b>Average</b>			<b>44.8 (24)</b>
Havaux <i>et al.</i> (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)
<b>Average</b>			<b>48.7 (31)</b>
Végh <i>et al.</i> (2018)	Europe	<i>T. aestivum</i> L.	<b>41.8 (5)</b>
Rekika <i>et al.</i> (1997)	Africa	<i>T. turgidum</i> L., ssp. <i>durum</i> Desf.	37.0 (1)
	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 (wild wheat)	<i>Aegilops species</i>	38.2 (5)
<b>Average</b>			<b>37.5 (11)</b>
Unpublished data from our group	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)
<b>Average</b>			<b>44.2 (133)</b>

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

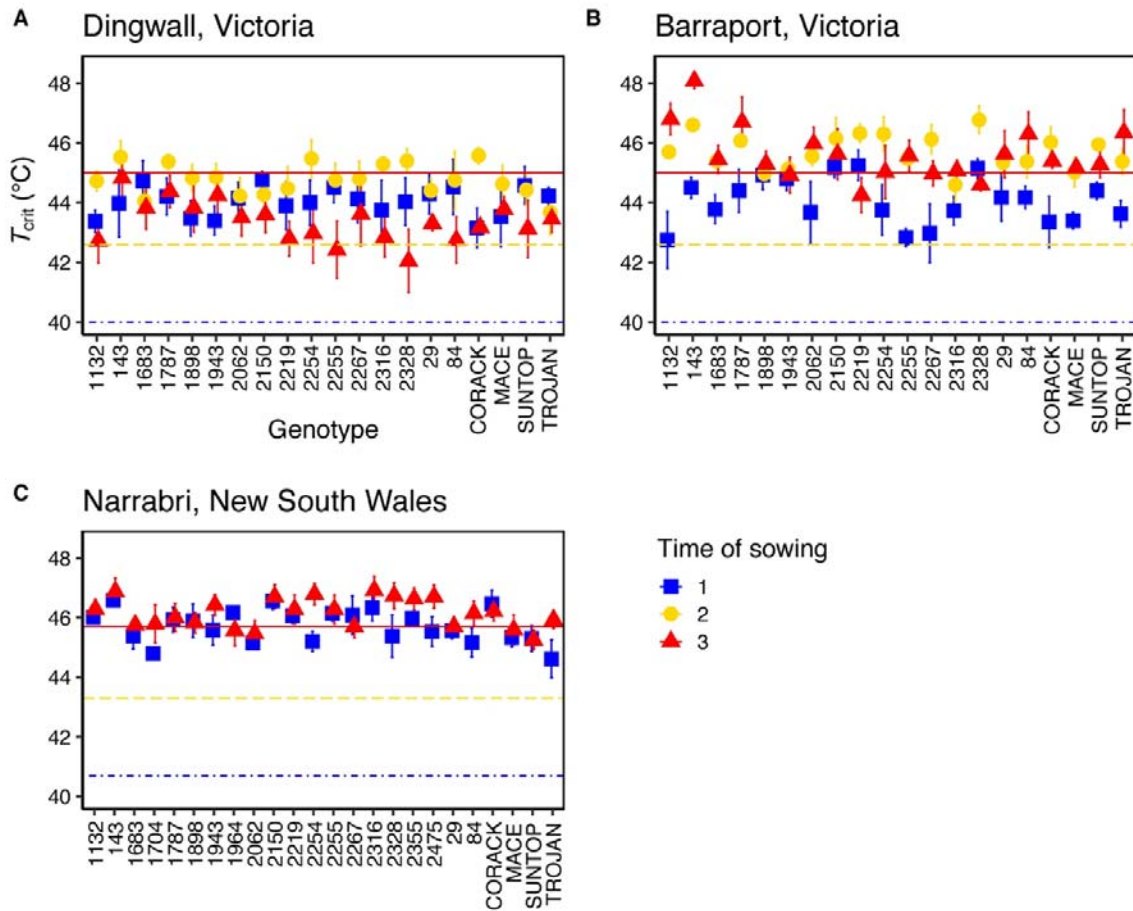


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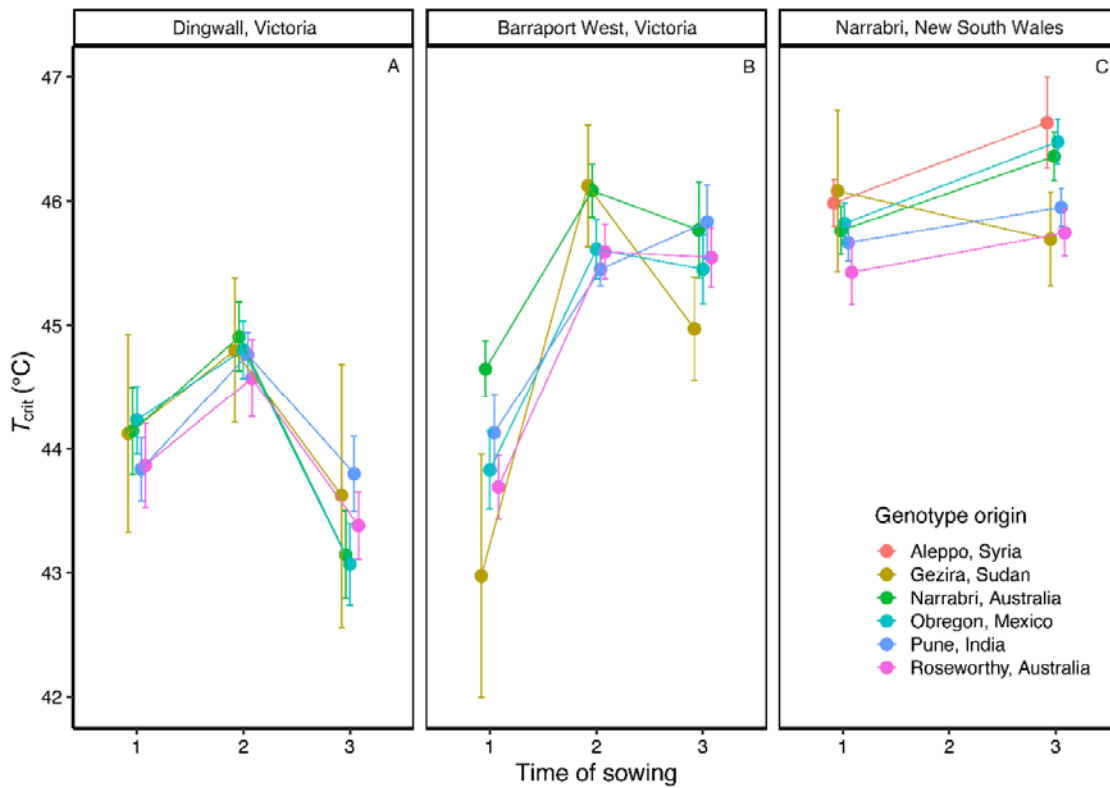
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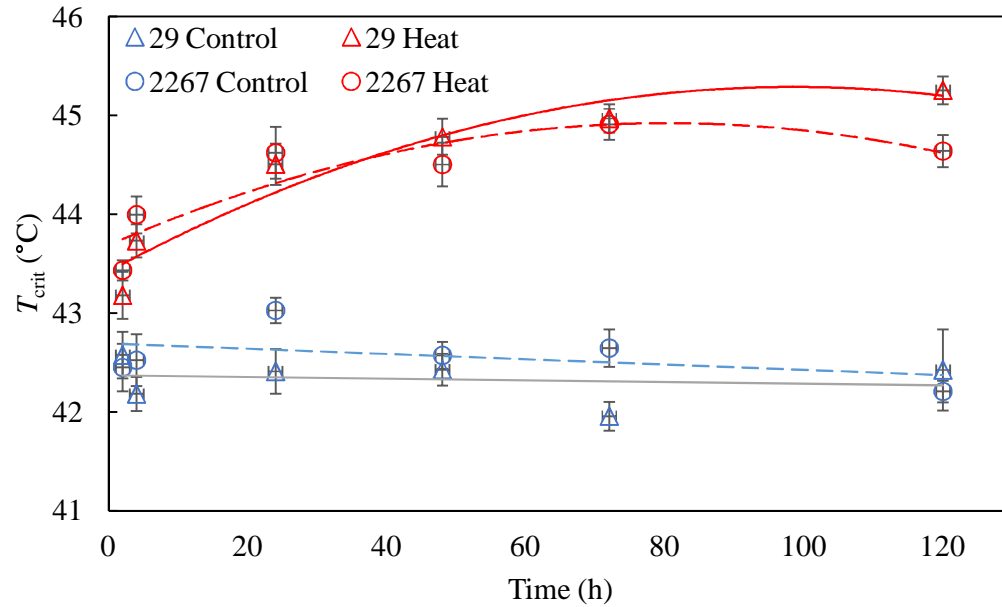


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2 **Figure 2.** Phenotypic plasticity of leaf  $T_{crit}$  and thermal safety margins of 20–24 wheat  
3 genotypes. The genotypes were sown at either the locally recommended time of year (time of  
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10 additional four genotypes were sown at Narrabri in 2019. The dash-dot blue lines mark the  
11 hottest recorded maximum temperature during the typical anthesis month (October) at each  
12 field site (40.7°C for Narrabri, and 40°C for Dingwall, data from the Australian Bureau of  
13 Meteorology; due to the close proximity of Dingwall and Barraport West we used the same  
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15 RCP 4.5 IPCC and RCP 8.5 IPCC emission scenarios (+2.6 and +5°C), respectively. The  
16 difference between the observed  $T_{crit}$  and these current and future maximum temperatures is  
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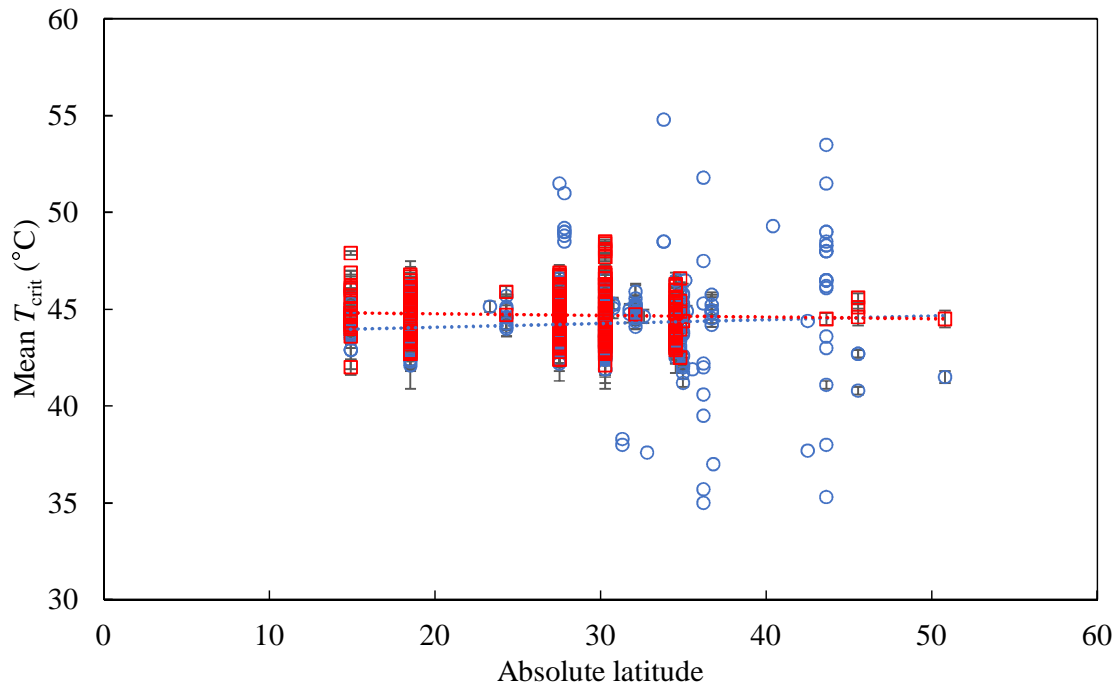


2  
3 **Figure 3.** Phenotypic response of wheat flag leaf photosystem II heat tolerance ( $T_{crit}$ ) to time  
4 of sowing at three Australian field sites: (A) Dingwall, Victoria; (B) Barraport West,  
5 Victoria; and (C) Narrabri, New South Wales. Genotypes are grouped according to the six  
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7 grown at Dingwall in 2017 and at Barraport West in 2018, while the same 20 plus an  
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1  
2 **Figure 4.** Leaf  $T_{crit}$  (°C) of two wheat genotypes – 29 (triangles and solid lines) and 2267  
3 (circles and dashed lines) exposed to 24°C (control; blue shapes and lines) or 36°C (heat; red  
4 shapes and lines) for varying durations (2, 4, 24, 48, 72, or 120 h) in a growth cabinet. Leaf  
5 samples for  $T_{crit}$  were from the third fully extended leaves on the main stem. Equations for the  
6 curvilinear relationships between  $T_{crit}$  at 36°C ( $T_{crit}^{36}$ , °C) and time ( $t$ ; hour) under heat for  
7 genotype 29 is  $T_{crit}^{36} = 43.42 + 0.038t - 0.00019t^2$  and for genotype 2267 is  $T_{crit}^{36} = 43.69 +$   
8  $0.031t - 0.00019t^2$ . Points represent mean  $\pm$  se,  $n = 4$ .

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**Figure 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. Data obtained from 183 wheat genotypes (3223 measurements of leaf  $T_{crit}$  overall) from experiments in Australia (this study) and published literature (Havaux *et al.*, 1988; Rekika *et al.*, 1997; Végh *et al.*, 2018). Data points represent mean  $T_{crit}$  ( $\pm$  SE where visible) for each genotype.