



HAL
open science

How does post-flowering heat impact grain growth and its determining processes in wheat?

Christine Girousse, Lauren Inchboard, Jean-Charles Deswarte, Karine Chenu

► To cite this version:

Christine Girousse, Lauren Inchboard, Jean-Charles Deswarte, Karine Chenu. How does post-flowering heat impact grain growth and its determining processes in wheat?. *Journal of Experimental Botany*, 2021, 72 (18), pp.6596-6610. 10.1093/jxb/erab282 . hal-03293623

HAL Id: hal-03293623

<https://hal.inrae.fr/hal-03293623>

Submitted on 9 Dec 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1
2 How does post-flowering heat impact grain growth and its
3 determining processes in wheat?
4
5

6 Christine Girousse^{1+*}, Lauren Inchboard¹⁺, Jean-Charles Deswarte², Karine Chenu³
7
8
9

10 ¹ INRAe, UCA, UMR 1095 GDEC, 5 Chemin de Beaulieu, F-63000 Clermont-Ferrand, France.

11 ² Arvalis Institut du Végétal, Route de Chateaufort, ZA des graviers, F-91190 Villiers-le-Bâcle,
12 France.

13 ³ The University of Queensland, Queensland Alliance for Agriculture and Food Innovation
14 (QAAFI), 13 Holberton street, Toowoomba, QLD 4350, Australia.
15

16 ⁺: these authors contributed equally to this work

17 * Correspondence: christine.girousse@inrae.fr– Tel: +33 443 76 15 75
18
19

20 Date of submission:

21 Number of tables: 4

22 Number of figures: 8

23 Word count: 6474
24

25 How does post-flowering heat impact grain growth and its
26 determining processes in wheat?

27

28 Running title

29

30 Impacts of post-flowering heat on grain growth in wheat

31

32 Highlight

33

34 Wheat yield is increasingly constrained by post-anthesis heat. Temperature response curves
35 were established for grain growth and expansion. Different heat sensitivities were revealed
36 among processes involved in grain elaboration.

37

38 Abstract

39

40 Wheat grain yield is anticipated to suffer from the increased temperatures expected under
41 climate change. In particular, the effects of post-anthesis temperatures on grain growth and
42 development must be better understood to improve crop models. Grain growth and
43 development involve several processes and we hypothesized that some of the most important
44 processes, *i.e.* grain dry matter and water accumulation, grain volume expansion and
45 endosperm cell proliferation, will have different thermal sensitivity. To assess this, we
46 established temperature response curves (TRC) of these processes for steady post-anthesis
47 temperatures between 15°C and 36°C. From anthesis to maturity, grain dry mass, water mass,
48 volume and endosperm cell number were monitored, whilst considering grain temperature.
49 Different sensitivities to heat of these various processes were revealed. The rate of grain dry
50 biomass accumulation increased linearly up to 25°C while the reciprocal of its duration linearly
51 increased up to at least 32°C. By contrast, the growth rates of traits contributing to grain
52 expansion, *e.g.* increase in grain volume and cell numbers, had higher optimum temperatures,

53 while the reciprocal of their durations were significantly lower. These TRC can contribute to
54 improve current crop models, and allow to target specific mechanisms for genetic and
55 genomic studies.

56

57 Keywords

58

59 Wheat grain, grain development, growth rate, growth duration, water accumulation,
60 endosperm cell number, grain filling, temperature response curve, high temperature, thermal
61 stress.

62

63 Introduction

64
65 Temperature is one of the main drivers of plant growth and development. In plant sciences,
66 temperature response curves (TRC) also referred as temperature performance curves; Schulte
67 *et al.*, 2011) are used to evaluate how natural variations in temperature (excluding sub- and
68 supra-temperatures) affect the rates of processes such as enzyme activity (Hawker and
69 Jenner, 1993), photosynthesis (both at the molecular level (Rubisco: Bernacchi *et al.*, 2001)
70 and at the plant level (Nagai and Makino, 2009)) and organ growth (e.g. Parent *et al.*, 2010).
71 In simulation studies, TRC are integrated into mechanistic models to account for the effects of
72 temperature (e.g. Granier and Tardieu, 1998; Wang *et al.*, 2017).

73 TRC typically describe (i) the rate or (ii) the reciprocal of the duration of processes related to
74 plant growth and development. The reciprocal of duration is a mean developmental rate that
75 characterizes the percentage of achievement rate of the process under concern. TRC typically
76 have an asymmetrical and left-skewed shape (Dowd *et al.*, 2015; Schulte *et al.*, 2011) but are
77 commonly characterized by two linear relationships with three cardinal temperatures: a
78 minimal temperature T_0 below which there is neither growth nor development; an optimal
79 temperature T_{opt} at which rates reach a maximum; and a maximal temperature T_{max} at which
80 growth and/or development are impeded by extreme temperatures (Monteith, 1984; Parent
81 *et al.*, 2010). In general, TRC are established and cardinal temperatures estimated in growth
82 chamber conditions (Poorter *et al.*, 2010) where the temperature is controlled as well as
83 possible. Responses to temperature are often evaluated with measurements of local air
84 temperature, though they should ideally be evaluated by the organ temperature (Bonhomme,
85 2000).

86 TRC are commonly used in plant or crop models that predict integrated traits such as grain
87 yield (Porter and Semenov, 2005; Liu *et al.*, 2020). Today, these predictions are of paramount
88 importance as it is anticipated that production of crops such as wheat will be negatively
89 affected by the increased frequency of high temperatures expected under climate change (;
90 Zheng *et al.*, 2012; Rosenzweig *et al.*, 2013; Asseng *et al.*, 2015; Lobell *et al.*, 2015; Zheng *et al.*
91 *et al.*, 2016). However, while TRC are known to be genotype- and process-dependent (Slafer and
92 Rawson, 1995a, b; Luo, 2011), research on the responses of wheat to temperature has mainly
93 focused on (i) the duration of pre-flowering phenological phases (e.g. Slafer and Rawson,
94 1995a, b) and (ii) the rate and/or duration of -germination, emergence and root elongation,

95 cell multiplication and/or tissues expansion in stems or leaves (e.g. Porter and Gawith, 1999;
96 Granier *et al.*, 2002; Parent and Tardieu, 2012). Few studies deal with TRC on the growth and
97 development of grains, despite their importance to the establishment of grain yield (Reynolds
98 *et al.*, 2011). Due to recent and foreseen increase in high temperature episodes during grain
99 filling (IPCC, 2019), and their impact on productivity (Ababaei and Chenu, 2020), it is crucial to
100 consider and improve the description of the effects of high temperatures on grain filling
101 (Porter and Semenov, 2005; Challinor *et al.*, 2014; Chenu *et al.*, 2017).

102 A few post-anthesis studies have established TRC on rate and duration of biomass
103 accumulation during grain filling from wheat grown in fields (Angus *et al.*, 1981; Slafer and
104 Savin, 1991) and controlled conditions (Sofield *et al.*, 1977; Chowdhury and Wardlaw, 1978).
105 All these studies focused on a range of temperatures where the maximum daily temperature
106 was below 30°C and where TRC can be considered as linear. In addition, these few studies on
107 post-flowering have focused on biomass accumulation in grains over the whole grain filling
108 period. The reduction of grain dry biomass in response to high temperature has been related
109 to a reduction in grain starch synthesis (Bhullar and Jenner, 1986; Spiertz *et al.*, 2006). Among
110 the enzymes regulating starch synthesis, the soluble starch synthase (SSS) is the most sensitive
111 one to heat stress (Jenner, 1994; Zahedi *et al.*, 2003) and TRC of SSS activity have been
112 established (e.g. Keeling *et al.*, 1993).

113 However post-anthesis temperatures are likely to impact grain growth and development
114 differently depending upon the grain development phase and on the underlying processes
115 involved (Slafer and Rawson, 1995a, b). Indeed, grain growth and development is generally
116 divided into three phases during which different processes occur: a rapid cell proliferation
117 phase called 'lag-phase', an effective filling phase and a maturation phase (Egli, 1998). Cell
118 proliferation within the grain endosperm begins just after fertilization. During this first phase,
119 water is rapidly accumulated inside the grain, contributing most to the volumic growth of the
120 grain. At the end of the lag-phase, the grain length is set (Lizana *et al.*, 2010; Nadaud *et al.*,
121 2010), the maximum number of endosperm cells is attained, and correlates positively with the
122 grain filling capacity (Brocklehurst, 1977). The second phase consists of the accumulation of
123 assimilates into the endosperm cells. Water mass during this phase is maintained constant
124 and dry matter content increases linearly over time (Egli, 1998). Finally, maturation of the
125 grain begins when the final dry biomass and lateral dimensions of the grain are set. During

126 the maturation phase, grains desiccate while the polymerization of storage proteins and
127 assembly with starch within the endosperm cells contribute to the final grain quality. If TRC
128 have already been established for one of the main processes related to grain dry biomass
129 accumulation (SSS synthesis), no such curves are available for the main processes related with
130 grain expansion (cell proliferation and water accumulation).

131 We hypothesized that the different processes involved in the successive phases of grain filling
132 may have different temperature responses, and that this may contribute to the response of
133 final grain dry biomass. Therefore, we established TRC for some of the main processes
134 involved in final grain size and biomass, namely grain dry biomass and water accumulation,
135 grain volume expansion, and endosperm cell proliferation. This included (i) parameterizing
136 cardinal temperatures for response curve of studied processes, (ii) testing the range of
137 temperatures where linear responses to temperature could be considered, and (iii) evaluating
138 whether the processes involved in the constitution of the final grain mass shared a common
139 sensitivity to temperature. TRC were established based on results of five experiments where
140 constant grain temperature (from 15°C to 36°C) was applied during the grain filling period, for
141 a unique genotype, and on grains at a fixed position on the spike. Here we show that the
142 different processes do have different temperature responses. These TRC can contribute to
143 bring more robustness into the calculation of post-anthesis thermal time in crop models, and
144 allow to target specific mechanisms for genetic and genomic studies and finally for plant
145 breeding.

146

147 Material and methods

148

149 *Plant material and growth conditions*

150 Seeds of spring wheat (*Triticum aestivum* L.), genotype SxB049 (Pinto *et al.*, 2010 and provided
151 by CIMMYT) were sown in 50 mL pots and placed in a greenhouse. At the three-leaf stage, the
152 plants were transplanted by pairs into PVC tubes (inner diameter 7.5 cm; length 50 cm) filled
153 with compost enriched with 2.5 kg m⁻³ of fertiliser 9:12:16 (N:P:K), and iron (Fe). The PVC tubes
154 were arranged into two contiguous containers to form a homogeneous canopy surrounded by
155 green and perforated screens to reduce edge effects. All plants were cultivated at a 16h

156 photoperiod, and watered daily in excess with a 0.5 strength Hoagland nutrient solution to
157 maintain the soil water potential above -0.5 MPa.

158 Both containers were first located in a “control” growth chamber (set at 19°C) during the
159 vegetative phase. A few days before anthesis, when the entire spikes had emerged from the
160 sheath, the main stem of each plant was labelled. Their number of spikelets was counted, and
161 only spikes with similar number of spikelets were retained. Anthesis was recorded as the date
162 when anthers of the basal florets of the middle spikelets of the main stem appeared. Each PVC
163 tube from one of the containers was transferred in the “treatment” growth chamber two days
164 after the averaged anthesis date of its two plants, in order to avoid or limit the impact of high
165 temperature on final grain number. If the time between the anthesis dates of the two plants
166 in the same tube was more than 2 days, only the second plant to reach anthesis was
167 considered for the study.

168 Five temperature treatments constant over 24h were applied: 36, 32, 29, 24 and 15°C in five
169 successive experiments conducted in the “treatment” chamber (Table 1). In each experiment
170 a control treatment at 19°C was conducted in the control chamber, so that there were 5
171 replicates of the control treatment at 19°C. In each growth chamber, the air temperature next
172 to the wheat spikes was measured using copper-constantan thermocouples placed under a
173 shield screen. Grain temperature was also measured using 0.2 mm copper-constantan
174 thermocouples inserted into basal grains of central spikelets of tillers (different than those
175 sampled for measurements). As the insertion of the thermocouples into the grain caused local
176 necrosis, thermocouples were moved to different grains every two days. In each chamber,
177 four thermocouples for measurement of air temperature and four thermocouples for
178 measurement of grain temperature were equally spaced into the plants. Air and grain
179 temperatures were recorded every 10s on a CR 1000 datalogger (Campbell Scientific Ltd,
180 Logan, UT, USA) and averaged over 15min. The mean daily temperature variation within the
181 growth chambers was less than 1.5°C. Plants received throughout the experiment a mean total
182 daily photosynthetic photon flux (PPF) of 250 ± 57 and $354 \pm 81 \mu\text{mol m}^{-2} \text{d}^{-1}$ in “control” and
183 “treatment” growth chambers, respectively. Relative humidity varied between 66.6 and
184 79.0 % depending on experimented temperature leading to an average VPD of 1.7 kPa for the
185 36°C treatment, and 1.1 kPa or lower for all the other treatments. Environmental conditions

186 in each growth chamber and for each experiment are reported in Table 1 and the variations
187 of air and grain temperatures in the five experiments are presented in Figure S1.

188

189 *Grain measurements*

190 For each experiment and each temperature treatment (i.e. in both control and treatment
191 growth chambers), sampling was conducted on 12 to 16 dates from anthesis to maturity. At
192 each date and for each temperature, six tagged main spikes were cut and placed in plastic
193 bags with a damp paper and transferred rapidly to the laboratory for measurements. For each
194 spike, the two basal grains of the two middle spikelets (i.e. four grains) were sampled to
195 measure fresh biomass and volume of individual grains. One grain from each spikelet was
196 dried at 70°C for two days in order to measure dry biomass. Water mass was calculated as the
197 difference between the fresh and dry biomass. The second grain from each spikelet was
198 frozen, then dissected to isolate the endosperm that was prepared for cell count. The
199 endosperm cell number was determined following the method described by Singh and Jenner
200 (1982). At each sampling date and for each temperature treatment, the values of the traits
201 measured on either two (for dry biomass and endosperm cell number) or four (fresh biomass
202 and grain volume) grains of the same spike were averaged before doing further analysis on
203 the six independent repetitions (i.e. six plants).

204

205 *Analysis of trait dynamics over time and final value*

206 The effects of high temperatures on grain volume, dry biomass and water mass at maturity,
207 and on the endosperm cell number at around 300°Cd after anthesis were statistically tested
208 followed by a Student-Newman-Keuls (SNK) test at the 5% level of significance for mean
209 comparison.

210 Growth kinetics were studied for four traits (dry biomass, volume, water mass and endosperm
211 cell number) describing mean grain growth for a range of temperatures. In a first step, classical
212 candidate growth functions (Hunt, 1979; Ratkowsky, 1990) were fitted to the change over
213 time of each studied trait for plants grown at 19°C (control) in each experiment with the
214 nonlinear least squares *nls* procedure. The growth function describing most adequately the
215 observations was chosen based on the homogeneity and values of residuals, biological
216 coherence (e.g. shape, starting values, durations), and the Akaike information criterion (AIC).

217 When the data were heteroscedastic, non-homoscedastic variance structures were tested and
 218 compared (Robert *et al.*, 1999). For each trait, the chosen function (Table 2) was then used to
 219 fit the observations from all the temperature treatments (second step, see below).

220 In the second step, to account for variations observed across experiments, the chosen growth
 221 functions were fitted for each trait, except endosperm cell number, on the complete dataset
 222 with a nonlinear mixed model. Each function parameter was considered as the sum of a fixed
 223 effect dependant on the temperature treatment, and a random effect dependant on the
 224 experiment as to account for bias observed among controls of the different experiments.
 225 When an ‘experiment’ random term was negligible, it was removed from the model, and the
 226 resulting model was compared to the former with the Fisher statistic to ensure that it was not
 227 significantly different. These non-linear mixed models were fitted using the R package *nlme*
 228 (Pinheiro *et al.*, 2018), which optimises parameters based on optimisation-maximisation of the
 229 log-likelihood. Endosperm cell number, on the other hand, was too variable to include a
 230 random ‘experiment’ effect within each parameter, and thus, the adjustment included only
 231 temperature-dependant fixed parameters and was carried out with *nls* (Bates *et al.*, 2007).

232

233 *Temperature response curve*

234 Maximum values, average growth rates and durations of the studied processes were defined
 235 from the selected growth functions as described in Table 2. For each trait, two TRC were
 236 established by plotting (i) the growth rate and (ii) the reciprocal of the duration against the
 237 mean grain temperature (Table 1). Standard deviations were provided by the mixed models
 238 or calculated using the multivariate delta method (Cox, 2005).

239 A simplified Arrhenius-type function developed for responses to temperature and depending
 240 on minimum (T_{min}), optimum (T_{opt}) and maximal (T_{max}) temperatures (Wang *et al.*, 2017), was
 241 fitted on the TRC [Eq.1].

242

$$243 \quad f(T) = a \cdot f_{Wang17}(T) = a \cdot \left(\frac{2(T-T_{min})^\alpha (T_{opt}-T_{min})^\alpha - (T-T_{min})^{2\alpha}}{(T_{opt}-T_{min})^{2\alpha}} \right)^\beta \quad [\text{Eq.1}].$$

244 with the α coefficient given by:

$$245 \quad \alpha = \frac{\ln 2}{\ln \left(\frac{T_{max} - T_{min}}{T_{opt} - T_{min}} \right)}$$

246

247 For each trait, a coefficient a was added to normalise the function and obtain dimensionless
248 values varying between 0 and 1. T_{opt} , T_{max} and a were estimated statically using nls , while T_{min}
249 was set at 0°C, as commonly used in the literature for wheat (e.g. Slafer and Rawson, 1995b)
250 and β was set at 1 as in Wang *et al.* (2017).

251 All statistical analyses were conducted with R 3.4.4 (R Core Team, 2018).

252 Results

253 *High temperatures affect grain growth in various ways*

254 Six steady temperature treatments (15, 19, 24, 29, 32, 36°C) were applied on plants during the
255 whole grain growth duration. Different traits related to grain growth, namely the grain dry
256 biomass, the grain volume, the grain water mass and the endosperm cell number were
257 recorded from basal grains from central spikelets. Throughout the experiments, the target
258 temperatures within the grains were consistent with the required temperatures (Fig. S1A).
259 While the grain and air temperatures were highly correlated throughout the experiments (Fig.
260 S1B), air temperature was significantly greater than grain temperature with a mean difference
261 of 0.29°C (SD=0.42, $T = 11.9$, $P < 0.0001$, one tailed). This small difference is probably due to
262 the growth cabinet conditions in which the instantaneous light flux on the spike is moderate.

263

264 As expected, high temperatures resulted in a strong significant reduction ($P < 0.001$) of the final
265 observed values of the different studied traits (Fig. 1). The maximal percentages of reduction
266 on final values calculated between 15°C and 36°C were 81.0, 74.8, 91.5 and 52.9 % for grain
267 dry biomass, grain volume, grain water mass and endosperm cell number, respectively.

268 The kinetics of the different traits were followed from 2 days after anthesis to maturity. For
269 each trait, the growth curves had a similar pattern for the different temperature treatments
270 (Fig. 2). Growth functions that best fitted the observations were chosen (Table 2) based on
271 data from the control temperature (19°C) and were then applied to data from all temperature
272 treatments. High correlations were found between observations and predicted values from
273 the statistical model (Table 3) and the standardized residuals were evenly distributed,
274 comforting the good adequation of the selected growth function at 19°C for all the

275 temperatures (Fig. S2). The adjusted growth functions are presented in Figure 3 for all traits
276 and temperature treatments.

277 For all temperatures, grain dry biomass accumulation followed a classic sigmoid pattern (Fig.
278 2A and 3A). Final grain biomass began to be affected by high temperature for a threshold
279 between 24 and 29°C (Fig. 1A). The dynamics of dry biomass accumulation was however
280 different (not necessarily significantly) for all the temperatures tested (i.e. 15 to 36°C) with a
281 greater impact observed for the highest temperatures.

282 Grain volume increased almost linearly up to a plateau under optimal temperatures (15°C; Fig.
283 2B and 3B). However, for most tested temperatures (19 to 36°C) and probably also for 15°C
284 (although not apparent in Fig. 3B), grain volume increased up to a maximum before decreasing
285 before the end of the grain filling period. This final volume (at maturity) decreased with
286 increase in temperature up to 29°C, while the final volume from the 29, 32 and 36°C
287 treatments were similar (Fig. 1B and 2B). The dynamics of the grain volume was also highly
288 impacted by temperature, with the maximum volume being reached earlier and being lower
289 for higher temperatures.

290 Similar trends were found for grain water mass, which increased up to a plateau before the
291 end of biomass accumulation and then decreased during a dehydration phase up to a
292 minimum content (Fig. 2C and 3C). The level of the water mass plateau decreased from 15 to
293 32°C and then increased substantially between 32 and 36°C, for which the plateau was close
294 to that of the control (19°C) (Fig. 3C). The final water mass was similar for most treatments
295 (from 24°C to 36°C, Fig. 1C and 2C) and may not have been reached in the 15°C treatment
296 when the experiment ended. Note that the growth function used did not capture when water
297 content stabilised to a final level (Fig. 3C).

298 Finally, for all temperatures up to 32°C, cell proliferation over time (Fig. 2D and 3D) increased
299 at the beginning of grain development up to a maximum value, which was followed by a slight
300 decrease and then a stabilization of the cell number defining the final cell number. Increased
301 temperatures tended to accelerate early cell multiplication, reduce the duration of cell
302 proliferation and ultimately result in a lower final cell number. Cell proliferation and cell
303 number decrease was different for the extreme treatment at 36°C, for which cell proliferation
304 occurred at a low rate and for a comparatively long period.

305

306 *Increased growth rate partly compensated shorter duration of grain growth*
307 *under high temperatures*

308 Fitting the growth functions allowed the estimation of the maximal value of each trait in each
309 temperature treatment, as well as the growth rate and the duration of the growth processes
310 (Fig. 4). Higher temperature accelerated the accumulation of biomass, the increase in volume
311 and water accumulation, and cell proliferation in the grain up to a temperature threshold
312 ranging from 24 to 32°C depending on the process considered (Fig. 4A, D, G, J). At the same
313 time, the duration of these processes was reduced by higher temperatures (Fig. 4B, E, H, K).
314 Overall, maximum estimated values were reduced by higher temperatures (Fig. 4C, F, I, L).
315 Hence, estimated final dry biomass of grains from middle spikelets at the end of the grain
316 filling decreased from 44 mg at 15°C to 6 mg at 36°C. Grain volume reached an estimated
317 maximum from 67 mm³ at 15°C to 40 mm³ at 36°C, during the grain filling. Grains accumulated
318 up to a maximum of 35 mg of water at 15°C, 24 mg of water at 32°C. Under the extreme
319 treatment of 36°C, a surprisingly important water accumulation was observed, with a
320 maximum of 31 mg per grain. The number of cells in the endosperm increased up to a greatest
321 maximum of 90,000 at 15°C to a lowest maximum of 40,000 at 36°C.

322

323 *Different temperature responses across traits*

324 The temperature response of the growth rate and the reciprocal of the duration of each
325 considered process were well fitted (R^2 in Table 4) by the same response function [Eq.1] (Fig.
326 5; Table 4). This function estimated optimal temperatures (T_{opt}) between 25.0 and 29.3°C for
327 the growth rates of the studied processes and between 29.9 and 45.0°C for the reciprocals of
328 their durations. Maximal estimated temperatures (T_{max}) varied between 37.2 and 43.7°C for
329 growth rates and between 39.4 and 66.1°C for duration reciprocals. Note that for all the
330 studied traits, the temperature responses could reasonably be deemed linear between 15°C
331 and 30°C or a bit more in some cases (Fig. 5), thus allowing the use of the usual additive
332 formalism of thermal time in these conditions.

333 The response of duration reciprocal for grain biomass accumulation is of particular interest to
334 crop modellers and it can be used to deduce the formalism for thermal time during the grain
335 filling period. The optimum temperature could not be properly estimated in this study and
336 may be outside our experimental temperature range, but no clear change in the duration of

337 biomass accumulation was observed between 32°C and 36°C, and no treatment over 36°C was
338 tested (Fig. 5B). This explains why the standard deviations for the estimated optimum and
339 maximum temperatures are important compared to standard deviations estimated for the
340 other traits (Table 4).

341 To enable comparisons between different physiological processes, the temperature responses
342 of growth rates and of duration reciprocals of the four processes studied were normalized at
343 19°C (Fig. 6). The temperature responses of both growth rates and reciprocals of duration had
344 similar values of optimum and maximum temperatures for grain volume, water mass and
345 endosperm cell proliferation (Fig. 6A and 6B; Table 4). The lowest growth rate was found for
346 biomass accumulation with an optimal temperature of 24.9°C and a maximal temperature of
347 38.2°C. Cardinal temperatures of the reciprocal of the duration for the biomass accumulation
348 could not be estimated properly as treatment with higher temperatures would have been
349 required.

350 Regardless of the trait, the amplitudes of the temperature responses were greater for
351 reciprocals of duration (Fig. 6B) than for growth rates (Fig. 6A) with a normalisation at 19°C.
352 For example, for biomass accumulation at the optimum temperature, reciprocals of duration
353 were 2.7-fold than at 19°C (Fig. 6B), compared to < 1.2-fold greater for the growth rate (Fig.
354 6A). Variations of amplitude were also observed among traits. For instance, the reciprocal for
355 the duration of endosperm cell proliferation was 1.9-fold greater than the one at 19°C (Fig.
356 6B) whereas for biomass accumulation it was 2.7-fold greater than the one at 19°C.

357 Discussion

358
359 High temperatures were applied from anthesis to maturity on whole plants. Thus, all
360 processes at the whole plant level (e.g. leaf photosynthesis, senescence, global respiration,
361 remobilization of stem reserves...) are likely to have been affected directly and thus may have
362 impact grain growth indirectly. While the objective of the study is to investigate the sensitivity
363 to high temperatures of grain growth and associated processes, the results will be discussed
364 independently of the direct or indirect causal physiological sources of the response and
365 focused on the grain scale.

366

367 *The rate of grain biomass accumulation linearly increased up to 25°C while the*
368 *reciprocal of its duration linearly increased up to at least 32°C*

369 In our experimental conditions, when temperature increased from 15°C to 36°C, the final dry
370 biomass of the grains continuously decreased (Fig. 1A, Fig. 4A). This result was expected and
371 consistent with the literature (e.g. Sofield *et al.*, 1977; Wardlaw *et al.*, 1980; Farooq *et al.*,
372 2011). The optimal temperature for the rate of grain filling was estimated to 24.9°C (Table 4),
373 which is higher than those reported in the literature: 20.7°C (Porter and Gawith, 1999, who
374 summarized the results from 7 studies), or between 18 and 22°C (Farooq *et al.*, 2011). The
375 possible discrepancy between the literature and the present study may be related to (i)
376 possible differences due to consideration of air or grain temperatures (but it is unlikely to fully
377 explain such differences), (ii) the great heterogeneity of temperature treatments among the
378 different studies, and especially the timing, duration and intensity (moderate vs heat shock)
379 of applied high temperatures, or possibly (iii) genotypic differences, as genetic variability has
380 been reported in wheat for cardinal temperatures of response curves for grain filling (Wardlaw
381 *et al.*, 1989 ; Slafer and Rawson, 1995b;). In our experimental conditions, the higher optimum
382 temperature response of grain filling may be inherent to the genotype SxB049, a warm-
383 adapted genotype that has been shown to tolerate thermal and water stress (Pinto *et al.*,
384 2010). It is important to note however that in all these previous studies, the optimal
385 temperature for grain filling was determined from the comparison between only 2 to 3
386 temperature treatments. To our knowledge, no meta-analysis, generalizing in a formal way
387 across a number of independent experiments (such as in Poorter *et al.*, 2010) is available for
388 the effect of temperature on wheat grain dry biomass accumulation.

389 The response of the grain final dry biomass to high temperature is the result of the response
390 of both duration and rate of dry mass accumulation to heat. Between 15°C and 25°C, the
391 duration of the dry biomass accumulation duration was shortened by increased temperatures
392 while the rate of dry matter accumulation increased (Fig. 4; Table 4). This was however not
393 enough to compensate the shortening of the grain filling period, and overall higher
394 temperatures resulted in smaller final grain biomass. On the contrary, above 25°C, both the
395 duration and the rate of grain dry biomass accumulation decreased. The duration of grain dry
396 biomass actually decreased constantly between 15 and 32°C. This result is consistent with the
397 literature (Sofield *et al.*, 1977; Jenner, 1994) showing that duration and rate of grain filling

398 display different types of response to variation in temperature in a range between 20 and
399 40°C. Moreover, the genotypic tolerance to high temperatures during grain filling has been
400 associated with an increased rate of grain filling compensating the reduced duration of grain
401 filling (Wardlaw and Moncur, 1995). This underlines the need to study specifically the
402 response to elevated temperatures of both rate and duration of the grain filling.

403 The estimated optimal temperature for the grain growth rate was 24.9°C (Table 4). Sofield *et*
404 *al.* (1977) suggest that the sensitivity to temperature of the grain filling rate could be
405 influenced by the number of grains. In our experimental conditions, the grain number per
406 spike was not modified by temperature as the plants were moved to the “treatment” chamber
407 2 days after anthesis when the effect of elevated temperatures on grain number per spike is
408 null or very weak (Prasad *et al.*, 2015). Unfortunately, for the duration of grain dry biomass
409 accumulation, our data do not allow to determine with accuracy whether the grain filling
410 duration still decreased or stagnated over 32°C. Moreover, note that our data do not allow
411 the estimation of the base temperature reliably, as the minimum temperature tested was
412 15°C.

413 One of the aims of our study was to define the temperature range where the rate and the
414 reciprocal of duration of grain dry matter accumulation increase linearly with the
415 temperature. This is the most important assumption for the use of the linear “thermal time
416 model” (Monteith, 1984) to determine the duration of the grain filling period. While there is
417 a statistical uncertainty around the estimated optimum temperature and the response for a
418 temperature above 32°C (Fig. 5B), our results clearly show that the response of grain dry
419 biomass accumulation to temperature can be considered linear between 15 and 32°C (Fig. 5B).

420

421 *Processes related to dry matter accumulation and expansion in the grain have*
422 *different sensitivities to heat*

423

424 Apart from accumulation of dry matter in the grain, other processes contribute to grain
425 growth. These processes include cell proliferation in the endosperm that takes place during
426 the early phase of grain development, and the accumulation of water which results in an
427 increase in the grain volume via cell expansion.

428 Temperature increases resulted in a decrease in traits relative to cell proliferation and
429 expansion growth, i.e. the maximum values of the volume, water quantity and number of cells

430 in the endosperm (Fig. 4A). Similar effects of temperature were previously observed for the
431 maximum number of cells in the albumen (Commuri and Jones, 1999; Girousse *et al.*, 2018).
432 In our experimental conditions, both the rate of increase and the reciprocal of duration for all
433 the traits relative to cell proliferation and grain expansion had an optimal temperature around
434 30°C (from 27.8 to 31.9°C; Table 4; Fig. 5). Above this optimum temperature, the rate slowed
435 down while the duration increased but allowing some compensation (Fig. 4). To our
436 knowledge, this is the first time that TRC have been established for such processes. Overall,
437 the rate of all the processes relative to cell proliferation and organ expansion had a similar
438 sensitivity to temperature. The same was observed for the reciprocal of their duration, which
439 responded similarly to temperature. Physiological processes presenting a common response
440 to temperature have previously been found for other traits in various crops, including wheat
441 (Parent *et al.*, 2010; Parent and Tardieu, 2012).

442 The temperature response of grain dry matter accumulation (growth rate and reciprocal of
443 duration) adequately followed a modified Arrhenius function (Fig. 6A and 6B) as previously
444 assumed for the reciprocal of duration of the post-anthesis development phase that finished
445 when grains reach their final biomass (Fig. 4B of Wang *et al.*, 2017). In Wang *et al.* (2017), the
446 temperature response for post-anthesis development was obtained with data from field
447 experiments (Reynolds *et al.*, 1994; White *et al.*, 2011) and semi-controlled conditions in
448 outdoor climate chambers (Triboi *et al.*, 2003). In these experiments, temperatures ranged
449 between 12.5 and 31°C, which was not enough to properly assess the optimal temperature
450 (T_{opt}) that was estimated at 33°C (Fig. 7; Wang *et al.*, 2017). Data from the present study
451 suggest that the optimal temperature for the reciprocal of duration of grain growth is likely to
452 be above 33°C (Fig. 5A), at least for the genotype tested. But here too, treatments were not
453 hot enough to allow a proper estimate of T_{opt} .

454 In our experimental conditions, the cardinal temperatures of the response of dry matter
455 accumulation were substantially different from those of processes related to cell proliferation
456 and grain expansion (Fig. 6; Table 4). The growth rate of traits contributing to the grain cell
457 proliferation and expansion generally had a substantially higher optimum (between 27.8 and
458 29.3°C) and maximum (between 38.2 and 43.7°C) temperatures than the rate of dry biomass
459 accumulation (optimal temperature of 25.0°C and maximum temperature of 37.2°C). While
460 the optimum and maximum temperatures of the reciprocal of duration for dry matter

461 accumulation could not be estimated properly, they were much higher than for the variables
462 related to cell proliferation and expansive growth (between 29.9 and 31.9°C; Fig. 5; Table 4).
463 The contrast in temperature responses between traits relative to grain expansion and grain
464 biomass accumulation is particularly well illustrated at 36°C where the grain volume and the
465 accumulated water in the grain increased at rates only slightly lower than at 30-32°C even
466 though accumulation of dry matter in the grain severely dropped to close to zero at 36°C (Fig.
467 5). One may assume that this difference comes from the temperature dependency of later
468 processes related to the grain filling, and in particular starch synthesis. According to the model
469 proposed by Pan *et al.* (2007; Eq[2]), the rate of grain starch accumulation results from the
470 product of two components: (i) a developmental component that corresponds to a potential
471 accumulation rate times a factor that depends on the post-anthesis thermal time and (ii) a
472 direct effect of temperature on the biochemistry of starch accumulation. Following this
473 framework, we assumed i) the developmental part to be reflected by the rate of volume
474 expansion, which sets the capacity of the grain (in terms of both (a) the cytoplasmic volume
475 defining possible sterical/mechanical constraints on grain filling and starch accumulation, and
476 (b) the amount of cells and hence of glucose transport and starch synthesis machinery); and
477 (ii) the biochemical part to be substantially driven by the main enzyme controlling starch
478 synthesis (i.e. Soluble Starch Synthase) (Keeling *et al.*, 1993; Boehlein *et al.*, 2019), that is
479 highly temperature dependent above 30°C (Keeling *et al.*, 1993; 1994). We propose that the
480 rate of dry biomass accumulation ($\frac{dMg}{dt}(t, T)$) over time (t) and in response to the
481 temperature (T) depends on the developmental volume component ($\frac{dV}{dt}(t, T)$) and the
482 biochemical components ($f(T)$) as follows:

483

$$484 \quad \frac{dMg}{dt}(t, T) = \frac{dV}{dt}(t, T) \times f(T) \quad [\text{Eq. 2}]$$

485 The dependence to temperature of the developmental component is given by the relative
486 temperature response curve established in Fig.6 and the dependence to temperature of the
487 SSS relative activity is taken from Keeling *et al.* (1993; Fig.1, 120 min temperature treatment).
488 The prediction of the model in [Eq. 2], is close to the experimental curve of the temperature
489 response of the rate of grain dry biomass accumulation ($R^2=0.76$; Fig.8). While equation 2 does
490 not correspond to a process-based model nor a mechanistic one, this phenomenological
491 approach suggests that considering the temperature responses of the morphogenetic

492 processes in the early phase and the temperature response of the later grain filling processes
493 may confer a robust estimate of grain biomass kinetic in response to fluctuating temperatures
494 from outdoor conditions.

495 Differences in temperature response of different processes have been found in other studies.
496 For instance, some morphological and physiological processes differ in their temperature
497 responses in alfalfa and tall fescue (Zaka *et al.*, 2017). In wheat, Slafer and Rawson (1995a, b)
498 found that the cardinal temperatures of the growth rates of different processes (e.g. leaf
499 appearance, internode growth) tend to increase during the plant development. Our results
500 revealed that within the same organ, the wheat grain, and during the same development
501 phase (grain development), synchronized processes can have different temperature
502 sensitivities. In particular, the process of dry matter accumulation and water accumulation in
503 the grain appear to have different optimal temperatures for both their rate of accumulation
504 and the reciprocal of their duration.

505

506

507 *Challenges related to studies and simulations of heat stress impacts*

508 In addition to timing and intensity, plant responses to temperature depend on i) the duration
509 of exposure to high temperatures (Tashiro and Wardlaw, 1990; Stone and Nicolas, 1995;
510 Prasad and Djanaguiramam, 2014; Chenu and Oudin, 2019), and as discussed before, ii) the
511 physiological processes involved. Short-term exposures to high temperature, for example in
512 the case of heat shock, can trigger metabolic/physiological changes at all spatial scales
513 (molecular, cellular, tissue or organ) within a few hours (Wahid *et al.*, 2007; Wang *et al.*, 2011).
514 If happening relatively early during the plant growth cycle, these modifications may enhance
515 the plant ability to cope with higher temperature exposures at later stages, ability also known
516 as acclimation (Wang *et al.*, 2011; Barlow *et al.*, 2015). Under our experimental conditions,
517 temperature treatments were applied from 2 days after anthesis to the grain maturity, i.e.
518 durations of exposition lasted between 2 and 9 weeks at 36 and 15°C, respectively. The TRC
519 obtained were thus integrated responses to long-term high temperature, which can differ
520 from short-term responses. In addition to metabolic or physiological modifications (such as
521 photosynthesis, respiration, senescence...), such long-term exposures to high temperatures
522 may also induce a degree of acclimation, which can occur within a few days (Sage and Kubien,

523 2007) and result in morphological or anatomical changes (Atkin *et al.*, 2006; Gorsuch *et al.*,
524 2010). Our data do not allow to assess such modifications. However, the durations of high
525 temperature exposures were likely long enough to induce feedback between the various
526 processes occurring in the different organs and tissues (including photosynthesis and
527 respiration) and then to contribute to integrated responses (Atkin *et al.*, 2006). Although
528 difficult to demonstrate, this effect of acclimation cannot be discarded; ignoring the
529 acclimation potential of plants could lead to an overestimation of the responses to high
530 temperature on the various developmental processes contributing to final grain biomass
531 (Perdomo *et al.*, 2015).

532 Grains do not respond in the same way at high temperature as opposed to control
533 temperature depending on their position on the spike (Tashiro and Wardlaw, 1990). The
534 stability of the response curves should be checked as a function of the position of the grains
535 on the ear. Here, only the basal grains taken from the central spikes of the spikes were studied.
536 On a given spike, these grains are the largest (i.e. Bremner, 1972; Baillot *et al.*, 2018) and have
537 higher sink forces than other grains on the same spike. The response curves established may
538 thus vary with the position of the grains on the spike.

539 Another challenge when studying heat stress relates to simply measuring the temperature.
540 Most studies record air temperature, rather than the organ (grain) temperatures (Jamieson *et al.*
541 *et al.*, 1995; Bonhomme, 2000). Temperature responses presented in this study relate to grain
542 temperature, even if small yet statistically significant differences (the slope of the regression
543 between the two temperatures was equal to 0.96, with an intercept of 0.61°C) were observed
544 between atmospheric and organ temperature in our well-watered and low-VPD conditions
545 (Fig. S1B). However, this is unlikely to be the case in a large number of field conditions,
546 especially during the grain filling period when heat and drought are the most frequent (Chenu
547 *et al.*, 2013; Ababaei and Chenu, 2020).

548

549 Overall, this study provides for the first time response curves for temperatures between 15°C
550 and 36°C established under identical growing conditions, with steady temperatures (Fig. 5;
551 Table 4). Such results can be used to improve current crop models in regards to how
552 temperature impacts the grain filling (Chenu *et al.*, 2017). Besides by defining a novel
553 framework to model grain biomass accumulation based on the grain volume expansion and

554 biochemical grain filling processes, our findings can help to elaborate more robust models for
555 grain dry biomass accumulation in particular in non-steady conditions. This can also assist
556 mechanistic investigation and possible genetic selection. For instance, this focus should be not
557 only on the biochemistry of grain filling but also on the morphogenetic processes that lead to
558 volumic growth of the grain. Following this, more work is needed to fully understand and
559 simulate the physiological processes and mechanisms involved. Moreover, the dependency of
560 these processes on the timing, intensity and duration of the heat events and other influential
561 environment factors (e.g. edaphic and atmospheric water deficits, atmospheric CO₂ content).
562

563 Supplementary data

564

565 **Fig. S1.** Relationship between air and grain temperatures

566 **Fig. S2.** Relevance of the adjusted growth functions (analysis of residuals)

567 Acknowledgments

568 The authors want to thank N. Allard for her precious technical assistance, F. Piraux and E.
569 Héritier for their assistance in statistical analysis. The authors are grateful to Dr B. Moulia for
570 useful comments on the manuscript and to Dr M. Reynolds (CIMMYT) for his kind gift of
571 offspring SxB49. Partial financial support was provided by the FSOV “HeatWheat” research
572 project.

573

References

- 1 **Ababaei B, Chenu K.** 2020. Heat shocks increasingly impede grain filling but have little effect
2 on grain setting across the Australian wheatbelt. *Agricultural and Forest Meteorology*
3 **284**:107889.
- 4 **Angus JF, Mackenzie DH, Morton R, Schafer, C. A.** 1981. Phasic development in field crops II.
5 Thermal and photoperiodic responses of spring wheat. *Field Crops Research* **4**, 269–283.
- 6 **Asseng S, Ewert F, Martre P, et al.** 2015. Rising temperatures reduce global wheat
7 production. *Nature Climate Change* **5**, 143–147.
- 8 **Atkin OK, Loveys BR, Atkinson LJ, Pons TL.** 2006. Phenotypic plasticity and growth
9 temperature understanding interspecific variability. *Journal of Experimental Botany* **57**, 267-
10 281.
- 11 **Baillet N, Girousse C, Allard V, Piquet-Pissaloux A, Le Gouis J.** 2018. Different grain-filling
12 rates explain grain weight differences along the wheat ear. *PLoS ONE* **13**, e0209597.
- 13 **Barlow KM, Christy BP, O’Leary GJ, Riffkin PA, Nuttall JG.** 2015. Simulating the impact of
14 extreme heat and frost events on wheat crop production: A review. *Field Crops Research*
15 **171**, 109-119.
- 16 **Bates D, DebRoy S, Gay DM.** 2007. nls: Nonlinear Least Squares. *R Core Team*.
- 17 **Bernacchi CJ, Singsaas EL, Pimentel C, Portis AR, Long SP.** 2001. Improved temperature
18 response functions for models of Rubisco-limited photosynthesis. *Plant, Cell and*
19 *Environment* **24**, 253-259.
- 20 **Bhullar SS, Jenner CF.** 1986. Effects of a brief episode of elevated temperature on grain
21 filling in wheat ears cultured on solutions of sucrose. *Australian Journal of Plant Physiology*
22 **13**, 617-626.
- 23 **Bonhomme R.** 2000. Bases and limits to using ‘degree.day’ units. *European Journal of*
24 *Agronomy* **1**, 1-10.

- 27 **Bremner PM.** 1972. Accumulation of dry-matter and nitrogen by grains in different
28 positions. *Australian Journal of Biological Sciences* **25**, 657-668.
- 29 **Brocklehurst PA.** 1977. Factors controlling grain weight in wheat. *Nature* **266**, 348-349.
- 30 **Boehlein SK, Liu P, Webster A, et al.** 2019. Effects of long-term exposure to elevated
31 temperature on *Zea mays* endosperm development during grain fill. *The Plant Journal* **99**,
32 23-40.
- 33 **Challinor AJ, Watson J, Lobell DB, Howden SM, Smith DR, Chhetri N.** 2014. A meta-analysis
34 of crop yield under climate change and adaptation. *Nature Climate Change* **4**, 287–291.
- 35 **Chenu K, Deihimfard R, Chapman SC.** 2013. Large-scale characterization of drought pattern:
36 a continent-wide modelling approach applied to the Australian wheatbelt spatial and
37 temporal trends. *New Phytologist* **198**, 801-820.
- 38 **Chenu K, Porter JR, Martre P, Basso B, Chapman SC, Ewert F, Bindi M, Asseng S.** 2017.
39 Contribution of Crop Models to Adaptation in Wheat. *Trends in Plant Science* **22**, 472–490.
- 40 **Chenu K, Oudin F.** 2019. Heat impact on yield components of fertile primary tillers in wheat
41 can inform crop modelling for future climates. Australian Agronomy Conference (25-29
42 August, Wagga Wagga, Australia).
- 43 **Chowdhury SI, Wardlaw IF.** 1978. The effect of temperature on kernel development in
44 cereals. *Australian Journal of Agricultural Research* **29**, 205-223.
- 45 **Commuri PD, Jones RJ.** 1999. Ultrastructural characterization of maize (*Zea mays* L.) kernels
46 exposed to high temperature during endosperm cell division. *Plant, Cell & Environment* **22**,
47 375-385.
- 48 **Cox C.** 2005. Delta Method. In: *Encyclopedia of Biostatistics*, p. American Cancer Society.
- 49 **Dowd WW, King FA, Denny MW.** 2015. Thermal variation, thermal extremes and the
50 physiological performance of individuals. *The Journal of Experimental Biology* **218**, 1956-
51 1967.
- 52 **Egli DB.** 1998. *Seed biology and the yield of grain crops*. Wallingford: CAB International.

53 **Farooq M, Bramley H, Palta JA, Siddique KHM.** 2011. Heat stress in wheat during
54 reproductive and grain-filling phases. *Critical Review in Plant Sciences* **30**, 491-507.

55 **Girousse C, Roche J, Guérin C, Le Gouis J, Balzeugue S, Mouzeyar S, Bouzidi MF.** 2018.
56 Coexpression network and phenotypic analysis identify metabolic pathways associated with
57 the effect of warming on grain yield components. *PLoS ONE* **13**, e0199434.

58 **Gorsuch PA, Pandey S, Atkin OK.** 2010. Temporal heterogeneity of cold acclimation
59 phenotypes in *Arabidopsis* leaves. *Plant, Cell and Environment* **33**, 244-258.

60 **Granier C, Tardieu F.** 1998. Is thermal time adequate for expressing the effects of
61 temperature on sunflower leaf development? *Plant, Cell and Environment* **21**, 695-703.

62 **Granier C, Massonnet C, Turc O, Muller B, Chenu K, Tardieu F.** 2002. Individual leaf
63 development in *Arabidopsis thaliana*: a stable thermal-time-based programme. *Annals of*
64 *Botany* **89**, 595-604.

65 **Hawker JS, Jenner CF.** 1993. High-temperature affects the activity of enzymes in the
66 committed pathway of starch synthesis in developing wheat endosperm. *Australian Journal*
67 *of Plant Physiology* **20**, 197-209.

68 **Hunt R.** 1979. *Plant Growth Analysis: The Rationale Behind the Use of the Fitted*
69 *Mathematical Function.* *Annals of Botany* **43**, 245–249.

70 **IPCC, 2019.** *Climate Change and Land. An IPCC Special Report on climate change,*
71 *desertification, land degradation, sustainable land management, food security, and*
72 *greenhouse gas fluxes in terrestrial ecosystems, 2019.*

73 **Jamieson PD, Brooking IR, Porter JR, Wilson DR.** 1995. Prediction of leaf appearance in
74 wheat – a question of temperature. *Field Crops Research* **41**, 35-44.

75 **Jenner CF.** 1994. Starch synthesis in the kernel of wheat under high-temperature conditions.
76 *Australian Journal of Plant Physiology* **21**, 791-806.

77 **Keeling CF, Bacon PJ, Holt DC.** 1993. Elevated temperature reduces starch deposition in
78 wheat endosperm by reducing the activity of soluble starch synthase. *Planta* **191**, 324-348.

79 **Keeling PL, Banisadr R, Barone L, Wasserman BP, Singletary GW.** 1994. Effect of
80 temperatures on enzymes in the pathway of starch biosynthesis in developing wheat and
81 maize grain. *Australian Journal of Plant Physiology* **21**, 807-827.

82 **Lebreton JD, Jolivet E, Millier C, Pavé A, Vila JP.** 1982. *Modèles dynamiques déterministes en*
83 *biologie*. Paris: Masson.

84 **Liu B, Liu L, Asseng S, Zhang D, Ma W, Tang L, Cao W, Zhu Y.** 2020. Modelling the effects of
85 post-heading heat stress on biomass partitioning, and grain number and weight of wheat.
86 *Journal of Experimental Botany* **71**, 6015-6031.

87 **Lizana XC, Riegel R, Gomez LD, Herrera J, Isla A, McQueen-Mason SJ, Calderini DF.** 2010.
88 Expansins expression is associated with grain size dynamics in wheat (*Triticum aestivum* L.).
89 *Journal of Experimental Botany* **61**, 1147-1157.

90 **Lobell DB, Hammer GL, Chenu K, Zheng B, McLean G, Chapman SC.** 2015. Global Change
91 *Biology* **21**, 4115-4127.

92 **Luo Q.** 2011. Temperature thresholds and crop production: a review. *Climatic Change* **109**,
93 583-598.

94 **Monteith JL.** 1984. Consistency and convenience in the choice of units for agricultural
95 science. *Experimental Agriculture* **20**, 105-117.

96 **Nadaud I, Girousse C, Debiton C, Chambon C, Bouzidi MF, Martre P, Branlard G.** 2010.
97 Proteomic and morphological analysis of early stages of wheat grain development.
98 *Proteomics* **10**, 2901-2910.

99 **Nagai T, Makino A.** 2009. Differences between rice and wheat in temperature responses of
100 photosynthesis and plant growth. *Plant and Cell Physiology* **50**, 744-755.

101 **Pan J, Zhu Y, Cao W.** 2007. Modeling plant carbon flow and starch accumulation in wheat.
102 *Field Crops Research* **101**, 276-284.

103 **Parent B, Turc O, Gibon Y, Stitt M, Tardieu F.** 2010. Modelling temperature-compensated
104 physiological rates, based on the co-ordination of responses to temperature of
105 developmental processes. *Journal of Experimental Botany* **61**, 2057-2069.

106 **Parent B, Tardieu F.** 2012. Temperature responses of developmental processes have not
107 been affected by breeding in different ecological areas for 17 crop species. *New Phytologist*
108 **194**, 760-774.

109 **Perdomo JA, Conesa MA, Ribas-Carbi M, Galmes J.** 2015. Effects of long-term individual and
110 combined water and temperature stress on the growth of rice, wheat and maize relationship
111 with morphological and physiological acclimation. *Physiologia Plantarum* **155**, 149-165.

112 **Pinheiro J, Bates D, DebRoy S, Sarkar D.** 2018. R Core Team (2018) nlme: linear and
113 nonlinear mixed effects models. R package version 3.1-131.1. *R software*.

114 **Pinto RS, Reynolds MP, Mathews KL, McIntyre L, Olivares-Villegas JJ, Chapman SC.** 2010.
115 Heat and drought adaptative QTL in a wheat population designed to minimize confounding
116 agronomic effects. *Theoretical and Applied Genetics* **121**, 1001-1021.

117 **Poorter H, Niinemets U, Walter A, Fiorani F, Schurr U.** 2010. A method to construct dose-
118 response curves for a wide range of environmental factors and plant traits by means of a
119 meta-analysis of phenotypic data. *Journal of Experimental Botany* **61**, 2043-2055.

120 **Porter JR, Gawith M.** 1999. Temperatures and the growth and development of wheat: a
121 review. *European Journal of Agronomy* **10**, 23-36.

122 **Porter JR, Semenov MA.** 2005. Crop responses to climatic variation. *Philosophical*
123 *Transactions of the Royal Society B-Biological Sciences* **360**, 2021–2035.

124 **Prasad PVV, Djanaguiraman M.** 2014. Response of floret fertility and individual grain weight
125 of wheat to high temperature stress: sensitive stages and thresholds for temperature and
126 duration. *Functional Plant Biology* **41**, 1261-1269.

127 **Prasad PVV, Djanaguiraman M, Perumal R, Ciampitti IA.** 2015. Impact of high temperature
128 stress on floret fertility and individual grain weight of grain sorghum: sensitive stages and
129 thresholds for temperature and duration. *Frontiers in Plant Science* **6**, 820.

130 **Ratkowsky DA.** 1990. *Handbook of nonlinear regression models*. New York: MARCEL DEKKER.

131 **Reynolds MP, Balota M, Delgado MIB, Amani I, Fischer RA.** 1994. Physiological and
132 morphological traits associated with spring wheat yield under hot, irrigated conditions.
133 *Australian Journal of Plant Physiology* **21**, 717-730.

134 **Reynolds M, Bonnett D, Chapman SC, Furbank RT, Manès Y, Mather DE, Parry MAJ.** 2011.
135 Raising yield potential of wheat. I. Overview of a consortium approach and breeding
136 strategies. *Journal of Experimental Botany* **62**, 439-452.

137 **Robert N, Huet S, Hennequet C, Bouvier A.** 1999. Methodology for choosing a model for
138 wheat kernel growth. *Agronomie* **19**, 405–417.

139 **Rosenzweig C, Jones JW, Hatfield JL, et al.** 2013. The Agricultural Model Intercomparison
140 and Improvement Project (AgMIP): Protocols and pilot studies. *Agricultural and Forest*
141 *Meteorology* **170**, 166–182.

142 **Sage RF, Kubien DS.** 2007. The temperature response of C-3 and C-4 photosynthesis. *Plant,*
143 *Cell and Environment* **30**, 1086-1106.

144 **Schulte PM, Healy TM, Fangué NA.** 2011. Thermal performance curves, phenotypic
145 plasticity, and the time scales of temperature exposure. *Integrative and Comparative Biology*
146 **51**, 691-702.

147 **Singh BK, Jenner CF.** 1982. A modified method for the determination of cell number in
148 wheat endosperm. *Plant Science Letters* **26**, 273-278.

149 **Slafer GA, Savin R.** 1991. Developmental base temperature in different phenological phases
150 of wheat (*Triticum aestivum* L.). *Journal of Experimental Botany* **42**, 1077-1082.

151 **Slafer GA, Rawson HM.** 1995a. Rates and cardinal temperatures for processes of
152 development in wheat: effects of temperature and thermal amplitude. *Australian Journal of*
153 *Plant Physiology* **22**, 913-926.

154 **Slafer GA, Rawson HM.** 1995b. Base and optimum temperatures vary with genotype and
155 stage of development in wheat. *Plant, Cell and Environment* **18**, 671-679.

156 **Sofield I, Evans LT, Cook MG, Wardlaw IF.** 1977. Factors influencing the rate and duration of
157 grain filling in wheat. *Australian Journal of Plant Physiology* **5**, 785-797.

158 **Spiertz JHJ, Hamer RJ, Xu H, Primo-Martin C, Don C, van der Putten PEL.** 2006. Heat stress
159 in wheat (*Triticum aestivum* L.) : effects on grain growth and quality traits. *European Journal*
160 *of Agronomy* **25**, 89-95.

161 **Stone PJ, Nicolas ME.** 1995. Effect of timing of heat stress during grain filling on two wheat
162 varieties differing in heat tolerance. 1. Grain growth. Australian Journal of Plant Physiology
163 **22**, 927-934.

164 **Tashiro T, Wardlaw IF.** 1990. The response to high-temperature shock and humidity changes
165 prior to and during the early stages of grain development in wheat. Australian Journal of
166 Plant Physiology **17**, 551-561.

167 **Triboi E, Martre P, Triboi-Blondel AM.** 2003. Environmentally-induced changes in protein
168 composition in developing grains of wheat are related to changes in total protein content.
169 Journal of Experimental Botany **54**, 1731-1742.

170 **Wahid A, Gelani S, Ashraf M, Foolad MR.** 2007. Heat tolerance in plants: an overview.
171 Environmental and Experimental Biology **61**, 199-223.

172 **Wang X, Cai J, Jiang D, Liu F, Dai T, Cao W.** 2011. Pre-anthesis high-temperature acclimation
173 alleviates damage to the flag leaf caused by post-anthesis heat stress in wheat. Journal of
174 Plant Physiology **168**, 585-593.

175 **Wang E, Martre P, Zhao Z, et al.** 2017. The uncertainty of crop yield projection is reduced by
176 improved temperature response functions. Nature Plants **3**, 17102.

177 **Wardlaw IF, Sofield I, Cartwright PM.** 1980. Factors limiting the rate of dry-matter
178 accumulation in the grain of wheat grown at high-temperature. Australian Journal of Plant
179 Physiology **7**, 387-400.

180 **Wardlaw IF, Dawson IA, Munibi P.** 1989. The tolerance of wheat to high-temperatures
181 during reproductive growth. 2. Grain filling. Australian Journal of Agricultural Research **40**,
182 15-24.

183 **Wardlaw IF, Montcur L.** 1995. The response of wheat to high-temperature following
184 anthesis. 1. The rate and duration of grain filling. Australian Journal of Plant Physiology **22**,
185 391-397.

186 **Werker A.** 1997. Modelling Asymmetrical Growth Curves that Rise and then Fall:
187 Applications to Foliage Dynamics of Sugar Beet (*Beta vulgaris* L.). Annals of Botany **79**, 657–
188 665.

189 **White JW, Kimball BA, Wall GW, Ottman MJ, Hunt LA.** 2011. Responses of time of anthesis
190 and maturity to sowing dates and infrared warming in spring wheat. *Field Crops Research* **12**,
191 213-222.

192 **Winsor CP.** 1932. The Gompertz Curve as a Growth Curve: Proceedings of the National
193 Academy of Sciences of the United States of America **18**, 1-8.

194 **Zahedi H, Sharma R, Jenner CF.** 2003. Effects of high temperature on grain growth and on
195 the metabolites and enzymes in the starch-synthesis pathway in the grains of two wheat
196 cultivars differing in their responses to temperature. *Functional Plant Biology* **30**, 291-300.

197 **Zaka S, Ahmed LQ, Escobar-Guetirez AJ, Gastal F, Julier B, Louarn G.** 2017. How variable are
198 non-linear developmental responses to temperature in two perennial forage species?
199 *Agricultural and Forest Meteorology* **232**, 433-442.

200 **Zheng B, Chenu K, Dreccer MF, Chapman SC.** 2012. Breeding for the future: what are the
201 potential impacts of future frost and heat events on sowing and flowering time
202 requirements for Australian bread wheat (*Triticum aestivum*) varieties? *Global Change*
203 *Biology* **18**, 2899-2914.

204
205 **Zheng B, Chenu K, Chapman SC.** 2016. Velocity of temperature and flowering time in wheat
206 – assisting breeders to keep pace with climate change. *Global Change Biology* **22**, 921-933.

207
208

1 Tables

2

3 **Table 1.** Environmental conditions in each growth chamber. For control treatment (19°C), the
4 values correspond to the average of the five successive experiments conducted in the same
5 growth chamber. Average and standard deviations of air and grain temperatures, relative
6 humidity (RH), vapour pressure deficit (VPD) during the day were calculated from anthesis to
7 maturity.

8

9

Temperature setting (°C)	Air temperature (°C)	Grain temperature (°C)	Air RH (%)	Mean VPD (kPa)
15	15.5 ± 1.3	15.5 ± 1.1	76.3 ± 3.1	0.4 ± 0.1
19	19.4 ± 1.3	19.1 ± 1.9	77.2 ± 3.0	0.5 ± 0.1
24	24.8 ± 1.5	24.4 ± 1.5	79.0 ± 3.4	0.7 ± 0.1
29	29.4 ± 0.9	28.9 ± 0.9	77.5 ± 3.4	0.9 ± 0.1
32	31.9 ± 1.3	31.7 ± 1.2	76.5 ± 4.2	1.1 ± 0.2
36	36.1 ± 0.7	35.2 ± 0.9	66.6 ± 12.4	1.7 ± 0.3

10

11

1

2 **Table 2.** Characteristics of the growth functions used to fit observations from anthesis to maturity for each trait and each temperature. For each
 3 trait, the function and variance structure used to model the trait response to temperature are presented as well as the equations used to calculate
 4 the growth rate, duration and maximum value.

5

Trait	Type of function	Equation	Variance structure	Growth rate	Duration	Maximum value
Grain dry biomass	Gompertz ¹	$ae^{-e^{-\frac{t-b}{c}}}$	Power	$\frac{a}{4c}$	$4c$	a
Grain volume	Gamma with constant ²	$ae^{-ct}t^{b-1} + d$	Exponential	$\frac{ac}{b-1}(e^{1-b}\left(\frac{b-1}{c}\right)^{b-1} + d)$	$\frac{b-1}{c}$	$ae^{1-b}\left(\frac{b-1}{c}\right)^{b-1} + d$
Grain water mass	Segmented Linear function	$\begin{cases} ax, & 0 \leq x \leq b \\ ab, & b < x \leq c \\ ab + d(x-c), & x > c \end{cases}$	Exponential	a	b	ab
Endosperm cell number	Gompertz with maxima ³	$ae^{(b(x-c)-\frac{b}{d})(1-e^{-d(x-c)})}$	Exponential	$\frac{a}{c}$	c	a

6 ¹ Winsor (1932); ² Lebreton et al. (1982); ³ Werker (1997)

7

1

2 **Table 3.** Equation and coefficient of determination (R^2) for the fits between observations and
3 predictions from non-linear mixed models for the four studied traits in all temperature
4 treatments

5

Trait	Observed – predicted relationship	R^2
Grain dry biomass	$-0.12 + 1.01x$	0.92
Grain volume	$0.25 + 0.99x$	0.86
Grain water mass	$0.75 + 0.99x$	0.86
Endosperm cell number	$0.04 + 0.99x$	0.93

6

7

8

1

2 **Table 4.** Coefficient of determination (R^2), estimated optimum temperature (T_{opt}), maximum temperature (T_{max}) and normalisation coefficient
 3 a for the growth rate and the reciprocal of the duration for the studied traits. For a given trait, growth rate is expressed in unit per day and
 4 reciprocal of duration in day⁻¹. The estimated temperatures and the coefficient a are calculated with n/s according to [Eq.1] and presented with
 5 their standard deviations.

6

7

Trait (per grain)	R^2		T_{opt} (°C)		T_{max} (°C)		a (same unit as rate or 1/duration)	
	Growth rate	1/Duration	Growth rate	1/Duration	Growth rate	1/Duration	Growth rate	1/Duration
Dry biomass accumulation	0.90	0.99	25.0 ± 1.0	45.0 ± 12.0	37.3 ± 0.9	66.1 ± 21.2	1.21 ± 0.07	0.070 ± 0.02
Volume increase	0.96	0.98	29.3 ± 0.4	31.9 ± 0.6	39.2 ± 0.9	39.9 ± 1.3	4.0 ± 0.2	0.094 ± 0.004
Water accumulation	0.74	0.76	28.9 ± 1.4	29.8 ± 1.1	43.7 ± 4.8	38.6 ± 2.1	3.5 ± 0.3	0.14 ± 0.02
Endosperm cell proliferation	0.56	0.85	27.8 ± 1.5	29.9 ± 0.9	38.2 ± 2.3	39.4 ± 2.0	9936 ± 1360	0.16 ± 0.01

8

1
2

Figure legends

Fig. 1. Effects of high post-anthesis temperature on final grain dry biomass (A), volume (B), water mass (C), and endosperm cell number (D). Each bar represents the mean \pm standard deviation of final values (n=24 to 27 for grain volume, 10 to 17 for grain dry biomass, water mass and endosperm cell number); measurements were taken at grain maturity, except for endosperm cell number. For this trait, final values were obtained during the filling phase when cell number is set (between 300 and 400 °Cd after anthesis). For each trait, the different letters above vertical bars indicate significant differences between temperature treatments at a 5% level (SNK test).

Fig. 2. Impact of temperature on the normalized grain dry biomass (A), volume (B), water mass (C) and endosperm cell number (D) during grain development. Each point corresponds to the mean of measurements on 12 grains (2 grains and 6 spikes) for grain dry biomass and endosperm cell number, or on 24 grains (4 grains and 6 spikes) for grain water mass and volume. All control treatments (19°C) were averaged together. Within each experiment, data were normalized by the maximum mean value of the control (19°C) of the respective experiments.

Fig. 3. Growth functions fitted to observed values of grain dry biomass (A), volume (B), water mass (C), and endosperm cell number (D) over time after anthesis. Growth functions were selected on the base of growth curves obtained at 19°C (control temperature), and applied to other temperatures (Table 2) in a non-linear mixed model fitted on the whole dataset (i.e. all treatments at once).

Fig. 4. Temperature responses of growth rates, durations and maximal values for grain dry biomass (A to C), volume (D to F), water mass (G to I) and endosperm cell division (J to L). The values of these traits were estimated based parameters from the growth curves (Table 2) fitted with a non-linear mixed model. Lines in A, D, G and J represent the fit of the response function [Eq.1]. Error bars correspond to two times the standard deviation, on either side of the mean.

1

2 **Fig. 5.** Temperature response of growth rates and reciprocals of the duration for the grain dry
3 biomass (A,B), cell volume (C,D), water mass (E,F), and endosperm cell number (G,H). Solid
4 lines represent the fit of the response function [Eq.1]. Dashed lines (B, D, F, H) represent the
5 linear regression fitted between 15°C to 32°C, for which the equation is given. Error bars
6 correspond to two times the standard deviation, on either side of mean.

7

8 **Fig. 6.** Normalized temperature responses of growth rates (A) and reciprocal of growth
9 durations (B) according to the response function [Eq.1] for the grain dry biomass
10 accumulation, increase in volume, water accumulation and endosperm cell proliferation.
11 Responses were normalized at 19°C. Solid lines indicate the range of temperatures for which
12 observations were collected.

13

14 **Fig. 7.** Normalized temperature response of the reciprocal of duration of grain growth
15 processes according to the response function [Eq.1]. Data concerning grain dry biomass
16 accumulation, grain volume, water mass and endosperm cell numbers are those obtained
17 from the experiments from this study. The post-anthesis development phase from Wang *et*
18 *al.* (2017) corresponds to the duration between anthesis and when grains reach their final dry
19 biomass. Response were normalized at 19°C. Solid lines indicate the range of temperatures
20 for which observations were collected.

21

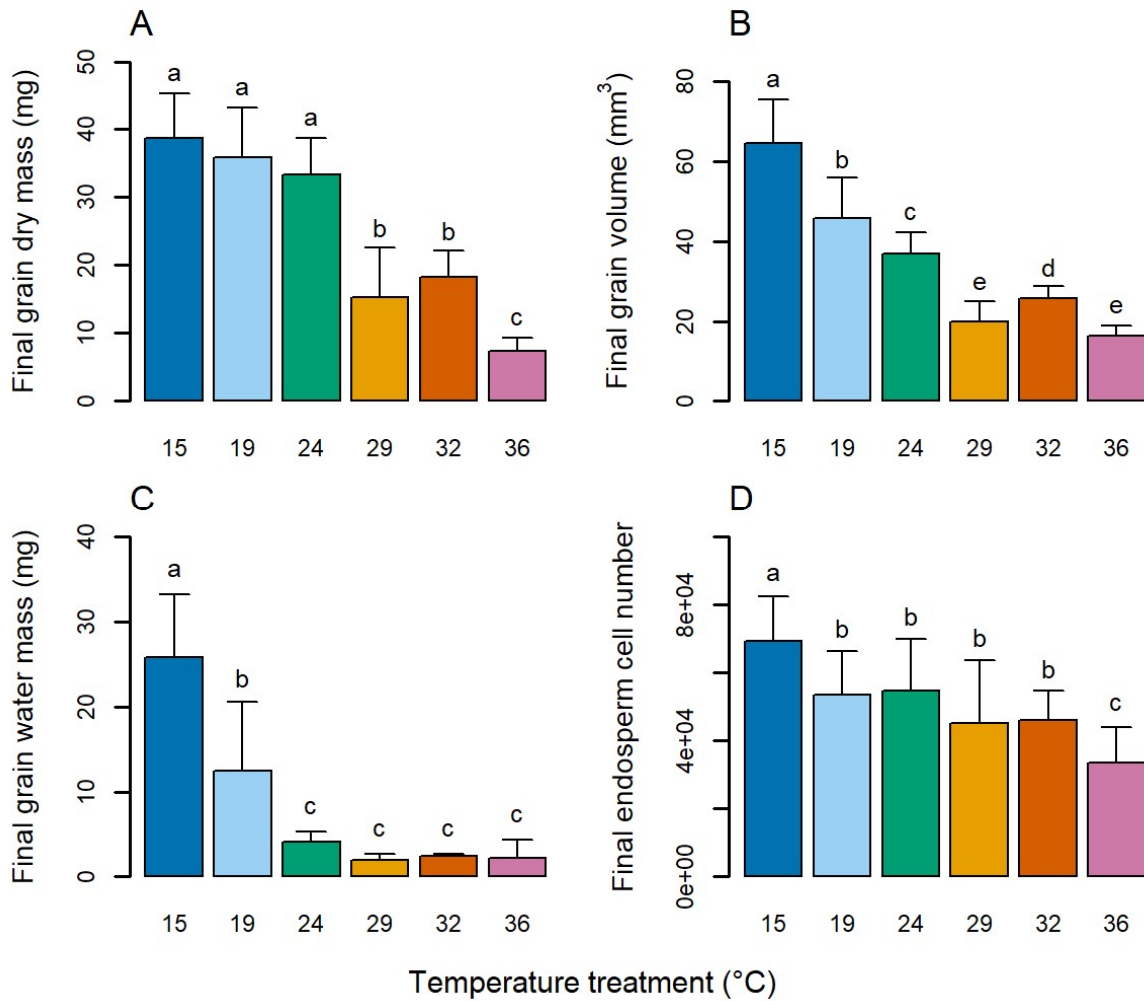
22

23 **Fig. 8.** Comparison between the normalized rate of grain dry-biomass accumulation estimated
24 based on direct observations ([Eq.1]) or predicted from observed grain volumes and published
25 temperature response for the relative activity of Starch Soluble Synthase enzyme ([Eq.2]). The
26 comparison is presented in terms of (A) normalized temperature responses and (B) predicted
27 values against experimental fitted values. Data concerning grain dry biomass accumulation
28 used in [Eq.1] are those obtained from the experiments from this study. Predicted values from
29 [Eq.2] derived from (i) data concerning grain volume increase obtained from the experiments
30 of this study and (ii) the relative activity of the Starch Soluble Synthase enzyme, extracted

- 1 from Figure 1 (120 min temperature treatment) of Keeling *et al.* (1993) and normalized at
- 2 19°C.
- 3

1 **Figures**

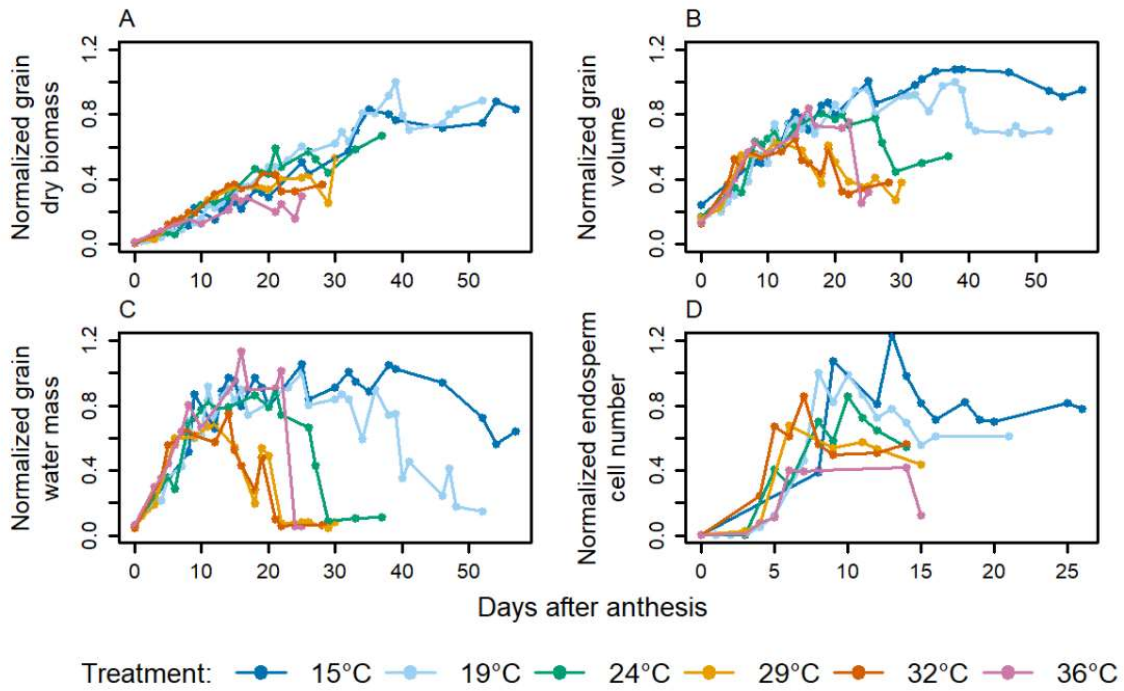
2
3



4
5
6
7
8
9
10
11
12
13
14
15
16

Fig. 1. Effects of high post-anthesis temperature on final grain dry biomass (A), volume (B), water mass (C), and endosperm cell number (D). Each bar represents the mean \pm standard deviation of final values ($n=24$ to 27 for grain volume, 10 to 17 for grain dry biomass, water mass and endosperm cell number); measurements were taken at grain maturity, except for endosperm cell number. For this trait, final values were obtained during the filling phase when cell number is set (between 300 and 400 °Cd after anthesis). For each trait, the different letters above vertical bars indicate significant differences between temperature treatments at a 5% level (SNK test).

1



2

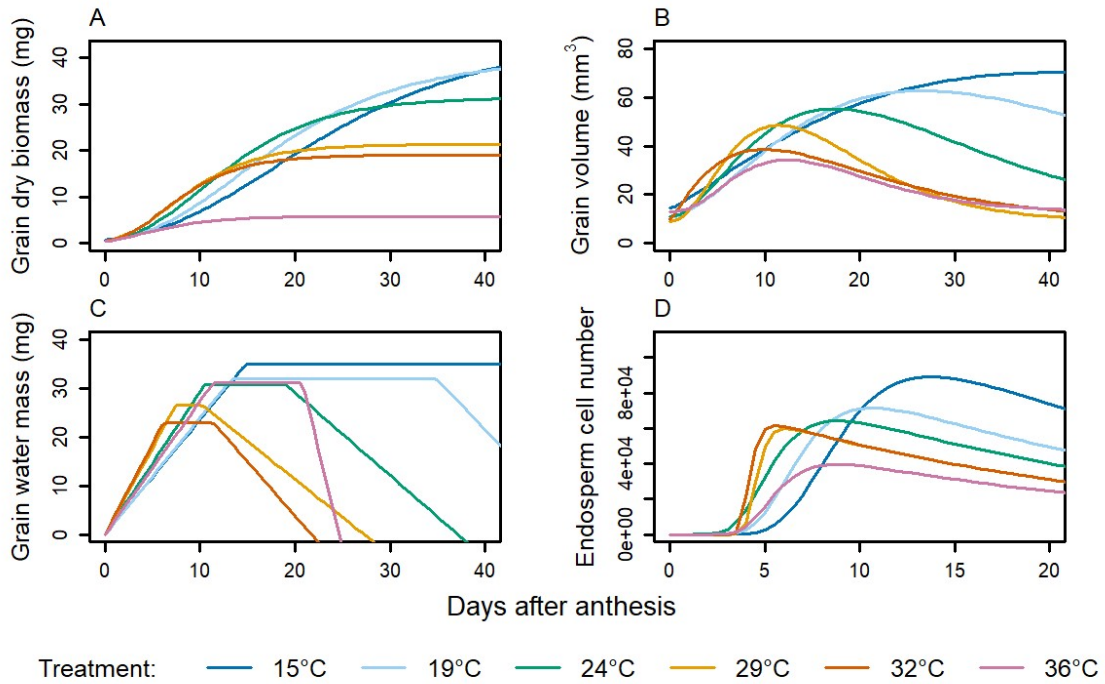
3

4

5 **Fig. 2.** Impact of temperature on the normalized grain dry biomass (A), volume (B), water mass
6 (C) and endosperm cell number (D) during grain development. Each point corresponds to the
7 mean of measurements on 12 grains (2 grains and 6 spikes) for grain dry biomass and
8 endosperm cell number, or on 24 grains (4 grains and 6 spikes) for grain water mass and
9 volume. All control treatments (19°C) were averaged together. Within each experiment, data
10 were normalized by the maximum mean value of the control (19°C) of the respective
11 experiments.

12

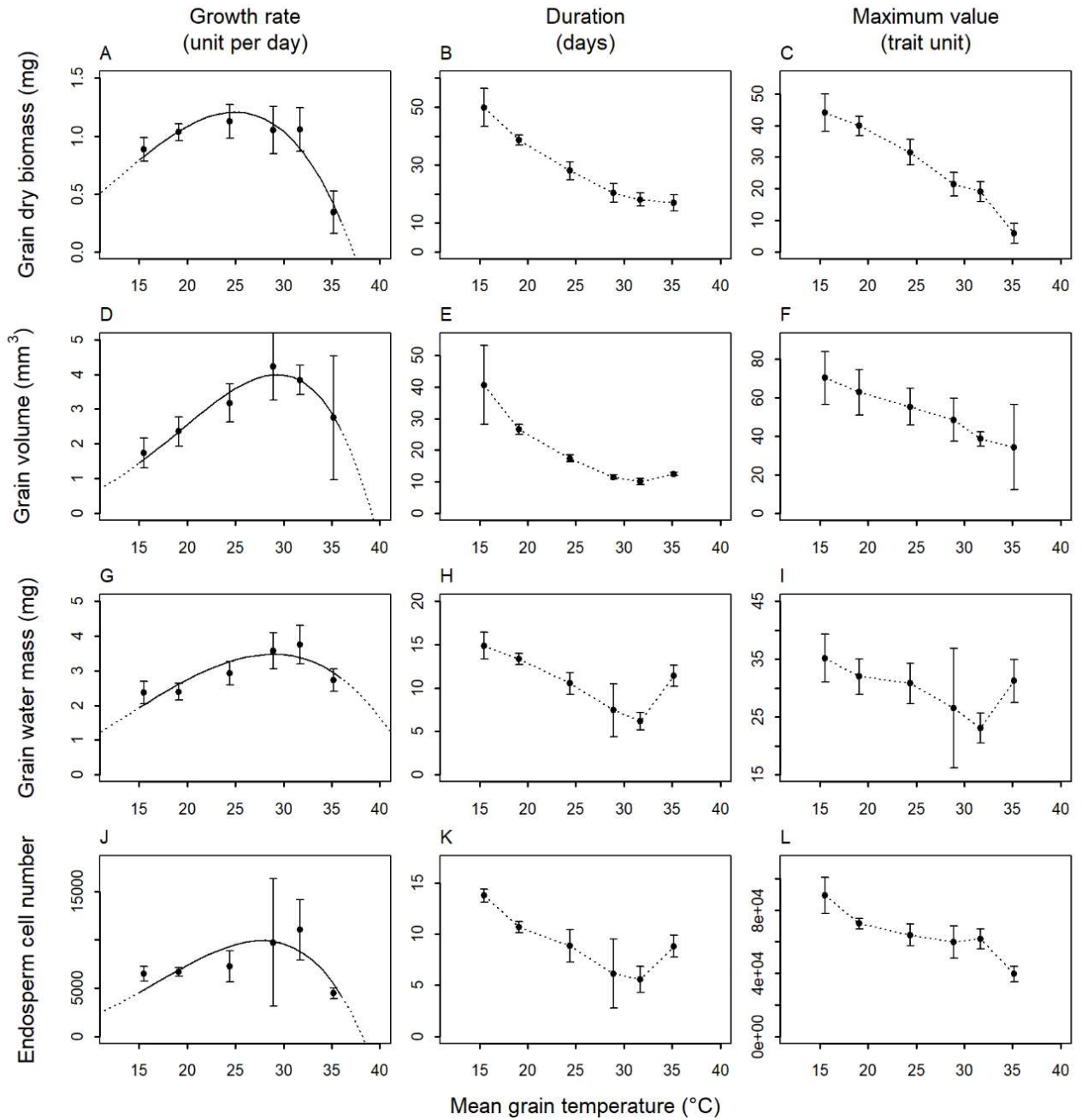
13



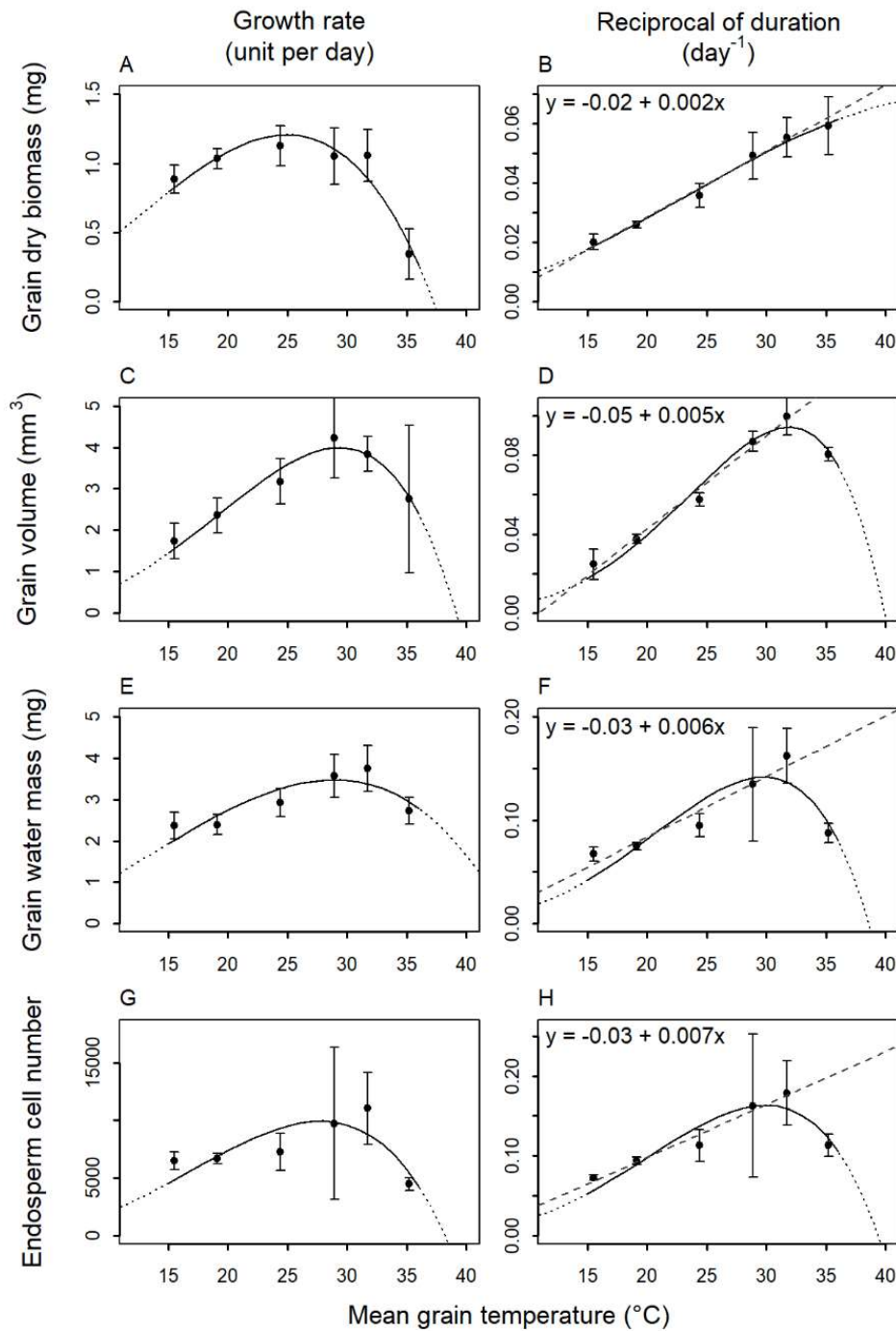
1
2
3
4
5
6
7
8

Fig. 3. Growth functions fitted to observed values of grain dry biomass (A), volume (B), water mass (C), and endosperm cell number (D) over time after anthesis. Growth functions were selected on the base of growth curves obtained at 19°C (control temperature), and applied to other temperatures (Table 2) in a non-linear mixed model fitted on the whole dataset (i.e. all treatments at once).

1



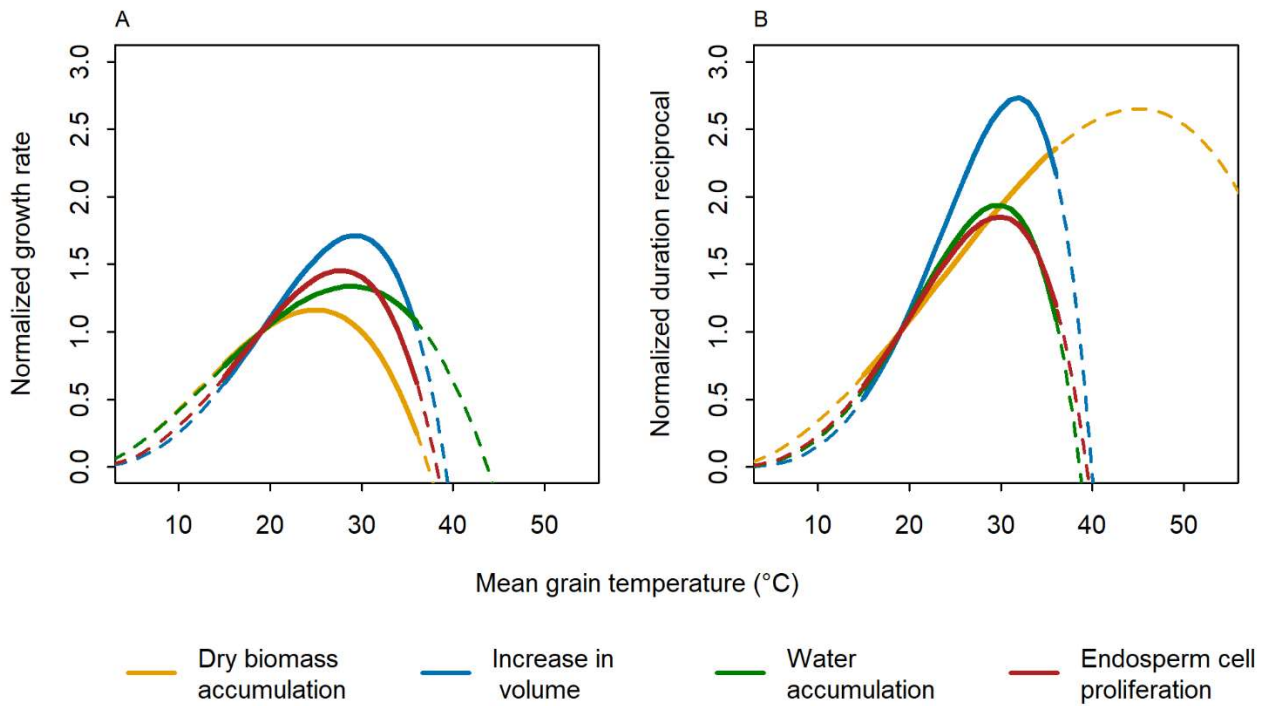
1
2



3
4
5
6
7
8
9
10

Fig. 5. Temperature response of growth rates and reciprocal of the durations for the grain dry biomass (A,B), cell volume (C,D), water mass (E,F), and endosperm cell number (G,H). Solid lines represent the fit of the response function [Eq.1]. Dashed lines (B, D, F, H) represent the linear regression fitted between 15°C to 32°C, for which the equation is given. Error bars correspond to two times the standard deviation, on either side of mean.

1



2

3

4

5

6

7

8

9

10

11

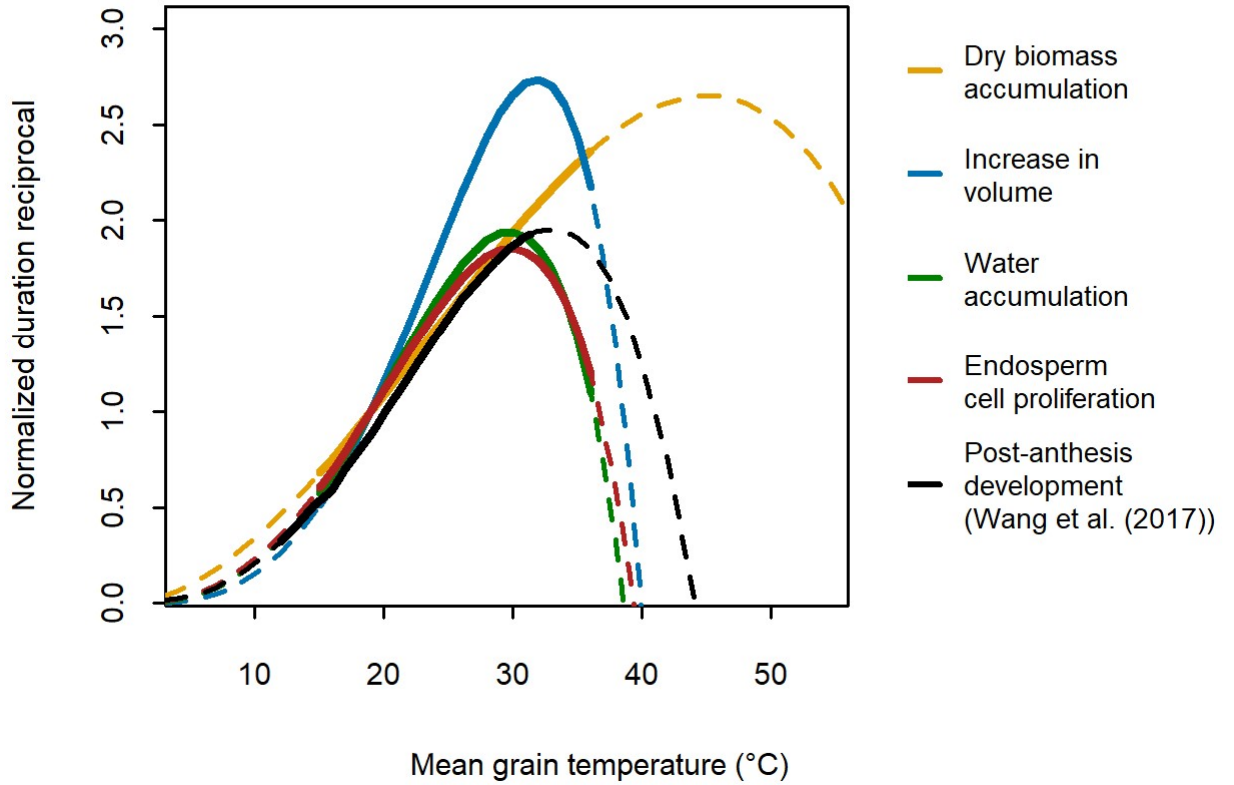
12

13

14

Fig. 6. Normalized temperature responses of growth rates (A) and reciprocal of growth durations (B) according to the response function [Eq.1] for the grain dry biomass accumulation, increase in volume, water accumulation and endosperm cell proliferation. Responses were normalized at 19°C. Solid lines indicate the range of temperatures for which observations were collected.

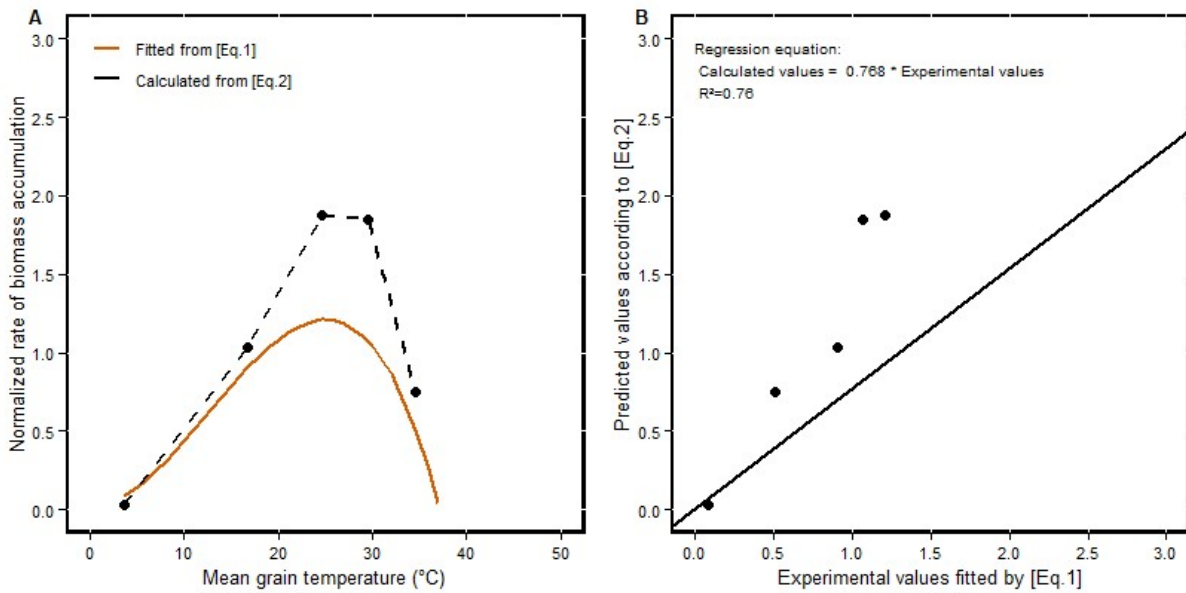
1
2
3



4
5
6
7
8
9
10
11
12
13
14
15

Fig. 7. Normalized temperature response of the reciprocal of duration of grain growth processes according to the response function [Eq.1]. Data concerning grain dry biomass accumulation, grain volume, water mass and endosperm cell numbers are those obtained from the experiments from this study. The post-anthesis development phase from Wang *et al.* (2017) corresponds to the duration between anthesis and when grains reach their final dry biomass. Response were normalized at 19°C. Solid lines indicate the range of temperatures for which observations were collected.

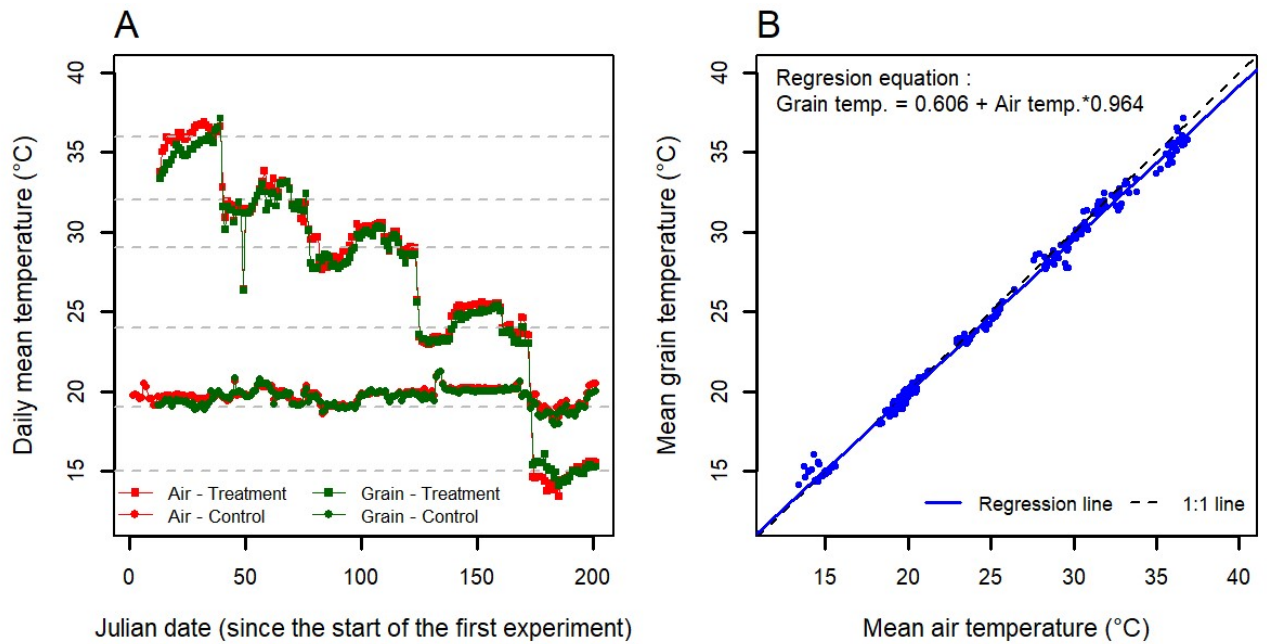
1
2



3
4
5
6
7
8
9
10
11
12
13
14
15

Fig. 8. Comparison between the normalized rate of grain dry-biomass accumulation estimated based on direct observations ([Eq.1]) or predicted from observed grain volumes and published temperature response for the relative activity of Starch Soluble Synthase enzyme ([Eq.2]). The comparison is presented in terms of (A) normalized temperature responses and (B) predicted values against experimental fitted values. Data concerning grain dry biomass accumulation used in [Eq.1] are those obtained from the experiments from this study. Predicted values from [Eq.2] derived from (i) data concerning grain volume increase obtained from the experiments of this study and (ii) the relative activity of the Starch Soluble Synthase enzyme, extracted from Figure 1 (120 min temperature treatment) of Keeling *et al.* (1993) and normalized at 19°C.

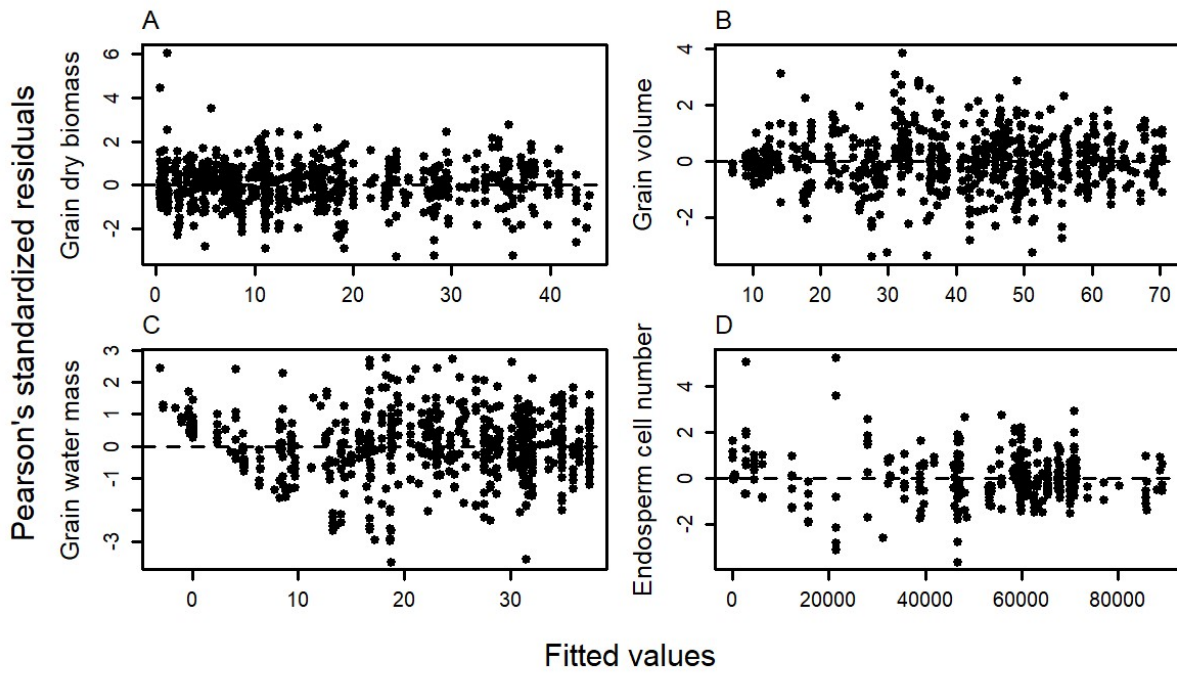
1
2



3
4
5
6
7
8
9
10
11
12
13
14

Fig. S1. Air and grain temperatures in the two growth chambers for the five successive experiments presented over time (A) and for grain temperature against air temperature (B). In (A), square symbol, control temperature; circle, temperature treatment; red, air temperature; green, grain temperature; the horizontal grey lines indicate the set temperatures for each experiment, i.e. 19°C for the control chamber, and alternatively 15, 24, 29, 32, and 36°C in the other chamber. In (B) daily mean grain temperature and air temperatures were considered for all the experimental data pooled together (B). Temperature was set to be constant over the whole days.

1



2

3

4 **Fig. S2.** Standardized residuals against fitted values of the adjusted growth functions
5 describing the change over time in grain dry biomass (A), volume (B), water mass (C) and
6 number (D). All temperature treatments were pooled. The data are presented in Figure 2. The
7 growth functions are described in Table 2 and were fitted on the whole dataset using a non-
8 linear mixed model, as described in the Material and Methods.

9

10