



*Quantifying the relationship between temperature regulation in the ear and floret development stage in wheat (*Triticum aestivum* L.) under heat and drought stress*

Article

Accepted Version

Steinmeyer, F. T., Lukac, M., Reynolds, M. P. and Jones, H. E. (2013) Quantifying the relationship between temperature regulation in the ear and floret development stage in wheat (*Triticum aestivum* L.) under heat and drought stress. *Functional Plant Biology*, 40 (7). pp. 700-707. ISSN 1445-4408 doi: <https://doi.org/10.1071/FP12362> Available at <http://centaur.reading.ac.uk/32198/>

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To link to this article DOI: <http://dx.doi.org/10.1071/FP12362>

Publisher: CSIRO Publishing

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1 **Quantifying the relationship between temperature regulation in the ear and floret**
2 **development stage in wheat (*Triticum aestivum* L.) under heat and drought stress**

3
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13
14 Keywords: Wheat, anthesis, temperature depression, controlled environment, screening

15
16 **Summary Text for Table of Contents:**

17
18 The relationship between temperature depression in the ears of *Triticum aestivum* L. and
19 flower development stage under heat and drought stress was examined. The early stages of
20 anthesis were associated with a lower ear temperature than the latter stages, indicating that
21 temperature depression occurs in the ear under stressed conditions, and potentially during the
22 heat sensitive flower development stages. This pioneering study provides a framework for
23 correlating ear temperature and grain yield under stressed conditions.

35 **Abstract**

36

37 Thermal imaging is a valuable tool for the elucidation of gas exchange dynamics between a
38 plant and its environment. The presence of stomata in wheat glumes and awns offers an
39 opportunity to assess photosynthetic activity of ears up to and during flowering. The
40 knowledge of spatial and temporal thermodynamics of the wheat ear may provide insight into
41 interactions between floret developmental stage (FDS), temperature depression (TD) and
42 ambient environment, with potential to be used as a high-throughput screening tool for
43 breeders. A controlled environment study was conducted using six spring wheat (*Triticum*
44 *aestivum* L.) genotypes of the elite recombinant inbred line Seri/Babax. Average ear
45 temperature (AET) was recorded using a hand held infrared camera and gas exchange was
46 measured by enclosing ears in a custom built cuvette. FDS was monitored and recorded daily
47 throughout the study. Plants were grown in pots and exposed to a combination of two
48 temperature and two water regimes. In the examined wheat lines, TD varied from 0.1°C to
49 0.6°C according to the level of stress imposed. The results indicated that TD does not occur
50 at FDS F3, the peak of active flowering, but during the preceding stages prior to pollen
51 release and stigma maturity (F1-F2). These findings suggest that ear temperature during the
52 early stages of anthesis, prior to pollen release and full extension of the stigma, are likely to
53 be the most relevant for identifying heat stress tolerant genotypes.

54

55

56 **Introduction**

57

58 On-going alteration of the global climate is predicted to lead to an increase in the frequency
59 of extreme weather events such as heat waves and droughts (IPCC 2007). The challenge
60 facing crop breeders is to create food crops with increasing resilience to environmental stress,
61 whilst producing ever higher yields. The full exploitation of the crop's genetic potential is
62 vital to achieve optimal crop performance. However, the ability of a plant to yield under
63 extreme or variable environmental conditions is actually mediated by a more complex
64 phenotype. Multiple points in the plant's development may exhibit various forms of
65 resilience, including early flowering (Acevedo *et al.* 2002), deep rooting (Hurd 1968), waxy
66 leaves (Cameron *et al.* 2006), as well as pollen production (Bita *et al.* 2011).

67

68 In wheat, anthesis is thought to be especially vulnerable to environmental stress (Saini and
69 Aspinall 1982). There are two approaches that wheat breeders can utilise to increase the
70 resilience of a wheat crop to environmental stress during anthesis: *avoidance/escape* or
71 *tolerance*. Lukac *et al.* (2012) concluded that by extending the period of flowering in wheat,
72 plants may be able to mitigate the effect of adverse environmental conditions at flowering by
73 staggering floret development. A plant can reduce the risk of a high temperature incident
74 (above 28°C) occurring and damaging all florets simultaneously by extending the flowering
75 period. Losses can be limited by having only a few florets at sensitive stages of development
76 at any one time. Adapting the flowering phenology to cope with environmental stress utilises
77 the *avoidance/escape* mechanism. Alternatively, *tolerance* allows a plant to develop in
78 conditions of environmental stress through mechanisms that actively shield key processes
79 from abiotic stresses (Wahid *et al.* 2007). Prior to anthesis, the process most sensitive to
80 environmental stress is the development of the male reproductive gamete. Pollen formation is
81 severely impaired by temperatures above 30°C for as little as 72 hours (Saini *et al.* 1983).
82 Heat tolerant lines of wheat have been shown to possess lower canopy temperature (CT) than
83 susceptible lines, achieved by a higher rate of transpiration in the canopy (Pinto *et al.* 2010).
84 The cooling of the canopy may be an active process and has evolved to shield the plants from
85 extreme temperatures during the most sensitive stages of development, or be merely a passive
86 indicator of improved physiology under stress environments. An increase in evaporative
87 cooling by the rest of the plant, however, will not have a direct effect on the temperature of
88 florets.

89

90 Under drought stress, the photosynthetic activity within the awns has been found to make a
91 significantly greater contribution to the total assimilate within the ear (Evans *et al.* 1972). The
92 same study demonstrated that the contribution of the awns to total grain yield only occurred
93 when plants were grown under stress. Photosynthesis in the glumes and awns of a wheat plant
94 can provide up to 30% of total grain carbon under ambient conditions and it has been
95 suggested that increases in ear photosynthesis will result in increased yield (Parry *et al.*
96 2011). The physiological effects of heat and drought stress on canopy leaves are numerous
97 and have been well documented (Al-Katib and Paulsen 1984; Berry and Bjorkman 1980;
98 Blum 1986). However, in the view of the potential contribution of spikes to the overall gas
99 exchange of the plant and a direct link to heat sensitive processes during anthesis, spike
100 temperature dynamics may offer a great potential in identifying phenotypes specific to stress
101 tolerance at anthesis. Significant gaps exist in our knowledge of the interaction between the
102 floret temperature and ambient environment.

103

104 Flowering in wheat is not a uniform process that occurs at an even rate along the ear. In order
105 to conserve resources, temperature depression (TD) may only occur in different sections of
106 the ear when the critical stages of stigma and anther development are occurring. Lukac *et al.*
107 (2012) identified significant differences in the pattern and rate of floret development within
108 and between spikes on the same plant. If TD takes place in the ear, one explanation for its
109 temporal variation may be the total number of florets at a critical floret development stage
110 (FDS) in the ear. This suggestion is supported by findings by Karimizadeh and Mohammadi
111 (2011), who concluded that canopy temperature depression (CTD) takes place at varying
112 rates depending on the growth stage of the plant. Although the vast majority of studies
113 investigating photosynthetic rate and environmental stress have been conducted on plant
114 canopy, their conclusions should be applicable to the photosynthetic tissue of the ear. Ear
115 temperature depression (ETD) denotes the difference between the air temperature and ear
116 temperature and may be expressed by the following formula;

117

118

$$ETD = T_a - T_e$$

119

120 where T_a is the air temperature and T_e is the ear temperature. ETD will have a positive value
121 when the ear temperature is lower than that of the air. ETD is a physiological trait potentially
122 useful to breeders aiming to screen genotypes for their ability to protect crucial stages of
123 development from environmental stress.

124 Lawlor (2009) postulated that impacts of environmental stress on plants result in a number of
125 short and long-term responses, all with the ultimate goal of acclimatising the plant and
126 ensuring its survival. The effect of heat and drought stress on the physiological and metabolic
127 activities in plants has been studied in great detail in recent years (Chaves *et al.* 2009; Lu and
128 Zang 2000; Mittler 2006; Wang *et al.* 2003). Many controlled environment studies have
129 focused on the effects of a single abiotic stress factor on the plant (Mittler 2006). Under field
130 conditions, however, multiple stress factors affecting plant development and photosynthesis
131 are compounded. The occurrence of abiotic stress is difficult to forecast more than a few
132 weeks in advance and may occur both early and late in the season. Breeders currently use a
133 range of screening methods, such as root morphology, CT, photosynthetic activity and days
134 until maturity, to select for stress tolerance (Reynolds 2002). A screening tool for stress
135 tolerance based on floret and/or ear temperature regulation does not currently exist, but may
136 be relevant when breeding plants for stress tolerance during anthesis. Before such a
137 potentially effective high-throughput screening tool for breeders is developed, it is crucial to
138 quantify the strength of interactions between the ear and the ambient environment. In order to
139 assess the scope of using ETD as a screening tool, this study sets out to detail the interaction
140 between floret development stage (FDS) and environmental stress and to study the
141 mechanisms of temperature depression (TD) utilized by the ear in stressed conditions. Four
142 key hypotheses were tested in this study; (1) genotypes tolerant to abiotic stress will have a
143 lower AET and therefore minimise damage to florets during anthesis resulting in higher grain
144 yields; (2) the basal section of the ear will be cooler than the middle section, which in turn is
145 cooler than the apical section due to its proximity to the terminal node on the stem; (3) stress
146 tolerant lines will increase the photosynthetic rate of the ear when the florets are at FDS F3;
147 and (4) in stress tolerant lines, the expected increase in photosynthetic activity of the ear at
148 FDS F3 will minimise damage to the plants reproductive organs resulting in higher grain
149 yields.

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158 **Materials and methods**

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160 *Plant material and controlled environment description*

161

162 Six recombinant inbred lines (RIL) of Mexican spring wheat were studied in controlled
163 environment (CE) conditions at the Plant Environment Laboratory, University of Reading
164 (UK). The plant material originated from a reciprocal crossing of two related parent lines,
165 namely ‘Seri M82’ (IWIS CODE (Fox *et al.* 1996), selection history: M31 IBWSN S-1
166 MXI96-97) and ‘Babax’ (IWIS CODE (Fox *et al.* 1996), selection history: CM92066-J-0Y-
167 0M-0Y-4M-0Y-0MEX-48BBB-0Y). Both are considered to be highly adapted semi-dwarf
168 lines (CIMMYT Wheat Personnel 1986), with Babax being highly tolerant to severe drought
169 whereas Seri M82 is moderately susceptible to severe drought (Pfeiffer 1988). Known as
170 Seri/Babax, this cross is widely used for phenotyping studies in heat and drought stress
171 environments. Seri/Babax has a relatively short period of flowering between 10 and 15 days,
172 making it ideal for this type of work (Olivares-Villegas *et al.* 2007). The lines used in this
173 study were Seri/Babax SB009, SB020, SB087, SB118, SB155 and SB165. Based on their
174 contrasting performance in field conditions under heat stress, as well as their similar
175 phenology and field performance without stress, Pinto *et al.* (2010) suggested pairing the
176 following contrasting lines of Seri/Babax: SB009/SB118, SB020/SB087 and SB155/SB165.
177 As this was a pioneer study, such pairs with contrasting phenology and performance were
178 utilised due to the fact that any observed differences are likely to be more informative of the
179 studied mechanism than when comparing lines with different phenologies.

180

181 Three seeds from each of the six lines were sown into 180mm plastic pots containing a
182 sterilised mixture of vermiculite, sand, gravel and compost (2: 1: 2: 0.5 ratio) as well as 2
183 kg/m³ Osmocote slow release granules containing N:P₂O₅:K₂O:MgO (15: 11: 13: 2 ratio).
184 Half of the pots were irrigated to field capacity (FC) three times daily (Irr) by an automated
185 drip system. The other half received minimal water to simulate drought conditions (Dro),
186 which was defined as ‘infrequent irrigation such that the water applied to the pot resulted in
187 the potting mix reaching no more than 25% of the FC at any given time’. The drought
188 treatment averaged 75 ml of irrigation every two days. Soil moisture content was monitored
189 by rotating twelve automatically logged theta probes (Delta-T Devices, Cambridge, UK)
190 between pots in all four cabinets on a daily basis. In the drought treatment, soil was
191 considered sufficiently dry when the voltmeter readings were between 100 and 120mV (18.7-

192 22.4% of FC). The soil in the Irr treatments was considered wet when the soil had a voltmeter
193 reading of between 275 and 500mV (51.4-93.5% of FC), with field capacity (FC) being
194 identified as being at 535mV. The plants were irrigated with an acidified complete nutrient
195 solution, containing 100mgL⁻¹ inorganic nitrogen. Although the potting mix selected for this
196 experiments means that results may be difficult to translate to field conditions, the intention
197 was to ensure free drainage of water from the pots in the growth cabinets so that drought
198 conditions can be easily simulated. A drying out curve of the potting mix in controlled
199 environment conditions was plotted (Supplementary Figure S1) under constant abiotic
200 conditions of 20°C. The water retention capacity of the potting mix mediated a ca. 25%
201 decline from FC over the initial 24 h period, whilst over 48 h the pots lost 32% of FC.
202 Electrical conductivity (EC) was followed during the drying process and remained within the
203 acceptable range for suitably wet soil over a 24 h period and did not reach values indicative
204 of water stress. Given that pots were irrigated to FC every 24 hours, the observed pattern of
205 water loss indicates that sufficient water remained accessible to plants between irrigation
206 events in the Irr treatment.

207

208 The plants were grown outdoors under bird netting until GS39 (Zadoks *et al.* 1974), when the
209 growth of plants in each pot was restricted to two plants per pot and two tillers per plant.
210 Once 50% of tillers had reached GS58-59, the pots were randomly allocated to four 1.37 x
211 1.47 m² Saxcil growth cabinets. Two cabinets were maintained at 28°C/18°C day/night cycle
212 ('Hot' treatment) and the other two were maintained at 22°C/14°C day/night cycle ('Cool'
213 treatment), with a margin of error of ±0.5°C. The photoperiod lasted for 16h at 650µmol m⁻²
214 s⁻¹. The plants were kept in the growth cabinets until flowering was complete (Zadoks
215 Growth Stage 69) and senescence had begun (Zadoks Growth Stage 70).

216

217 *Flowering and ear physiology measurement*

218

219 Due to flowering synchrony between the sides of the ear (Lukac *et al.* 2012) only the florets
220 on the even side were scored to determine the floret development stage (FDS). The
221 developmental stages of individual florets were scored after Lukac *et al.* (2012), with four
222 stages of anther development (Supplementary Fig. S2) and three stages of stigma
223 (Supplementary Fig. S3) identified in each floret at each sampling date. The method allows
224 for a quick identification of the stages of floral development for both the stigma and anther.
225 Pollination occurs when the stigma is at stage F and the anther is at stage 3 (FDS F3). The

226 odd side of the ear was not scored during any stage of the growth cycle and was reserved for
227 infrared (IR) imaging. This was done to prevent damage to the glumes and interference with
228 the temperature readings of the florets. IR images were taken daily using a hand held, thermal
229 imaging camera (FLIR Systems, Oregon, USA) between 09.00h and 12.00h for a total of six
230 days (until the end of anthesis). The IR camera used (FLIR model T335) operated in a
231 spectral range of 7.5 to 13 μm and was accurate to $\pm 2\%$ of the reading (FLIR 2013). In order
232 to avoid any interference with the temperature of the ear, the pot was turned within the
233 cabinet so that the odd side faced the camera whilst ensuring that the ear was not touched.
234 The camera was held horizontally between 30 and 35cm away from the ear in the growth
235 cabinet when the reading was taken. Thermal image background did not interfere with the ear
236 temperature readings.

237

238 Gas exchange measurements at ear level were conducted using CIRAS 1 (PP Systems,
239 Ayrshire, UK), a portable gas exchange analyser. Net carbon dioxide flux and relative
240 humidity were recorded in a specially constructed, clear and sealed cuvette placed around the
241 ear during analysis. Measurements of CO_2 concentration and relative humidity inside the
242 cuvette took place at 10s intervals, for a total of 100s (10 readings in total). In each growth
243 cabinet, four second order tillers per line were randomly selected and followed throughout the
244 photosynthesis recordings. Only ears that had not been scored were used for gas exchange
245 measurement. Recordings were taken for three consecutive days during morning (09.00h-
246 11.00h), midday (12.00h-14.00h) and afternoon (15.00h-17.00h). This terminology was
247 chosen to denote distinct periods within the diurnal cycle. The plants in controlled
248 environment cabinets experienced a significant temperature and light gradient during the
249 day/night transitions, analogous to ambient conditions.

250

251 *Statistical data analysis*

252

253 Ear temperature analysis of the IR images was carried out using FLIR Quick Report 1.2 SP1
254 (FLIR Systems, Oregon, USA). Exploratory data analysis, including ANOVA, REML, time
255 series analysis and regression analysis were performed using Genstat version 13.1 (VSN
256 International Ltd., UK). Bonferroni correction was applied to ANOVA post-hoc tests in pair-
257 wise comparisons. Separate pots within growth cabinets were considered independent
258 replicates. A comparison of hot and cool treatments was not carried out due to insufficient
259 replication of this factor. Effects were considered significant at $P < 0.05$.

260 **Results**

261

262 *Ear temperature*

263

264 Water availability did not have a significant effect on ear temperature depression (ETD) of
265 the wheat genotypes utilised in this study ($P=0.075$, Figure 1). In the ‘Cool’ environment, the
266 difference in mean ETD between SB020 and SB087 was statistically significant ($P=0.029$),
267 whereas no significant difference was identified between SB155 and SB165 ($P=0.083$, Figure
268 2). In the ‘Hot’ environment there was no statistically significant difference between
269 SB020/SB087 ($P=0.112$) whereas SB155/SB165 showed significant differences in the mean
270 ETD ($P=0.015$). Genotype SB118 was not included in the IR analysis because growth did not
271 advance beyond GS45. Due to a technical fault with the thermographic equipment used,
272 SB009 was recorded incorrectly and this genotype was also excluded from IR analysis.

273

274 SB020 and SB087 only were selected for detailed ear temperature analysis on the basis of
275 Pinto *et al.* (2010) having identified SB020 as the higher yielding genotype of the pair under
276 conditions of heat stress, drought and irrigation (Supplementary Fig. S4). The ETD of
277 genotype SB020 decreased by 2.46°C i.e. the spike got warmer, between the period that the
278 plants were placed in the growth cabinets until the end of anthesis, with a concurrent decline
279 in florets at FDS F3 of 42%. Over that same period, the highest ETD was observed when
280 florets at FDS HF1 and HF2 were at a maximum. At FDS F3, there was no clear correlation
281 between the proportion of florets at this stage and a higher ETD (data not shown). However, a
282 decrease in ETD was observed to coincide with increasing number of florets at FDS F3 and
283 PF4 both in SB020 ($P=0.012$) and SB087 ($P=0.032$, Figure 3). There was no difference in the
284 slope of the linear relationship between the two genotypes in cool ($P=0.090$) or hot ($P=0.303$)
285 treatments.

286

287 Further, in genotypes SB020 and SB087, data relating to IR imaging and FDS of each ear
288 were evenly split into three sections, namely the ‘basal’, ‘middle’ and ‘apical’ sections
289 according to the spikelet distribution. For example, if an ear had 12 spikelets on both the even
290 and odd sides, spikelets 1-4 were labelled as ‘basal’, spikelets 5-8 were labelled as ‘middle’
291 and spikelets 9-12 were labelled as ‘apical’. No other alternative standardised method
292 currently exists for dividing the ear into different sections. A linear regression was fitted for
293 each section of the ear to pooled data from both genotypes. There were no statistically

294 significant differences between SB020 and SB087 in the relationships between FDS and ETD
295 in any of the three ear sections ($P=0.124$). Similarly, there was no difference in the slopes of
296 the linear fits between basal, middle and apical regions in cool ($P=0.163$) and hot ($P=0.974$)
297 treatments.

298

299 *Gas exchange*

300

301 Carbon dioxide and water vapour exchange of four replicate ears in genotypes SB009,
302 SB087, SB155 and SB165 was measured daily at three set time intervals for a total of three
303 days. There was no significant difference in the carbon dioxide uptake between the
304 genotypes, except during the midday session ($P=0.019$). Irrigation was not identified as
305 having a significant effect on the carbon dioxide uptake at any stage (Table 1). There was no
306 significant difference in the water vapour exchange between the genotypes or as a result of
307 varying levels of irrigation (Table 1). Genotypes SB009 and SB087 were utilised to study the
308 interaction between the percentage of florets at FDS F3 and the rate of gas exchange of wheat
309 ears. No significant differences were identified in either the carbon dioxide uptake or the
310 water vapour exchange between the genotypes in both the ‘Cool’ and the ‘Hot’ environments.
311 The results from genotype SB087 indicated a trend correlation between CO_2 uptake and
312 florets at F3 in the ‘Cool’ environment, but this was not statistically significant ($P=0.054$).

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328 Discussion

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330 Early methods of breeding for crop yield improvement were based on indicators of crop
331 performance, such as ear density, fertility and grain size. These highly integrative agronomic
332 traits while being plastic in their response to environment, do not offer any information on
333 factors affecting their expression in season. However, in recent decades a number of
334 physiological processes in wheat have been linked to yield, including osmotic adjustment
335 (Blum 1988; Morgan and Condon 1986), maintaining root development to maximise soil
336 moisture extraction (Lopes and Reynolds 2010) and delaying leaf senescence (Hsiao *et al.*
337 1984). Under irrigated conditions Fischer *et al.* (1998) found that grain yields associated well
338 with canopy temperature depression (CTD), leaf conductance (LC) and leaf photosynthetic
339 rate (LPR) in genotypes developed over a 26 year period in Mexico. Canopy temperature has
340 been identified as being indicative of heat tolerance (Reynolds *et al.* 1998), drought tolerance
341 (Blum *et al.* 1989) and plant water status (Blum *et al.* 1982). As with most physiological
342 processes in plants, the genetic basis of CTD is likely to be complex and involve a large
343 number of interacting genes. Therefore, selecting genotypes based on genetic screening is a
344 fraught and costly approach. Reynolds (2002) concluded that screening based on CTD allows
345 for early removal of genetically inferior genotypes, which increases the accuracy and the
346 speed of the breeding process.

347

348 The lack of detailed studies identifying the exact mechanisms controlling CTD means that
349 there is an equally great gap in our understanding of the mechanisms regulating TD in the
350 ear. Teare *et al.* (1972) postulate that higher grain yields observed in long awned cultivars of
351 wheat, compared to short awned cultivars, can in part be explained by differences in stomatal
352 density. This is closely linked to the gaseous exchange capacity of the glumes which, due to
353 the presence of stomata, have the potential to cool the ear during sensitive periods. As glumes
354 transpire at a rate similar to that of the flag leaf, there is a possibility that the mechanism of
355 TD in the ear is regulated in a similar manner to the mechanisms controlling TD in leaves
356 (Blum 1985). This leads to a suggestion that to protect heat sensitive processes such as
357 gametogenesis and fertilisation, heat tolerant populations are capable of maintaining lower
358 ear temperatures in stressed conditions than susceptible populations, similar to the plant
359 cooling the canopy to protect sensitive developmental stages (Bahar *et al.* 2008). Yield data
360 were not collected from plants utilised in this pilot study as most ears were damaged to a
361 certain extent during floret scoring. Pinto *et al.* (2010) utilised the same genotypes in trials

362 with combinations of water availability and heat stress. Yield data, and particularly the
363 sensitivity of wheat genotypes to the drought and heat stress informed the choice of
364 contrasting SB genotypes in this study (Supplementary Figure S4 & Table S1). Utilising such
365 a selection of genotypes, this study shows that wheat may be capable of thermoregulation in
366 the ear, and the rate of which may be modified by flowering stage. Nevo *et al.* (1992)
367 concluded that in a number of genotypes of the wild progenitors of wheat (*Triticum*
368 *dicoccoides*) and barley (*Horedeum spontaneum*), intense thermogenesis in the flowering
369 organs occurs when a plant is exposed to temperatures outside of its optimal growing
370 conditions. This is an active attempt by the plant to adjust and adapt in order to prevent
371 damage to reproductive processes. If plants retain the capability to actively produce heat
372 through mechanisms inherent to animals in order to shield their reproductive organs from
373 stress, it is equally feasible that plants utilise a cooling mechanism to shield the flowers from
374 high temperature (McDaniel 1982). In this study, the extent of thermoregulation varied
375 among the genotypes in the ‘hot’ treatment, with SB165 expressing a significantly lower
376 AET than the other genotypes. In the case of SB165, the AET was approximately 0.6°C
377 cooler than the mean AET of the other three genotypes. Figure 2 illustrates the differences in
378 AET between the genotypes in the ‘cool’ and ‘hot’ treatments. In this context, a better
379 understanding of the interactions which explain differences between genotypes might be
380 gained by including the root network (Hurd 1968), as well as pollen production and viability
381 (Bita *et al.* 2011) in the consideration. This study did not attempt to correlate ear temperature
382 with the corresponding canopy/flag leaf temperature, but solely attempted to establish
383 whether TD occurred between the six paired genotypes of Seri/Babax in a controlled
384 environment setting. However, correlating ear temperature and flag leaf temperature may be
385 a key indicator as it would shed light on preferential cooling of these organs at different
386 stages of development.

387

388 The findings of this study indicate that TD does occur during the early, but not in the late
389 stages of anthesis. In the examined genotypes, differences between AET and air temperature
390 were limited to between 1.5°C and 2.0°C. In heat stressed environments, this cooling of the
391 ear has the potential to help a plant maintain key processes which would otherwise be
392 disrupted and cause irreversible damage to the yield potential. A candidate mechanism
393 identified in rice is the dehiscence of the thecae leading to pollen release; this process is
394 particularly sensitive to heat stress (Matsui *et al.* 2000). In maize the equivalent process of
395 pollen release is the dehydration of the stomium which releases pollen (Keijzer 1996). Hence

396 the wheat may have the potential to maintain a cooler ear up to the point of pollen release.
397 With a 2°C to 4°C rise in average global temperatures predicted to occur as a result of climate
398 change by the end of this century (IPCC 2007), the cooling capacity of the wheat ear may
399 have the potential to maintain ear metabolic processes despite increasing temperature.

400

401 Water availability for transpiration from the glumes may be greater in the lower sections of
402 the ear because of their proximity to the xylem transport system contained within the stem.
403 The transportation stream consists of columns of internal water created by the losses of water
404 from above ground biomass. Maintaining this stream of water to the ear as a result of
405 increased transpiration from the glumes should create a corresponding cooling effect. In this
406 study, however, there was no difference in the temperature of the ear sections due to the
407 proximity to the stem. The observed temporal increase of ear surface temperature was solely
408 due to mean FDS of each section.

409

410 Several key factors interact with heat and drought stress and modify plant response and
411 eventual yield loss e.g. concentration of WSC in plant tissue, chlorophyll content and pollen
412 development. WSC can maintain plant function and grain yields. Waite and Boyd (1953)
413 identified significant differences in the concentrations of individual WSC between plant
414 organs depending on their growth stage. Drought stress tolerant populations of wheat are
415 likely to have higher concentrations of WSC (Xue *et al.* 2008), increasing the potential of
416 homeostasis being maintained in the growing ear.

417

418 Chlorophyll concentration varies between genotypes and its susceptibility to heat stress
419 differs accordingly (Graham and McDonald 2001). To date, the vast majority of studies
420 dealing with genotype and chlorophyll interactions have focused largely on chlorophyll
421 concentrations in the flag leaf, not the ear. Chlorophyll is closely related with photosynthetic
422 activity, which in turn has been linked to the ability of a plant to regulate CTD.

423

424 Finally, across a wide range of crops the critical threshold for pollen production and viability
425 varies only by 2°C to 4°C between semi-arid crops (ground-nut (Rasad *et al.* 1999)),
426 vegetable crops (tomato (Zinn *et al.* 2010)) and crops grown in a flooded environments (rice
427 (Nakagawa *et al.* 2002)). It appears that although pollen structure differs greatly between
428 crops (Edlund *et al.* 2004), a crops ability to tolerate heat and drought stress does not solely
429 lie in an ability to produce pollen capable of withstanding adverse environmental conditions.

430 It is likely that an ability to shield pollen from these adverse conditions is the vital feature
431 that allows plants to grow in environments where the critical threshold for pollen production
432 and viability in the early stages of development is reached on a regular basis.

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464 **Conclusion**

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466 This study has highlighted a number of key issues, namely that an active cooling mechanism
467 might have evolved in the ear to protect the heat sensitive stages of flower development and
468 that water availability. The results illustrates that TD does occur in the ear, with the potential
469 of significantly reducing the AET in stressed environments. No evidence was found to
470 support the hypothesis that the greatest TD would coincide with FDS F3, rather that it is FDS
471 HF1 and HF2 which show the greatest TD. Future work should focus on verifying the extent
472 to which the results are applicable to studies in the field: (i) do differences exist between base
473 cellular temperatures which significantly influence plant tolerance to stress; and (ii) at what
474 stage during anthesis TD in the ear is most critical. The results provide a strong platform
475 from which further work can be conducted. A much wider range of genetic material needs to
476 be fully screened in order to identify whether TD takes place in all genotypes, and to what
477 extent it correlates to stress tolerance as well as how alternative mechanisms may be used to
478 perform thermoregulation in the ear.

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498 **Acknowledgements**

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500 We would like to thank Mr. Laurence Hansen, Mrs. Caroline Hadley, Mr. Benn Taylor for
501 their technical support during this study. This work was funded by the UROP scheme of the
502 University of Reading.

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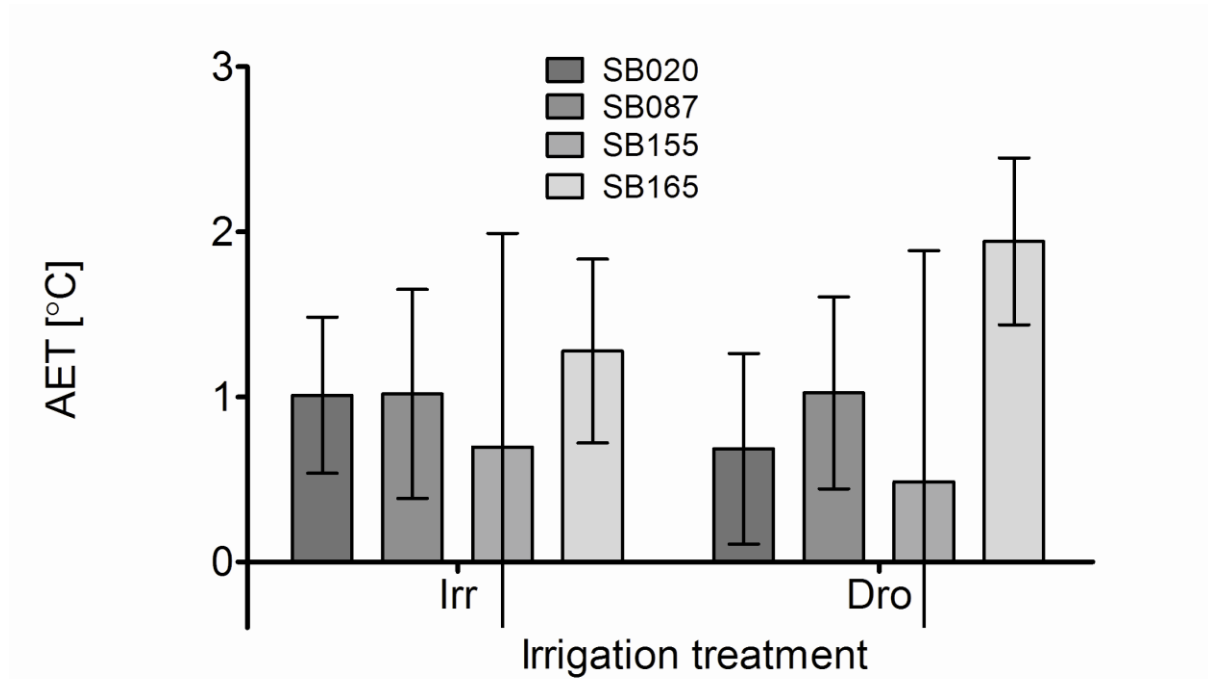
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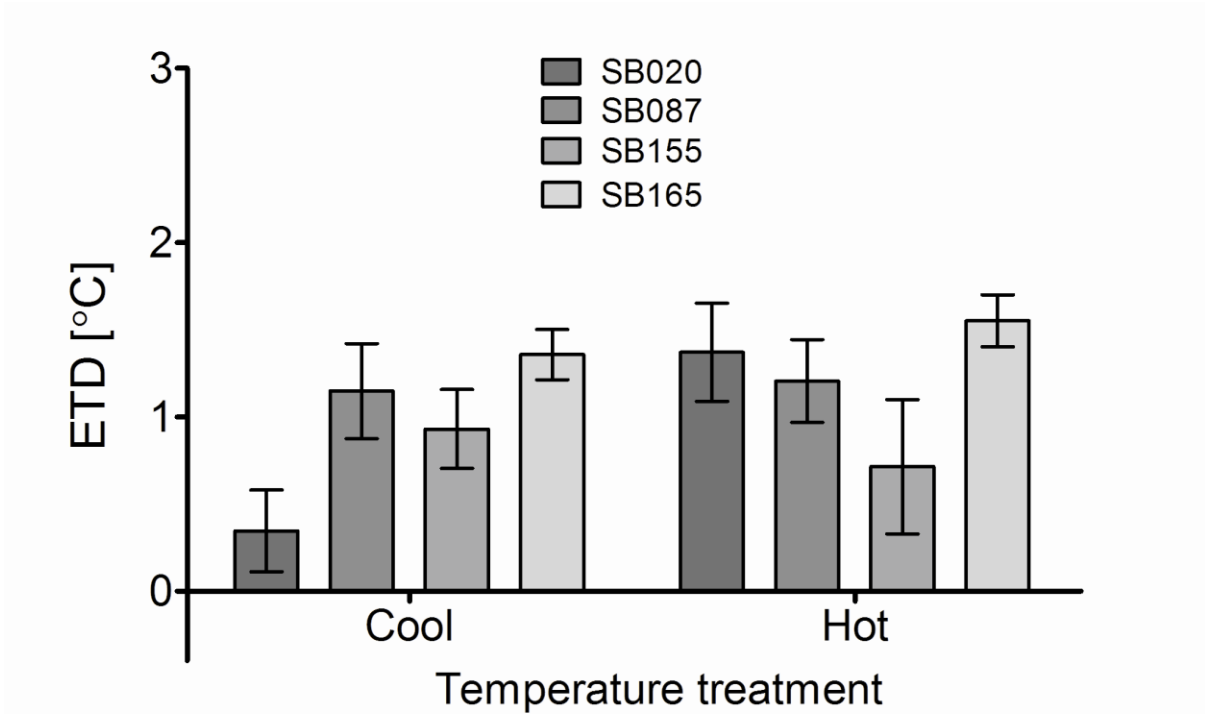
707 **Figure 1.** Ear Temperature Depression (ETD) of wheat genotypes SB020, SB087, SB155 and
708 SB165 grown in irrigated and drought conditions. ETD was defined as the difference between
709 ambient and mean ear temperatures. Positive ETD denotes cooling of the ear relative to
710 ambient air. Bars indicate standard error.



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730 **Figure 2.** Ear Temperature Depression (ETD) of wheat genotypes SB020, SB087, SB155 and
731 SB165 grown in cool (22/12°C) and hot (28/14°C) environments. ETD was defined as the
732 difference between ambient and mean ear temperatures. Positive ETD denotes cooling of the
733 ear relative to ambient air. Bars indicate standard error.



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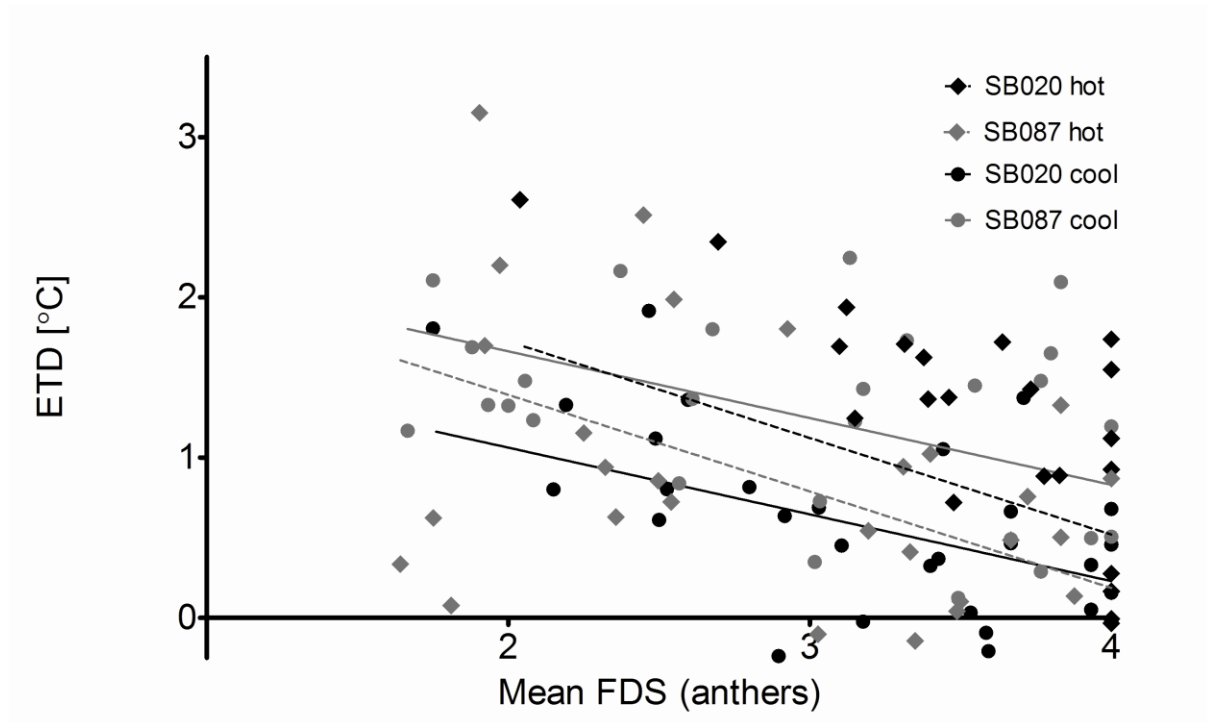
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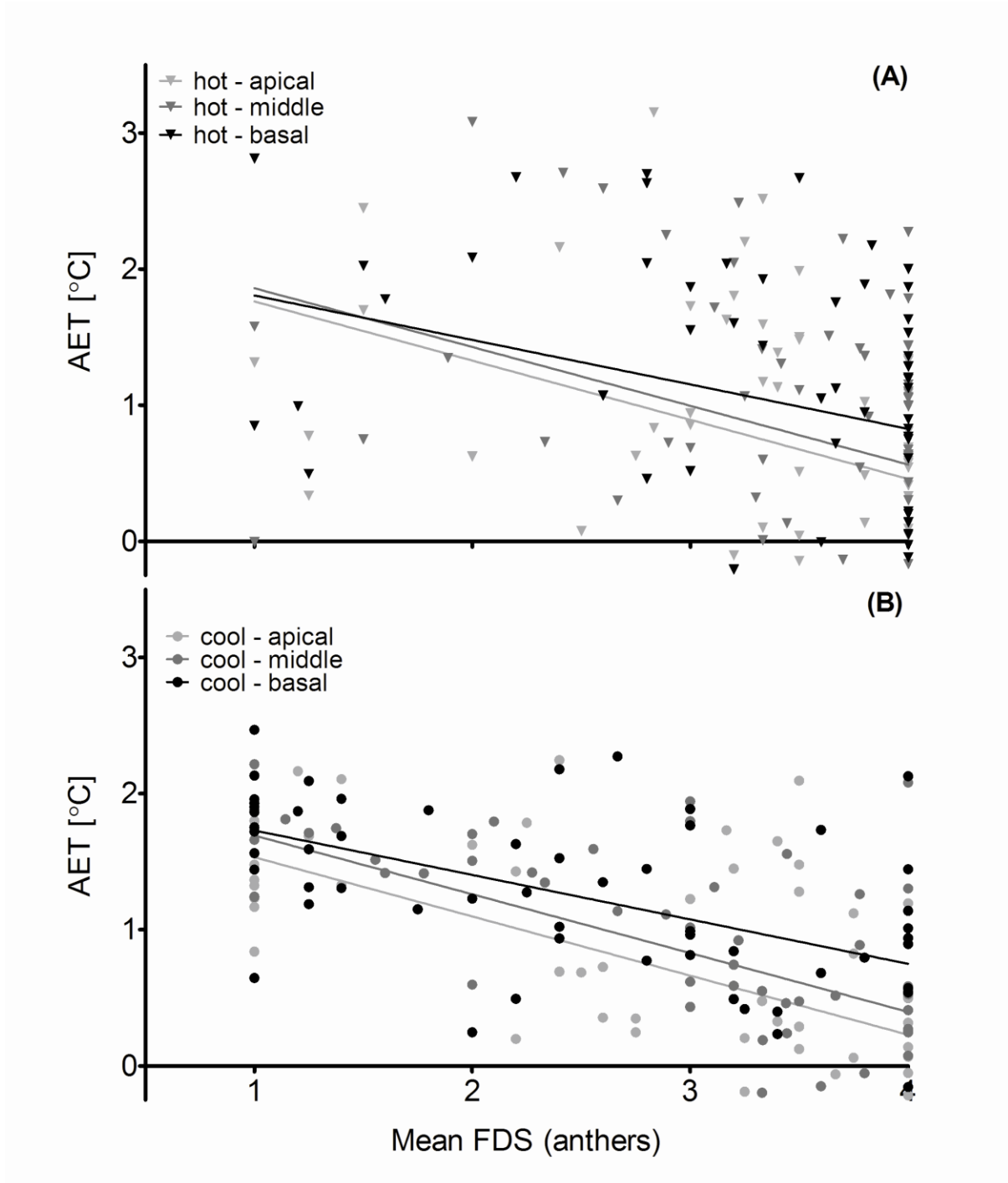
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747 **Figure 3.** The relationship between mean Flower Development Score (FDS) of male flower
748 parts (anthers) and Ear Temperature Depression (ETD) in genotypes SB020 and SB087 in
749 cool (22/12°C) and hot (28/14°C) environments.. The decreasing trend of ETD is significant
750 both in the ‘hot’ (dashed lines, P=0.003) and in the ‘cool’ (solid lines, P<0.001)
751 environments.



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768 **Figure 4.** The relationship between mean Flower Development Score (FDS) of male flower
769 parts (anthers) and Ear Temperature Depression (ETD) in apical, middle and basal ear
770 sections. Data for genotypes SB020 and SB087 were pooled, no statistically significant
771 differences between the ear sections were found in either the ‘hot’ (panel A, $P=0.975$) or
772 ‘cool’ (panel B, $P=0.163$) environments.



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776 Table 1 – *P*-values from a REML analysis of carbon dioxide uptake ($V\cdot CO_2$) and water
 777 vapour exchange (ΔH_2O) of the wheat ears in the experiment. Ear gas exchange was
 778 measured for 100 sec intervals on second order ears of SB020, SB087, SB155 and SB165.

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780 **Carbon dioxide uptake ($V\cdot CO_2$)**

781 *P*-values

	<u>Genotype</u>	<u>Irrigation</u>
782 Morning (09.00-11.00h)	0.266	0.413
783 Midday (12.00-14.00h)	0.019**	0.603
784 Afternoon (15.00-17.00h)	0.061	0.515
785		

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787 **Water vapour exchange (ΔH_2O)**

788 *P*-values

	<u>Genotype</u>	<u>Irrigation</u>
789 Morning (09.00-11.00h)	0.469	0.298
790 Midday (12.00-14.00h)	0.297	0.883
791 Afternoon (15.00-17.00h)	0.957	0.327
792		

793 *P*-value significance levels: * - $P < 0.001$, ** - $P > 0.01$, *** - $P > 0.05$.