

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

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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			
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14	Keywords: Wheat, anthesis, temperature depression, controlled environment, screening			
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16	Summary Text for Table of Contents:			
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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.			
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### 35 Abstract

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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 ambient environment, with potential to be used as a high-throughput screening tool for 42 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 throughout the study. Plants were grown in pots and exposed to a combination of two 47 48 temperature and two water regimes. In the examined wheat lines, TD varied from 0.1°C to 0.6°C according to the level of stress imposed. The results indicated that TD does not occur 49 at FDS F3, the peak of active flowering, but during the preceding stages prior to pollen 50 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.

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### 56 Introduction

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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 extreme or variable environmental conditions is actually mediated by a more complex 63 phenotype. Multiple points in the plant's development may exhibit various forms of 64 resilience, including early flowering (Acevedo et al. 2002), deep rooting (Hurd 1968), waxy 65 leaves (Cameron et al. 2006), as well as pollen production (Bita et al. 2011). 66

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In wheat, anthesis is thought to be especially vulnerable to environmental stress (Saini and 68 69 Aspinall 1982). There are two approaches that wheat breeders can utilise to increase the resilience of a wheat crop to environmental stress during anthesis: avoidance/escape or 70 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 conditions of environmental stress through mechanisms that actively shield key processes 78 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 at 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). The cooling of the canopy may be an active process and has evolved to shield the plants from 84 extreme temperatures during the most sensitive stages of development, or be merely a passive 85 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.

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 temperature dynamics may offer a great potential in identifying phenotypes specific to stress 100 101 tolerance at anthesis. Significant gaps exist in our knowledge of the interaction between the 102 floret temperature and ambient environment.

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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 rates depending on the growth stage of the plant. Although the vast majority of studies 112 113 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;

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 $ETD = T_a - T_e$ 

where  $T_a$  is the air temperature and  $T_e$  is the ear temperature. ETD will have a positive value when the ear temperature is lower than that of the air. ETD is a physiological trait potentially useful to breeders aiming to screen genotypes for their ability to protect crucial stages of 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 are compounded. The occurrence of abiotic stress is difficult to forecast more than a few 131 132 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 be relevant when breeding plants for stress tolerance during anthesis. Before such a 136 137 potentially effective high-throughput screening tool for breeders is developed, it is crucial to quantify the strength of interactions between the ear and the ambient environment. In order to 138 assess the scope of using ETD as a screening tool, this study sets out to detail the interaction 139 140 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 key hypotheses were tested in this study; (1) genotypes tolerant to abiotic stress will have a 142 143 lower AET and therefore minimise damage to florets during anthesis resulting in higher grain yields; (2) the basal section of the ear will be cooler than the middle section, which in turn is 144 145 cooler than the apical section due to its proximity to the terminal node on the stem; (3) stress tolerant lines will increase the photosynthetic rate of the ear when the florets are at FDS F3; 146 147 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 vields. 150 151 152

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- 158 Materials and methods
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#### 160 Plant material and controlled environment description

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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, namely 'Seri M82' (IWIS CODE (Fox et al. 1996), selection history: M31 IBWSN S-1 165 166 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 whereas Seri M82 is moderately susceptible to severe drought (Pfeiffer 1988). Known as 169 170 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, making it ideal for this type of work (Olivares-Villegas et al. 2007). The lines used in this 172 study were Seri/Babax SB009, SB020, SB087, SB118, SB155 and SB165. Based on their 173 174 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.

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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/m<sup>3</sup> Osmocote slow release granules containing N:P<sub>2</sub>0<sub>5</sub>:K<sub>2</sub>O:MgO (15: 11: 13: 2 ratio). 184 Half of the pots were irrigated to field capacity (FC) three times daily (Irr) by an automated drip system. The other half received minimal water to simulate drought conditions (Dro), 185 186 which was defined as 'infrequent irrigation such that the water applied to the pot resulted in the potting mix reaching no more than 25% of the FC at any given time'. The drought 187 188 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 (18.7192 22.4% of FC). The soil in the Irr treatments was considered wet when the soil had a voltmeter reading of between 275 and 500mV (51.4-93.5% of FC), with field capacity (FC) being 193 194 identified as being at 535mV. The plants were irrigated with an acidified complete nutrient solution, containing 100mgL<sup>-1</sup> inorganic nitrogen. Although the potting mix selected for this 195 196 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.

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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 x1.47 m<sup>2</sup> Saxcil growth cabinets. Two cabinets were maintained at 28°C/18°C day/night cycle 211 ('Hot' treatment) and the other two were maintained at 22°C/14°C day/night cycle ('Cool' 212 213 treatment), with a margin of error of  $\pm 0.5^{\circ}$ C. The photoperiod lasted for 16h at 650µmol m<sup>-2</sup> 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).

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## 217 Flowering and ear physiology measurement

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Due to flowering synchrony between the sides of the ear (Lukac *et al.* 2012) only the florets on the even side were scored to determine the floret development stage (FDS). The developmental stages of individual florets were scored after Lukac *et al.* (2012), with four stages of anther development (Supplementary Fig. S2) and three stages of stigma (Supplementary Fig. S3) identified in each floret at each sampling date. The method allows for a quick identification of the stages of floral development for both the stigma and anther. 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  $\mu$ m 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.

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Gas exchange measurements at ear level were conducted using CIRAS 1 (PP Systems, 238 Ayrshire, UK), a portable gas exchange analyser. Net carbon dioxide flux and relative 239 humidity were recorded in a specially constructed, clear and sealed cuvette placed around the 240 ear during analysis. Measurements of CO<sub>2</sub> concentration and relative humidity inside the 241 242 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-11.00h), midday (12.00h-14.00h) and afternoon (15.00h-17.00h). This terminology was 246 247 chosen to denote distinct periods within the diurnal cycle. The plants in controlled environment cabinets experienced a significant temperature and light gradient during the 248 249 day/night transitions, analogous to ambient conditions.

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## 251 Statistical data analysis

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Ear temperature analysis of the IR images was carried out using FLIR Quick Report 1.2 SP1 (FLIR Systems, Oregon, USA). Exploratory data analysis, including ANOVA, REML, time series analysis and regression analysis were performed using Genstat version 13.1 (VSN International Ltd., UK). Bonferroni correction was applied to ANOVA post-hoc tests in pairwise comparisons. Separate pots within growth cabinets were considered independent replicates. A comparison of hot and cool treatments was not carried out due to insufficient replication of this factor. Effects were considered significant at P<0.05. 260 **Results** 

261

262 *Ear temperature* 

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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 florets at FDS HF1 and HF2 were at a maximum. At FDS F3, there was no clear correlation 280 281 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

Further, in genotypes SB020 and SB087, data relating to IR imaging and FDS of each ear were evenly split into three sections, namely the 'basal', 'middle' and 'apical' sections according to the spikelet distribution. For example, if an ear had 12 spikelets on both the even and odd sides, spikelets 1-4 were labelled as 'basal', spikelets 5-8 were labelled as 'middle' and spikelets 9-12 were labelled as 'apical'. No other alternative standardised method currently exists for dividing the ear into different sections. A linear regression was fitted for each section of the ear to pooled data from both genotypes. There were no statistically significant differences between SB020 and SB087 in the relationships between FDS and ETD
in any of the three ear sections (P=0.124). Similarly, there was no difference in the slopes of
the linear fits between basal, middle and apical regions in cool (P=0.163) and hot (P=0.974)
treatments.

*Gas exchange* 

Carbon dioxide and water vapour exchange of four replicate ears in genotypes SB009, SB087, SB155 and SB165 was measured daily at three set time intervals for a total of three days. There was no significant difference in the carbon dioxide uptake between the genotypes, except during the midday session (P=0.019). Irrigation was not identified as having a significant effect on the carbon dioxide uptake at any stage (Table 1). There was no significant difference in the water vapour exchange between the genotypes or as a result of varying levels of irrigation (Table 1). Genotypes SB009 and SB087 were utilised to study the interaction between the percentage of florets at FDS F3 and the rate of gas exchange of wheat ears. No significant differences were identified in either the carbon dioxide uptake or the water vapour exchange between the genotypes in both the 'Cool' and the 'Hot' environments. The results from genotype SB087 indicated a trend correlation between CO<sub>2</sub> uptake and florets at F3 in the 'Cool' environment, but this was not statistically significant (P=0.054). 

#### 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 (Blum et al. 1989) and plant water status (Blum et al. 1982). As with most physiological 341 processes in plants, the genetic basis of CTD is likely to be complex and involve a large 342 number of interacting genes. Therefore, selecting genotypes based on genetic screening is a 343 344 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 ear. Teare et al. (1972) postulate that higher grain yields observed in long awned cultivars of 350 351 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 certain extent during floret scoring. Pinto et al. (2010) utilised the same genotypes in trials 361

with combinations of water availability and heat stress. Yield data, and particularly the 362 363 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 though 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 AET than the other genotypes. In the case of SB165, the AET was approximately 0.6°C 376 377 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 environment setting. However, correlating ear temperature and flag leaf temperature may be 384 385 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

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

Finally, across a wide range of crops the critical threshold for pollen production and viability
varies only by 2°C to 4°C between semi-arid crops (ground-nut (Rasad *et al.* 1999)),
vegetable crops (tomato (Zinn *et al.* 2010)) and crops grown in a flooded environments (rice
(Nakagawa *et al.* 2002)). It appears that although pollen structure differs greatly between
crops (Edlund *et al.* 2004), a crops ability to tolerate heat and drought stress does not solely
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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This study has highlighted a number of key issues, namely that an active cooling mechanism might have evolved in the ear to protect the heat sensitive stages of flower development and that water availability. The results illustrates that TD does occur in the ear, with the potential of significantly reducing the AET in stressed environments. No evidence was found to support the hypothesis that the greatest TD would coincide with FDS F3, rather that it is FDS HF1 and HF2 which show the greatest TD. Future work should focus on verifying the extent to which the results are applicable to studies in the field: (i) do differences exist between base cellular temperatures which significantly influence plant tolerance to stress; and (ii) at what stage during anthesis TD in the ear is most critical. The results provide a strong platform from which further work can be conducted. A much wider range of genetic material needs to be fully screened in order to identify whether TD takes place in all genotypes, and to what extent it correlates to stress tolerance as well as how alternative mechanisms may be used to perform thermoregulation in the ear. 

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



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



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



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



776	Table $1 - P$ -values from a REML analysis of carbon dioxide uptake (V.CO <sub>2</sub> ) and water			
777	vapour exchange ( $\Delta H_2O$ ) 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			
779				
780	Carbon dioxide uptake (V.CO <sub>2</sub> )			
781	P-values			
782		Genotype	Irrigation	
783	Morning (09.00-11.00h)	0.266	0.413	
784	Midday (12.00-14.00h)	0.019**	0.603	
785	Afternoon (15.00-17.00h)	0.061	0.515	
786				
787	<u>Water vapour exchange (ΔH<sub>2</sub>O)</u>			
788	P-values			
789		Genotype	Irrigation	
790	Morning (09.00-11.00h)	0.469	0.298	
791	Midday (12.00-14.00h)	0.297	0.883	
792	Afternoon (15.00-17.00h)	0.957	0.327	

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

0.957

Afternoon (15.00-17.00h)