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

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

## Abstract

Thermal imaging is a valuable tool for the elucidation of gas exchange dynamics between a plant and its environment. The presence of stomata in wheat glumes and awns offers an opportunity to assess photosynthetic activity of ears up to and during flowering. The knowledge of spatial and temporal thermodynamics of the wheat ear may provide insight into interactions between floret developmental stage (FDS), temperature depression (TD) and ambient environment, with potential to be used as a high-throughput screening tool for breeders. A controlled environment study was conducted using six spring wheat (*Triticum aestivum* L.) genotypes of the elite recombinant inbred line Seri/Babax. Average ear temperature (AET) was recorded using a hand held infrared camera and gas exchange was 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 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 at FDS F3, the peak of active flowering, but during the preceding stages prior to pollen release and stigma maturity (F1-F2). These findings suggest that ear temperature during the early stages of anthesis, prior to pollen release and full extension of the stigma, are likely to be the most relevant for identifying heat stress tolerant genotypes.

## Introduction

On-going alteration of the global climate is predicted to lead to an increase in the frequency of extreme weather events such as heat waves and droughts (IPCC 2007). The challenge facing crop breeders is to create food crops with increasing resilience to environmental stress, whilst producing ever higher yields. The full exploitation of the crop's genetic potential is 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 phenotype. Multiple points in the plant's development may exhibit various forms of resilience, including early flowering (Acevedo *et al.* 2002), deep rooting (Hurd 1968), waxy leaves (Cameron *et al.* 2006), as well as pollen production (Bita *et al.* 2011).

In wheat, anthesis is thought to be especially vulnerable to environmental stress (Saini and 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 *tolerance*. Lukac *et al.* (2012) concluded that by extending the period of flowering in wheat, plants may be able to mitigate the effect of adverse environmental conditions at flowering by staggering floret development. A plant can reduce the risk of a high temperature incident (above 28°C) occurring and damaging all florets simultaneously by extending the flowering period. Losses can be limited by having only a few florets at sensitive stages of development at any one time. Adapting the flowering phenology to cope with environmental stress utilises the *avoidance/escape* mechanism. Alternatively, *tolerance* allows a plant to develop in conditions of environmental stress through mechanisms that actively shield key processes from abiotic stresses (Wahid *et al.* 2007). Prior to anthesis, the process most sensitive to environmental stress is the development of the male reproductive gamete. Pollen formation is severely impaired by temperatures above 30°C for as little as 72 hours (Saini *et al.* 1983). Heat tolerant lines of wheat have been shown to possess lower canopy temperature (CT) than 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 extreme temperatures during the most sensitive stages of development, or be merely a passive indicator of improved physiology under stress environments. An increase in evaporative cooling by the rest of the plant, however, will not have a direct effect on the temperature of florets.

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

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

$$ETD = T_a - T_e$$

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.

Lawlor (2009) postulated that impacts of environmental stress on plants result in a number of short and long-term responses, all with the ultimate goal of acclimatising the plant and ensuring its survival. The effect of heat and drought stress on the physiological and metabolic activities in plants has been studied in great detail in recent years (Chaves *et al.* 2009; Lu and Zang 2000; Mittler 2006; Wang *et al.* 2003). Many controlled environment studies have focused on the effects of a single abiotic stress factor on the plant (Mittler 2006). Under field 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 weeks in advance and may occur both early and late in the season. Breeders currently use a range of screening methods, such as root morphology, CT, photosynthetic activity and days until maturity, to select for stress tolerance (Reynolds 2002). A screening tool for stress 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 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 assess the scope of using ETD as a screening tool, this study sets out to detail the interaction between floret development stage (FDS) and environmental stress and to study the 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 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 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; and (4) in stress tolerant lines, the expected increase in photosynthetic activity of the ear at FDS F3 will minimise damage to the plants reproductive organs resulting in higher grain yields.

## Materials and methods

### *Plant material and controlled environment description*

Six recombinant inbred lines (RIL) of Mexican spring wheat were studied in controlled environment (CE) conditions at the Plant Environment Laboratory, University of Reading (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 MXI96-97) and ‘Babax’ (IWIS CODE (Fox *et al.* 1996), selection history: CM92066-J-0Y-0M-0Y-4M-0Y-0MEX-48BBB-0Y). Both are considered to be highly adapted semi-dwarf 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 Seri/Babax, this cross is widely used for phenotyping studies in heat and drought stress 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 study were Seri/Babax SB009, SB020, SB087, SB118, SB155 and SB165. Based on their contrasting performance in field conditions under heat stress, as well as their similar phenology and field performance without stress, Pinto *et al.* (2010) suggested pairing the following contrasting lines of Seri/Babax: SB009/SB118, SB020/SB087 and SB155/SB165. As this was a pioneer study, such pairs with contrasting phenology and performance were utilised due to the fact that any observed differences are likely to be more informative of the studied mechanism than when comparing lines with different phenologies.

Three seeds from each of the six lines were sown into 180mm plastic pots containing a sterilised mixture of vermiculite, sand, gravel and compost (2: 1: 2: 0.5 ratio) as well as 2 kg/m<sup>3</sup> Osmocote slow release granules containing N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O:MgO (15: 11: 13: 2 ratio). 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), 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 treatment averaged 75 ml of irrigation every two days. Soil moisture content was monitored by rotating twelve automatically logged theta probes (Delta-T Devices, Cambridge, UK) between pots in all four cabinets on a daily basis. In the drought treatment, soil was considered sufficiently dry when the voltmeter readings were between 100 and 120mV (18.7-



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 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 experiments means that results may be difficult to translate to field conditions, the intention was to ensure free drainage of water from the pots in the growth cabinets so that drought conditions can be easily simulated. A drying out curve of the potting mix in controlled environment conditions was plotted (Supplementary Figure S1) under constant abiotic conditions of 20°C. The water retention capacity of the potting mix mediated a ca. 25% decline from FC over the initial 24 h period, whilst over 48 h the pots lost 32% of FC. Electrical conductivity (EC) was followed during the drying process and remained within the acceptable range for suitably wet soil over a 24 h period and did not reach values indicative of water stress. Given that pots were irrigated to FC every 24 hours, the observed pattern of water loss indicates that sufficient water remained accessible to plants between irrigation events in the Irr treatment.

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

#### *Flowering and ear physiology measurement*

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

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

Gas exchange measurements at ear level were conducted using CIRAS 1 (PP Systems, Ayrshire, UK), a portable gas exchange analyser. Net carbon dioxide flux and relative humidity were recorded in a specially constructed, clear and sealed cuvette placed around the ear during analysis. Measurements of  $\text{CO}_2$  concentration and relative humidity inside the cuvette took place at 10s intervals, for a total of 100s (10 readings in total). In each growth cabinet, four second order tillers per line were randomly selected and followed throughout the photosynthesis recordings. Only ears that had not been scored were used for gas exchange 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 chosen to denote distinct periods within the diurnal cycle. The plants in controlled environment cabinets experienced a significant temperature and light gradient during the day/night transitions, analogous to ambient conditions.

#### *Statistical data analysis*

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  $P < 0.05$ .

## Results

### *Ear temperature*

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

SB020 and SB087 only were selected for detailed ear temperature analysis on the basis of Pinto *et al.* (2010) having identified SB020 as the higher yielding genotype of the pair under conditions of heat stress, drought and irrigation (Supplementary Fig. S4). The ETD of genotype SB020 decreased by  $2.46^{\circ}\text{C}$  i.e. the spike got warmer, between the period that the plants were placed in the growth cabinets until the end of anthesis, with a concurrent decline 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 between the proportion of florets at this stage and a higher ETD (data not shown). However, a decrease in ETD was observed to coincide with increasing number of florets at FDS F3 and PF4 both in SB020 ( $P=0.012$ ) and SB087 ( $P=0.032$ , Figure 3). There was no difference in the slope of the linear relationship between the two genotypes in cool ( $P=0.090$ ) or hot ( $P=0.303$ ) treatments.

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  $\text{CO}_2$  uptake and florets at F3 in the ‘Cool’ environment, but this was not statistically significant ( $P=0.054$ ).

## Discussion

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

The lack of detailed studies identifying the exact mechanisms controlling CTD means that 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 wheat, compared to short awned cultivars, can in part be explained by differences in stomatal density. This is closely linked to the gaseous exchange capacity of the glumes which, due to the presence of stomata, have the potential to cool the ear during sensitive periods. As glumes transpire at a rate similar to that of the flag leaf, there is a possibility that the mechanism of TD in the ear is regulated in a similar manner to the mechanisms controlling TD in leaves (Blum 1985). This leads to a suggestion that to protect heat sensitive processes such as gametogenesis and fertilisation, heat tolerant populations are capable of maintaining lower ear temperatures in stressed conditions than susceptible populations, similar to the plant cooling the canopy to protect sensitive developmental stages (Bahar *et al.* 2008). Yield data 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

with combinations of water availability and heat stress. Yield data, and particularly the sensitivity of wheat genotypes to the drought and heat stress informed the choice of contrasting SB genotypes in this study (Supplementary Figure S4 & Table S1). Utilising such a selection of genotypes, this study shows that wheat may be capable of thermoregulation in the ear, and the rate of which may be modified by flowering stage. Nevo *et al.* (1992) concluded that in a number of genotypes of the wild progenitors of wheat (*Triticum dicoccoides*) and barley (*Horedeum spontaneum*), intense thermogenesis in the flowering organs occurs when a plant is exposed to temperatures outside of its optimal growing conditions. This is an active attempt by the plant to adjust and adapt in order to prevent damage to reproductive processes. If plants retain the capability to actively produce heat though mechanisms inherent to animals in order to shield their reproductive organs from stress, it is equally feasible that plants utilise a cooling mechanism to shield the flowers from high temperature (McDaniel 1982). In this study, the extent of thermoregulation varied 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 cooler than the mean AET of the other three genotypes. Figure 2 illustrates the differences in AET between the genotypes in the ‘cool’ and ‘hot’ treatments. In this context, a better understanding of the interactions which explain differences between genotypes might be gained by including the root network (Hurd 1968), as well as pollen production and viability (Bita *et al.* 2011) in the consideration. This study did not attempt to correlate ear temperature with the corresponding canopy/flag leaf temperature, but solely attempted to establish 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 a key indicator as it would shed light on preferential cooling of these organs at different stages of development.

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

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

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

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

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.

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



## Conclusion

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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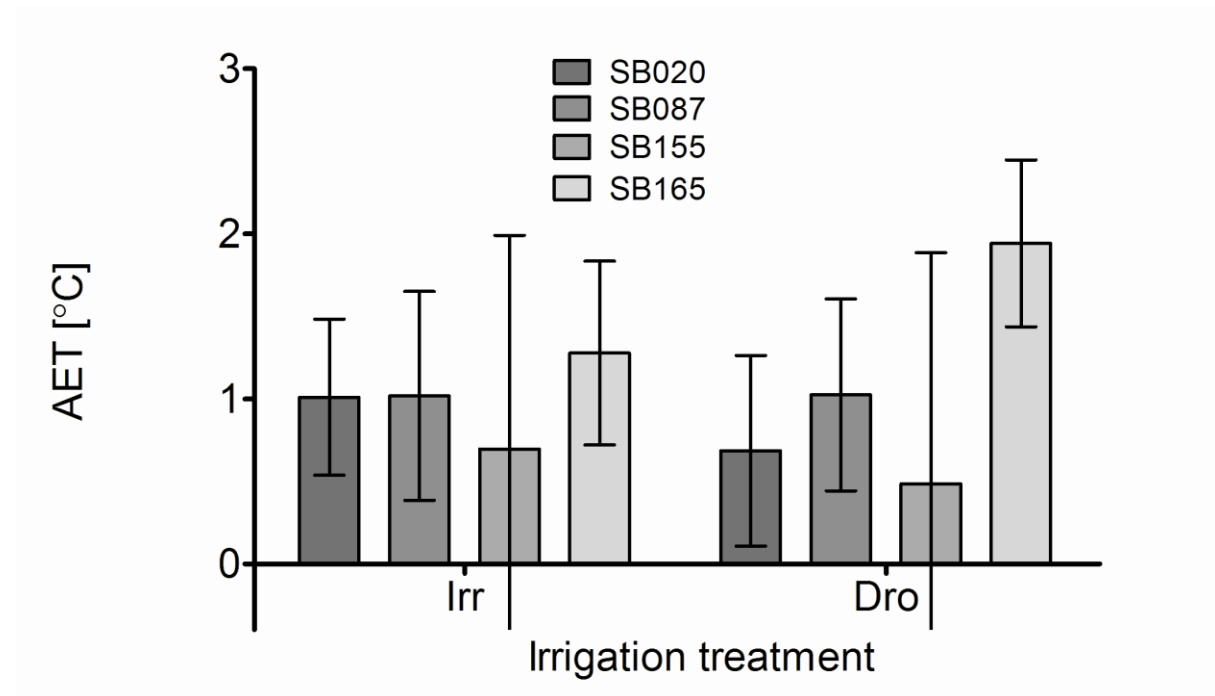
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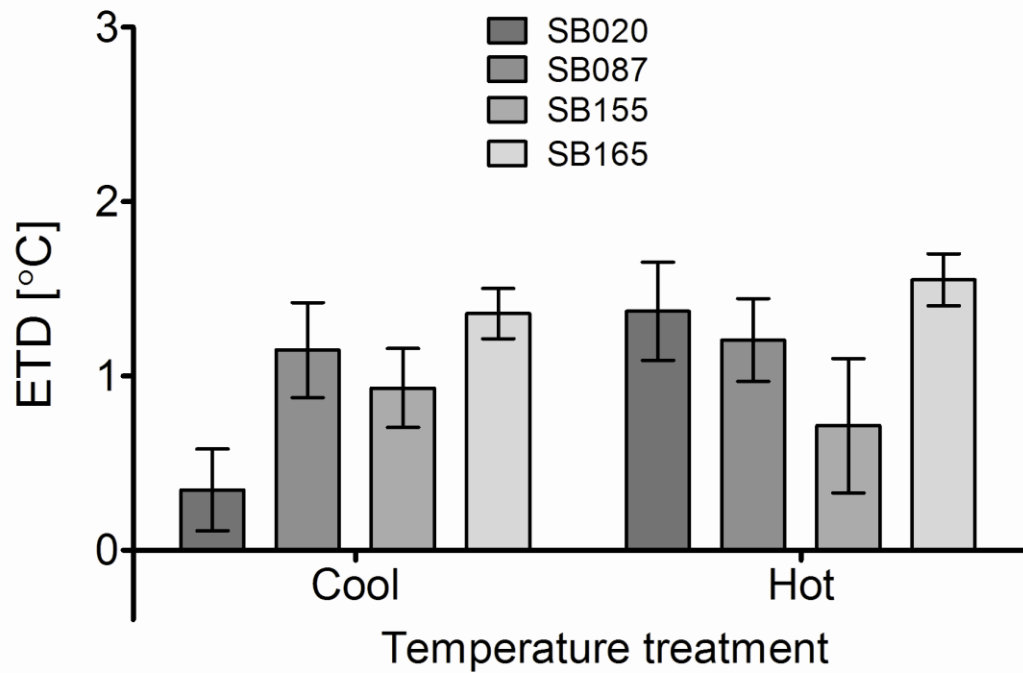
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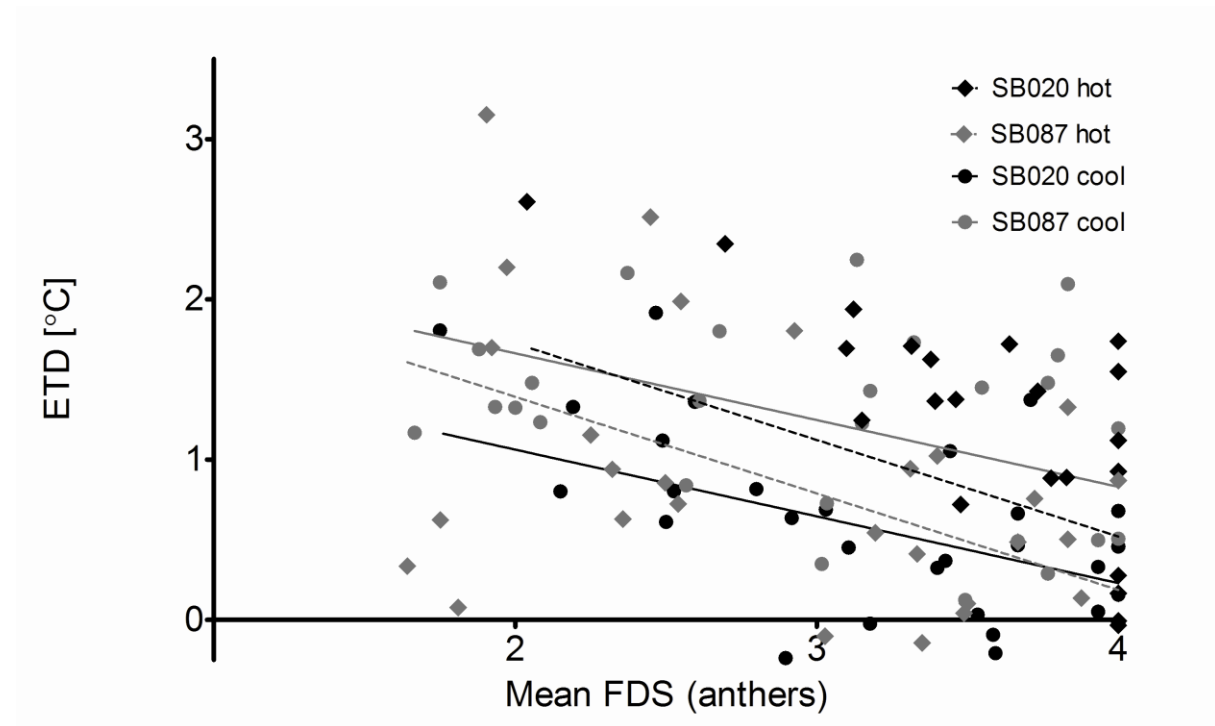
**Figure 1.** Ear Temperature Depression (ETD) of wheat genotypes SB020, SB087, SB155 and SB165 grown in irrigated and drought conditions. ETD was defined as the difference between ambient and mean ear temperatures. Positive ETD denotes cooling of the ear relative to ambient air. Bars indicate standard 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,  $P<0.001$ ) 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.

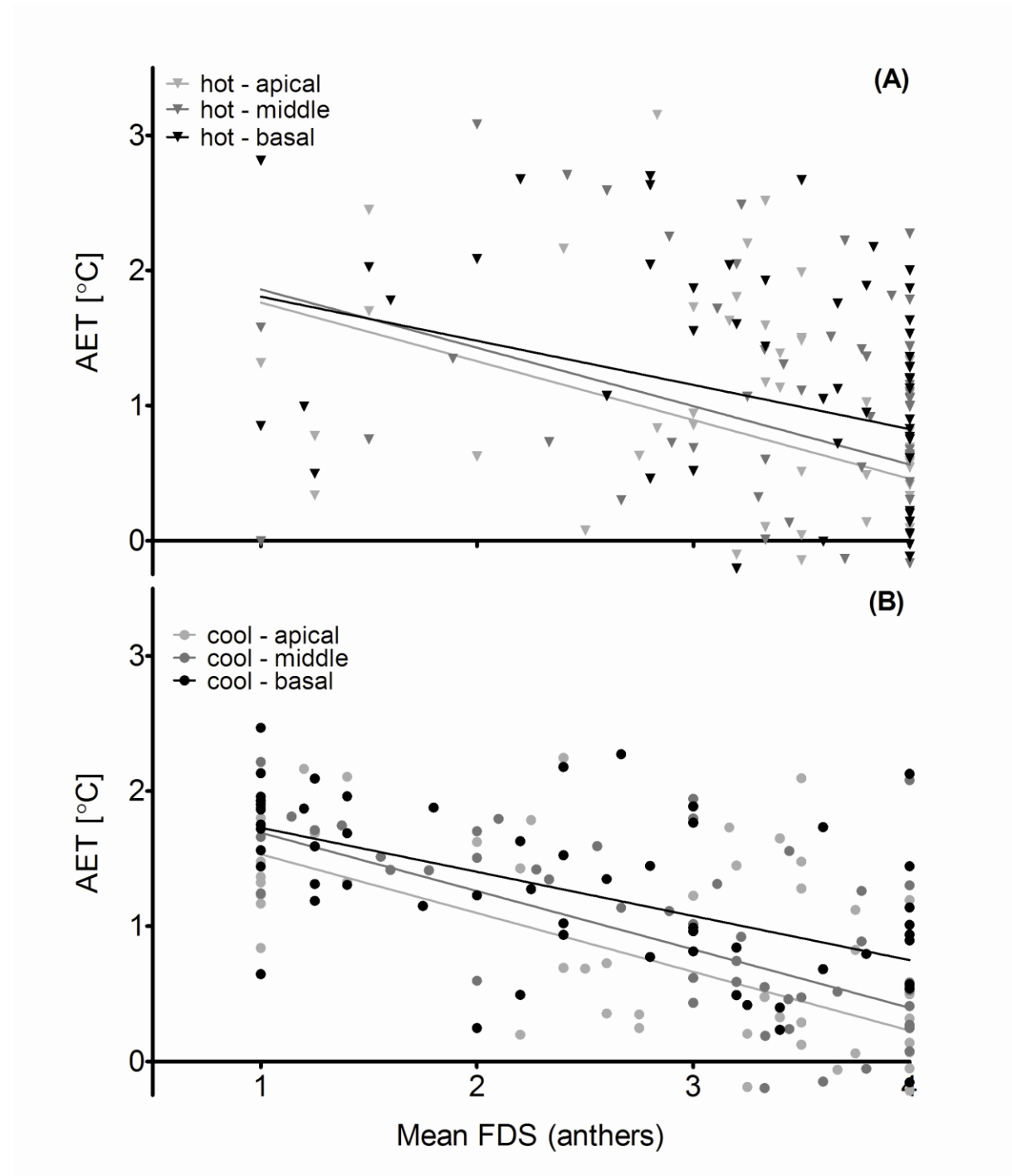


Table 1 – *P*-values from a REML analysis of carbon dioxide uptake ( $V\text{CO}_2$ ) and water vapour exchange ( $\Delta H_2O$ ) of the wheat ears in the experiment. Ear gas exchange was measured for 100 sec intervals on second order ears of SB020, SB087, SB155 and SB165.

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**Carbon dioxide uptake ( $V\text{CO}_2$ )**

*P*-values

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

**Water vapour exchange ( $\Delta H_2O$ )**

*P*-values

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

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