**Impact of post-flowering heat stress on stay-green and grain development in wheat**

Najeeb Ullah[1], Karine Chenu[1]

[1] The University of Queensland, Queensland Alliance for Agriculture and Food Innovation (QAAFI), Toowoomba, QLD, Australia
Email: n.ullah@uq.edu.au; karine.chenu@uq.edu.au

## Abstract

With recent and projected changes in climate, wheat crops are increasingly likely to experience post-flowering heat stress. An experiment was conducted to assess the impact of post-flowering heat on wheat crop. Recombinant inbred lines SB062 and SB003 were exposed to a 7-day heat shock (32.7/21.6°C day/night temperature) at different periods during the grain filling. These post-flowering heat shocks accelerated leaf senescence, with a greater impact on older leaves and for mid post-flowering stresses. Substantial genotypic differences were observed, with SB062 maintaining leaf greenness longer than SB003 especially when submitted to a heat stress. High temperature also reduced grain dimension and weight, especially for stresses applied during early-to-mid grain filling. SB062 was found as heat tolerant, as neither senescence of its two top leaves nor its grain size were significantly affected by heat in the tested conditions. Delayed leaf senescence appeared to play a role in maintaining grain size in SB062 under heat stress. The research findings will assist improving crop models for post-flowering heat effects and developing techniques for screening heat tolerant wheat lines.

## Key Words:

Heat stress, leaf senescence, chlorophyll, grain weight, grain size, genotypic variations.

## Introduction

Crop production and global food security are greatly threatened by global warming. Climatic models suggest a 2–5°C increase in the average atmospheric temperature by the end of the century, combined with an increased frequency of heat waves (IPCC, 2012). In many wheat-producing regions, the reproductive and grain filling phase of crop is often exposed to episodes of high temperature, which causes significant losses in grain yield (Barlow et al., 2015). High temperature is an abiotic stress that strongly influences wheat production in Australia (Zheng et al., 2012; Lobell et al., 2015). Field studies suggest a loss of 190 kg $ha^{-1}$ in wheat grain yield for each degree rise in the average day temperature (Bennett et al., 2012). Critical phases for grain yield formation, particularly at the time of meiosis and during early grain filling, are very sensitive to heat stress, as e.g. a temperature above 30ºC can induce pollen sterility, poor grain fill, reduced grain size and lower grain yield (Shirdelmoghanloo et al., 2016).

Developing grains require continuous supply of assimilates, which is met either through direct assimilation (photosynthesis from green parts of the plant) or through remobilisation from stored reserves (Pheloung and Siddique, 1991). As the photosynthetic machinery in plants is highly sensitive to heat, high temperature can truncate grain fill by initiating chlorophyll degradation – a process leading to leaf senescence (Shirdelmoghanloo et al., 2016). Stay-green, i.e. the ability of plants to maintain leaf greenness for a longer period after anthesis, has been considered as a superior trait of crops in a wide range of environments, including for drought and heat stress in wheat (Christopher et al., 2016; Shirdelmoghanloo et al., 2016).

This study investigates the impact of post-flowering heat shocks on leaf senescence, grain weight and grain size in two contrasting wheat genotypes.

## Methods

Two wheat recombinant inbred lines (RILs) from the Seri x Babax population (Olivares-Villegas et al., 2007), SB062 and SB003, were submitted to different heat shock treatments in a growth-room experiment. Plants were grown in pots at a density of 100 plants $m^{-2}$, in non-limiting conditions at 16h photoperiod. Two seeds of the same genotype were sown per pot at 3-cm depth in an ANOVApot® containing 1L of potting mix with 7 g of Osmocote® and 5 g of micromix. The experiment was laid out as randomised complete block design with six replicates (i.e. 6 pots) for each treatment (genotype x heat stress combinations). Plants were grown under optimum temperature (22.2/11.4°C day/night) up to flowering for all treatments, and up to maturity for the control. At 0 (0ºCd), 7 (143ºCd), 14 (285ºCd), 21 (431ºCd) and 28 (578ºCd) days after anthesis of the main stem, a set of plants was moved from the 'optimum-temperature room' to a 'high-temperature room'

32.7/21.6°C day/night temperature) for 7 days. After the heat treatment, plants were returned to the optimum-temperature room. Greenness of top two leaves of the main stem was measured on one plant per pot using a SPAD meter (SPAD-502 plus, Konica Minolta Inc.) before and after each heat treatment. Measurements were also made for six control plants at the same time. Post-anthesis senescence of the top two leaves was measured from the main tiller of all studied plants on a weekly basis through visual scoring of the proportion (0-100%) of leaf area that had senesced. At maturity, plants were harvested, and grains were dried at 70°C for 3 days. Grain dry weight was measured using an electronic balance and data on grain length, width and area was collected using a grain scanner (Epson Perfection® V550 Photo). Analyses were performed in R (R Core Team, 2018). Growing degree days (°Cd) were calculated as described by Zhou and Wang (2018). Statistical differences were tested with student's t-tests at a 5% level.

## Results

*Heat-induced leaf senescence*

While SB062 and SB003 flowered within 1-2 day(s) of each other, SB062 matured 4-5 days later than SB003 in unstressed conditions. Under control conditions, leaves of SB062 kept their greenness longer than SB003, especially for lower rank leaves (Fig. 1; unshown data). Post-anthesis heat significantly accelerated leaf senescence in SB003, especially for the penultimate leaf (Fig. 1 and 2). The timing of the heat stress had a significant impact on leaf senescence. A 7-d heat treatment applied at 7 day after anthesis (i.e 143-285°Cd after anthesis) or later shorten the lifespan of the penultimate leaf in SB003 by 2-3 weeks, while an early post-flowering stress applied at 0-7 days (0-143 °Cd) after anthesis had a relatively moderate effect on senescence (Fig. 1 and 2). Accordingly, the SPAD value of the SB003 penultimate leaf was reduced by 80% ($P < 0.05$) after the heat shock applied 7-14 days (143-285°Cd) after anthesis. The impact of heat stress at any growth stage was more moderate on the flag leaf than on penultimate leaf. While no effect on the flag-leaf greenness could be seen directly after a 7d heat stress (Fig. 1 and 2), a small but significant ($P < 0.05$) after-stress impact was observed for the flag leaf of SB003 at later developmental stages for early post-flowering heat stresses applied between 0 and 21 days (0 and 431°Cd) after anthesis (Fig. 2).

By contrast, a heat shock did not significantly affect the senescence rate of SB062 top two leaves, at any stage of the grain filling (Fig. 2). SB062 thus appeared as more stay-green than SB003, both under optimal and heat-stress conditions.

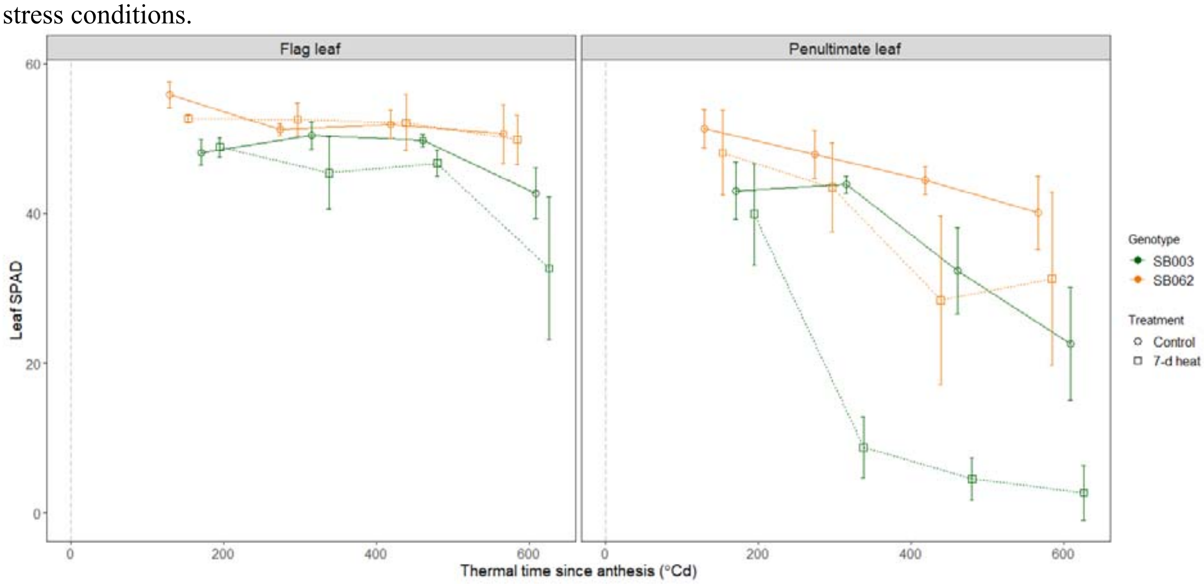


**Figure 1: Post-flowering heat-shock effect on SPAD values of top two leaves from the main stem in two contrasting genotypes (SB003 and SB062). Heat stress was applied at 0-7 (0-143 °Cd), 7-14 (143-285°Cd), 14-21 (285-431°Cd), 21-28 (431-578°Cd) and 28-35 (578-722 °Cd) days after anthesis of the main stem (dashed vertical grey line). SPAD measurements of heat-treated plants were taken at the end of the heat-stress treatment. Data presented for flag and penultimate leaf (flag leaf-1) of the main stem. Error bars correspond to confidence interval at *P*=0.05.**

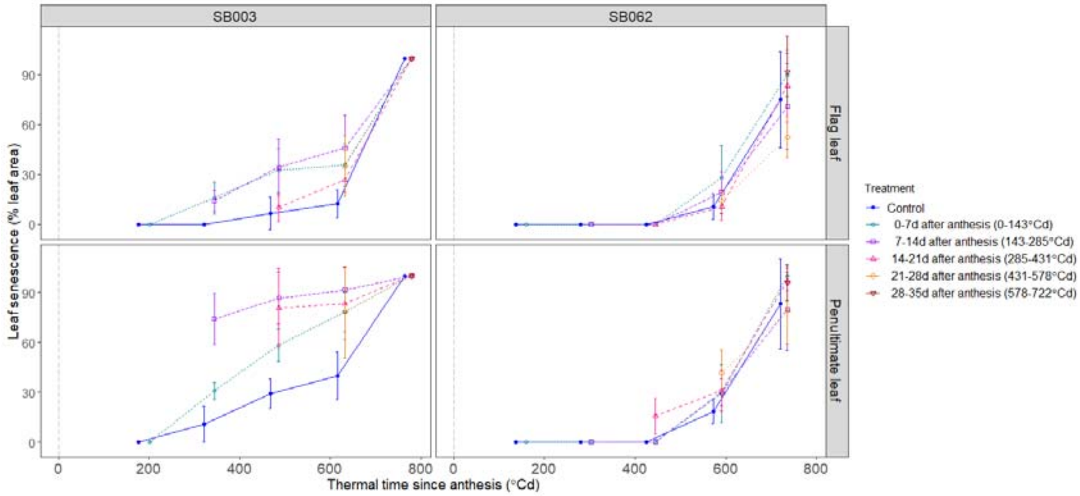


**Figure 2: Leaf senescence in response to a heat stress applied at 0-7 (0-143 ºCd), 7-14 (143-285ºCd), 14-21 (285-431ºCd), 21-28 (431-578ºCd) and 28-35 (578-722 ºCd) days after anthesis of the main stem (dashed vertical grey line) in two contrasting genotypes (SB003 and SB062). Data presented for flag and penultimate leaf (flag leaf-1) of the main stem. Error bars correspond to confidence interval at *P*=0.05.**

## *Post-flowering heat response of grain size*

Heat stress, during early-to-mid grain filling (143-431ºCd**)** significantly (P < 0.05) reduced individual grain weight and area in SB003 (Fig. 3). For example, when stressed between 7-14d (143-285ºCd), SB003 had 7% and 10% smaller grain width and area, respectively, compared to the control (averaged across all grains of the main stem, Fig. 3 B-C). Similarly, heat shock at this stage significantly reduced (P < 0.05) the individual grain weight of SB003 by 13% or more compared with controlled conditions (Fig 3 D). By contrast, no significant change in the grain dimension was observed in SB062 for any of the tested heat events, despite the fact that individual grain weight and width tended to be reduced by heat in SB062 too (Fig. 3).

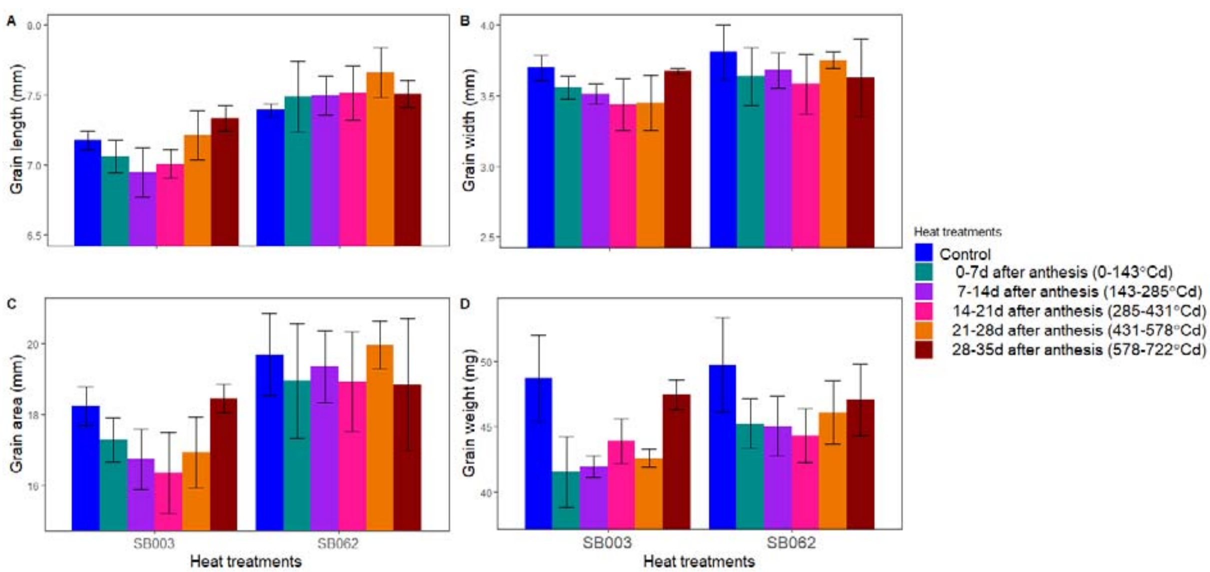


**Figure 3: Effect of heat stress on final length (mm), width (mm), area (mm$^2$) and dry weight (mg) of individual grains from the main stem in two genotypes (SB003 and SB062). A heat stress was applied at 0-7 (0-143 ºCd), 7-14 (143-285ºCd), 14-21 (285-431ºCd), 12-28 (431-578ºCd) and 28-35 (578-722 ºCd) days after anthesis of the main stem. Error bars correspond to confidence interval at *P*=0.05.**

## Discussion

Grain weight, an important determinant of yield and quality in cereals, is strongly controlled by the duration and rate of grain fill (Yang and Zhang, 2010). Post-anthesis supply of assimilates to developing grains is met either directly through assimilation or indirectly through remobilisation of reserves accumulated before anthesis. Our study indicated that short episodes of heat stress during the grain filling accelerate leaf senescence, influencing assimilate supply and grain development. These negative effects of heat were clear for SB003, which experienced earlier leaf senescence and produced reduced grain area and weight, especially for early-to-mid post-anthesis stress. SB062 appeared as a heat tolerant line in terms of both leaf senescence and grain development. Green leaves, one of the major assimilate suppliers to developing grains (Araus et al., 1993), are essential to the grain filling process, and a decline in leaf greenness can seriously impair grain size. Hence, maintaining leaf greenness longer after anthesis can contribute to better yield performance, in particular under stress (Lopes and Reynolds, 2012; Christopher et al., 2016). Apart from limiting current assimilate supply, high temperature may also induce senescence of grains, curtailing their ability to convert available sugars into starch (Shirdelmoghanloo et al., 2016). In addition, heat effects reported in this study are expected to substantially strengthen when crops are affected by heat and drought together, as frequently occurring in Australian production environments (Lobell et al., 2015).

## Conclusion

Grain filling process in wheat is sensitive to heat stress. Short episodes of heat during the grain filling period accelerate chlorophyll degradation and leaf senescence in wheat, thus impairing grain development. This study suggested that wheat lines with an ability to maintain leaf greenness longer after a heat event could better sustain grain development through increased assimilate supply. Stay-green appears as a promising adaptive trait to improve heat tolerance in wheat.

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## References

Araus JL, Brown HR, Febrero A, Bort J and Serret MD (1993). Ear photosynthesis, carbon isotope discrimination and the contribution of respiratory $CO_2$ to differences in grain mass in durum wheat. Plant Cell Environ. 16,383–392.

Bennett D, Izanloo A, Reynolds M, Kuchel H, Langridge P and Schnurbusch T (2012). Genetic dissection of grain yield and physical grain quality in bread wheat (*Triticum aestium* L.) under water-limited environments. Theor. Appl. Genet. 125, 255-271.

Christopher J, Christopher MJ, Borrell AK, Fletcher S and Chenu K (2016). Stay-green traits to improve wheat adaptation in well-watered and water-limited environments. J. Exp. Bot. 67, 5159-5172.

Barlow KM, Christy BP, O'Leary GJ, Riffkin PA, Nuttall JG (2015). Simulating the impact of extreme heat and frost events on wheat crop production: A review. Field Crops Res. 171,109-119.

IPCC (2012) – Field, C.B., V. Barros, T.F. Stocker, D. Qin, D.J. Dokken, K.L. Ebi, M.D. Mastrandrea, K.J. Mach, G.-K. Plattner, S.K. Allen, M. Tignor, and P.M. Midgley (Eds.) Available from Cambridge University Press, The Edinburgh Building, Shaftesbury Road, Cambridge England, 582 pp.

Lobell DB, Hammer GL, Chenu K, Zheng B, McLean G and Chapman SC (2015). The shifting influence of drought and heat stress for crops in northeast Australia. Global Change Biol. 21, 4115-4127.

Lopes MS and Reynolds MP (2012). Stay-green in spring wheat can be determined by spectral reflectance measurements (normalized difference vegetation index) independently from phenology. J. Exp. Bot. 63, 3789-3798.

Pheloung PC and Siddique KHM. (1991). Contribution of stem dry matter to grain yield in wheat cultivars. Func. Plant Biol. 18, 53-64.

Olivares-Villegas JJ, Reynolds MP and McDonald GK (2007). Drought-adaptive attributes in the Seri/Babax hexaploid wheat population. Funct. Plant Biol. 34, 189-203.

R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Shirdelmoghanloo H, Cozzolino D, Lohraseb I and Collins NC (2016). Truncation of grain filling in wheat (*Triticum aestivum*) triggered by brief heat stress during early grain filling: association with senescence responses and reductions in stem reserves. Funct. Plant Biol. 43, 919-930.

Zhou G and Wang Q (2018). A new nonlinear method for calculating growing degree days. Sci. Rep, 8, 10149.

Yang J and Zhang J (2010). Grain-filling problem in 'super' rice. J. Exp. Bot. 61, 1-4.

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