

Genetic variation in wheat pollen heat tolerance

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Abstract

Cereal crops will be exposed to heat waves more frequently in the future according to climate forecasts. When these periods of elevated temperature coincide with sensitive stages of reproductive development in wheat (*Triticum aestivum*) major yield loss often occurs. This investigation tested the hypothesis that there is genetic variation in pollen heat tolerance during the meiosis and anthesis stages of development in wheat. Studies were conducted on five genotypes in controlled environment greenhouse experiments in Sydney and Narrabri. Wheat genotypes were exposed to high temperature stress (35/22°C day/night) for 3 days at meiosis and anthesis and their response compared with unheated controls. Kernel weight per spike, kernel number per spike and 1000-kernel weight were measured as indicators of the heat stress response on pollen traits and grain yield. Crusader was the most sensitive to high temperatures during reproduction, while Sokoll performed best under heat stress, experiencing little reduction in kernel number and weight per spike. Traits contributing to grain yield were most reduced when heat stress coincided with anthesis rather than meiosis. High temperature stress caused abnormalities in the progression through meiosis, including arrested pollen development. The reduction in grain yield in greenhouse plants exposed to high temperature stress at anthesis provided similar trends to wheat grown under field conditions in Narrabri. This new information on the heat stress response of wheat at meiosis and anthesis could be exploited to develop varieties with superior reproductive stage heat tolerance for future warming and variable climates.

Key words

Wheat, heat, pollen, meiosis, anthesis, breeding

Introduction

Hotter and drier climate conditions anticipated in southern and western regions of Australia are predicted to reduce wheat yield by up to 15% (Kokic *et al.* 2005). Short duration heat shock, when maximum temperatures can exceed 35°C, are already quite common in the grain belt of Australia during spring when crops are approaching flowering. Pollen development has been identified as the most heat-sensitive process in plant sexual reproduction, with both meiosis and gametogenesis being thermosensitive (Bokszczanin and Frangkostefanakis 2013). Various degrees of tolerance to high temperatures (36/30°C) at anthesis were reported in wild wheats (*Aegilops* species) (Pradhan *et al.* 2012). Although previous research has identified wheat germplasm with superior heat tolerance during anthesis, few studies targeted the sensitive pollen development stage (e.g. meiosis). This study aims to test the hypothesis that there is genetic variation in pollen heat tolerance during meiosis and anthesis in wheat.

Materials and methods

Five wheat genotypes (Table 1) were grown at the Darlington polytunnel, the University of Sydney, Sydney and also repeated in a greenhouse at Narrabri in northern NSW. Five seeds per genotype per pot were sown across three temperature different treatments with four replicates in a completely randomised block design. Plants were heated (35°C/22°C day/night) for three days at either meiosis or anthesis and compared with an untreated control.

Table 1. List of varieties/lines, their pedigree and pre-determined tolerance or sensitivity to high temperature based on field experiments in Narrabri (*CIMMYT- International Maize and Wheat Improvement Centre)

Variety	Heat tolerance	Pedigree / Accession
Sokoll	Tolerant	Synthetic wheat derivative
WH542	Tolerant	CIMMYT* breeding line released as a variety in India
Atilla*2/PBW65	Sensitive	CIMMYT breeding line Atilla x PBW65 (Indian variety)
Berkut	Sensitive	CIMMYT Drought tolerant breeding line
Crusader	Sensitive	Pedigree Sunbrook/H45 LongReach Plant Breeders cultivar released in NSW

Temperature and relative humidity were controlled in the greenhouse facility before and after the imposition of heat treatments. Temperature was set at 23/15°C day/night with 50% humidity. Auricle distance was monitored once flag leaf emergence occurred in each plant. This is the distance measured between the auricle of the flag leaf and the auricle of the penultimate leaf (Fig. 1). An auricle distance of 4-8 cm was used as a morphological marker for meiosis (Barton *et al.* 2014).



Fig. 1. Auricle distance (cm): the distance (cm) between the auricle of the flag leaf and the auricle of the penultimate leaf.

Individual primary culms at the appropriate developmental stage for the heat treatment were tagged for later identification. Plants were transferred to growth cabinets, when the majority of culms in each pot had reached either meiosis or anthesis and exposed to high temperature for 3 days. Control plants remained in the greenhouse during this period under controlled temperatures (23/15°C day/night). Pots were returned to the greenhouse to mature following heat treatment. At physiological maturity, tagged culms were randomly selected from each pot and the spikes were removed. The grains from each spike were collected using a single head thresher and bagged separately. The kernel weight and number per spike and 1000-kernel weight was recorded for each pot. The percentage reduction in grain weight per spike was calculated for each variety in this experiment and compared with the percentage reduction in grain yield of those same genotypes evaluated in a late sowing field study at Narrabri in 2012 to determine the relationship between controlled environment and field screening. The field experiment was conducted in 2012 at the International Grains Research Centre in Narrabri using early (13 May 2012) and late (15 August 2012) sowings to compare the response to high temperature stress between genotypes. Statistical analysis was conducted by analysis of variance (ANOVA) for each character measured using Genstat 16th Ed.

Results and discussion

There was a general consistency in the ranking of genotypes for heat stress tolerance across the greenhouse studies in Sydney and Narrabri, although only the Sydney data is presented in this paper. All genotypes subjected to high temperature stress expressed a significant reduction in mean kernel number per spike and kernel weight per spike, compared with the control ($P < 0.01$) (Fig. 1). Anthesis was the stage of development most sensitive to high temperature stress. Average kernel weight per spike reduced by 55% when heat stress occurred at anthesis compared with 47% at meiosis. Crusader was the exception and temperature stress at meiosis had greater influence on kernel traits. The interaction between variety and treatment was significant

for all traits ($P < 0.01$), indicating that genotypes responded differently to high temperature stress applied at different development stages. The greatest difference in heat stress response, based on kernel number and weight per spike, was observed between Crusader and Sokoll. The heat sensitive cultivar Crusader had the most negative response of all genotypes to high temperature at both meiosis and anthesis. Heat stress reduced kernel weight up to 89% and greatly reduced kernel number per spike. In contrast, heat stress only slightly reduced kernel number and kernel weight per spike at meiosis in Sokoll. Of all varieties tested, Sokoll was most resilient to high temperature stress during the reproductive stages. Berkut also demonstrated some tolerance to heat stress. However, despite WH542 being classified as tolerant to high temperatures, a significant difference in kernel weight per spike between heated (meiosis = 1.38 g, anthesis = 1.41 g) and unheated controls (2.96 g) was observed. The effect of heat treatment on 1000-kernel weight was minor, but was most significant in sensitive genotypes. Highest 1000-kernel weight occurred in Attila*2/PBW65 heated at meiosis (62.7 g) and the lowest occurred in Crusader heated at anthesis (35.7 g). Heat stress at meiosis resulted in the highest 1000-kernel weight across treatments in most genotypes. Berkut was the only exception, recording higher 1000-kernel weight in the unheated control.

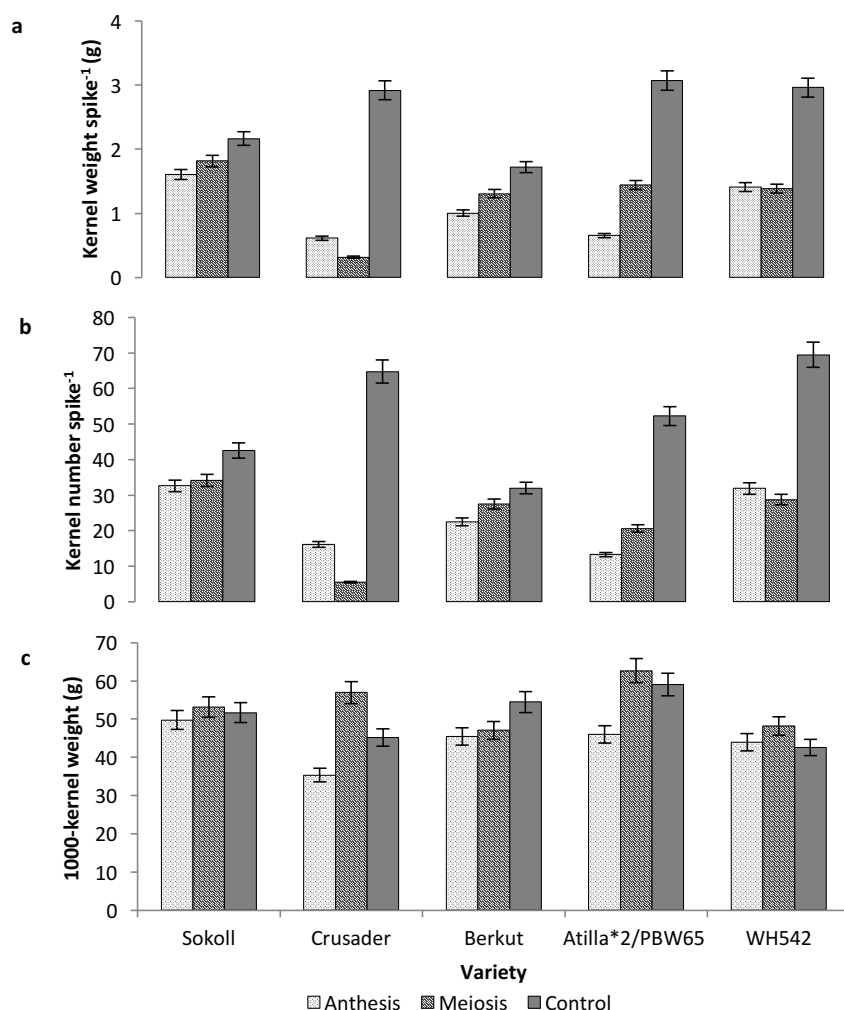


Figure 2. (a) Kernel weight per spike (g), (b) kernel number per spike and (c) 1000-kernel weight (g) of five varieties (Sokoll, Crusader, Berkut, Atilla*2/PBW65 and WH542) heated at meiosis and, anthesis (35°/22°C day/night for 3 days) and unheated control (Dots= anthesis, hashed= meiosis and solid= control). Error bars represent standard error of the mean.

A strong positive correlation ($r = 0.89$) was found between kernel number per spike and kernel weight per spike ($y = 0.0464x + 0.1032$). This relationship was strongest in plants that were heated at either meiosis or anthesis compared with the unheated control. 1000-kernel weight did not appear to have any impact on kernel weight per spike. Grain yield loss of the same five genotypes evaluated in late sown heat stress treatments in field experiments at Narrabri in 2012 correlated ($r = 0.76$) with the reduction in kernel weight

per spike in the polytunnel study (Fig. 3). Yield loss was calculated as the difference between optimally sown (13 May) and late sown (15 August) materials. The ranking of wheat genotypes for heat stress tolerance was consistent under field and polytunnel conditions; Crusader was the most sensitive and Sokoll was the most tolerant to high temperatures during the reproductive stage.

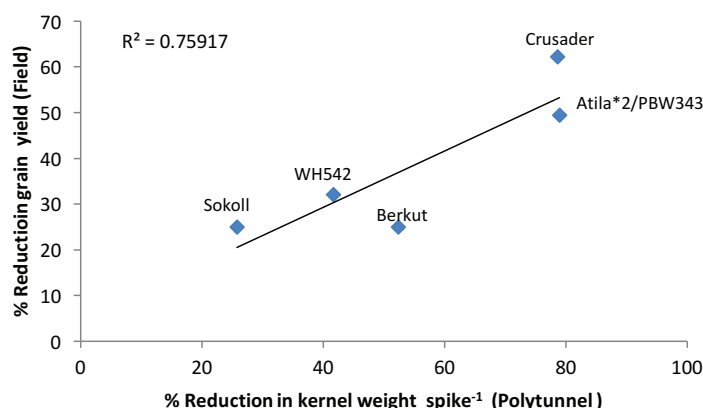


Figure 3. The relationship in average percentage reduction in grain yield between wheat grown in the polytunnel under high temperature stress at anthesis compared with unheated control and late sown compared with early sown wheat under field conditions in Narrabri for five wheat genotypes (Sokoll, WH542, Berkut, ATILLA*2/PBW343 and Crusader).

Sokoll was classified as the most heat tolerant genotype as no significant grain reduction occurred when exposed to high temperature at both anthesis and meiosis. This genotype is a synthetic derived line containing new D-genome diversity from *Aegilops tauschii* that may have been lost during the evolution of wheat or never present in the original ‘chance’ cross between ancestral tetraploid wheat and the D genome donor, which occurred some 8,000 years ago. Sokoll is therefore a genetic resource for pollen heat tolerance that can be used to develop more heat tolerant wheat cultivars. Kernel number per spike under temperature stress could be an effective selection criterion for crop improvement. As most of the variation in heat tolerance is additive, the breeder can identify the least related lines from among the best performing materials under heat stress to combine in crossing (Trethowan *et al.* 2010).

Conclusions

This study supports the hypothesis that there is genetic variation in pollen heat tolerance during meiosis and anthesis in wheat. Vulnerability to high temperature stress is heightened at anthesis for some genotypes and at meiosis in others, such as Crusader.

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