Lipid components of leaf and pollen tissue, a possible biochemical trait for breeding heat-tolerant wheat (*Triticum aestivum*)

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Abstract
Heat stress at reproductive development affects pollen viability of wheat in part due to biochemical changes in lipids. Hence, the lipid metabolism of wheat under heat stress could ultimately be used as a possible biochemical trait for breeding thermotolerant genotypes. Wheat genotypes of diverse heat-tolerance levels were heat-stressed at meiosis and analysed for leaf- and anther/pollen-lipid concentrations as well as pollen viability and grain formation. Lipid metabolism in the leaf was less affected by the heat stress than pollen. Majority of the anther/pollen unsaturated fatty acids were decreased by the heat stress but there was an increase in some saturated fatty acids. Heat-tolerance of the wheat genotypes was more related with the level of decrease of unsaturated fatty acids in anther/pollen tissue than in the leaf. Reduction in unsaturated fatty acid concentrations were associated with reduced pollen viability, poor grain formation and yield loss, to differing degrees in different genotypes. Identification of wheat genotypes with resilient lipid metabolism under heat stress that can maintain pollen viability may provide breeders with an additional tool for screening promising heat-tolerant breeding lines and facilitate the development of wheat varieties for future climate.

Keywords
heat stress, lipid metabolism, pollen viability, wheat breeding, grain formation.

Introduction

With changing global climates, heat waves have become a common abiotic stress to broadacre crops such as wheat, especially during the reproductive development. Heat shock disrupts photosynthesis, inhibits plant growth and affects reproductive development by limiting starch mobilization from leaf tissues to reproductive organs such as anther and pollen grains. Under stress, plants switch to utilizing fatty acids (FA) as respiratory substrates for normal growth and development due to the lipidome changing its content to implement defending actions in the process of adaptation (Yu et al. 2018). Plants alter lipid metabolism as a mechanism of resistance for their survival. However, this can lead to reduced pollen viability and high pollen mortality (Dwivedi et al. 2017). The size of alterations of lipid/FA composition, especially the level of unsaturation during abiotic stress, determines the stability of cell membranes (Narayanan et al. 2015). As a major component in the pollen wall, lipids form the hydrophobic exine layer for a protection during pollination (Zhang et al. 2016).

In response to heat stress with biological acclimation for adaptation, lipid composition alterations in both leaf and pollen tissue are common responses for wheat (Narayanan et al. 2018). Unlike vegetative tissue or the female gametophyte, the pollen grain (male gametophyte) is more vulnerable and sensitive to high temperature due to its unique physiological features (Muller and Rieu 2016). Female gametophyte (ovule) and pistil fertilizing ability can adapt and stay unaffected under high temperatures (33 - 40 °C), while the male gametophyte (pollen) is very heat-sensitive and may be unable to survive (Zhang et al. 2016). While pollen cells are highly sensitive to heat stress, its developmental process during meiosis is the most heat-sensitive. Identification and characterisation of those responsive lipid groups in wheat genotypes may help to uncover biochemical traits for heat-resistance and for breeding the heat-tolerant wheat varieties.

In order to benefit the breeding of heat-tolerant wheat, it is crucial to investigate the association between lipid alterations and pollen viability of wheat after a heat shock that affect pollination and grain formation. This research aims to identify the lipid species of leaf and anther/pollen tissue of wheat that could be biomarker trait and can be used in the advanced heat-tolerant wheat breeding program.

Materials and Methods

Wheat genotypes and plant growing conditions
The experiment was conducted with five genotypes of wheat (Table 1) at the Plant Breeding Institute of the University of Sydney located at Camden NSW, Australia with one heat tolerant (genotype number 45), two

moderately heat tolerant (genotypes 245 and 262), and two heat susceptible (genotypes 51 and 58) wheat genotypes. Wheat plants were grown at controlled temperatures in a microclimate room at 22°C/15°C day night conditions with artificial day-time lighting (300–400 µmol m^{-2} s^{-1}), planting 4 seeds in 4 L pots with standard potting mix and fertilisers. Plants were taken care as per routine cultural practices and irrigated manually when required.

Heat treatment and sampling for FA analysis

For heat treatment, plants were placed in a heat chamber (Thermoline plant growth cabinet, model: TPG-1260-TH-Co2) set at 35/22 ºC day/night, for 3 days at the meiosis stage of the plants (auricle distance of 4-8 cm). The control plants continued to grow at 22°C/15°C. Leaf samples for FA analysis were collected immediately after the heat treatment (0 d) with their corresponding controls, from middle half of the flag leaf and cut to small pieces before storing at -80°C. The anther/pollen samples for FA were collected at anthesis, pooling a total of 40 mature anthers before dehiscence. The FA contents were calculated to µg/g fresh weight.

Extraction of FA and GC-MS analysis

For the extraction and simultaneous esterification of wheat leaf and pollen lipids, we used acid-catalyzed transesterification. This procedure involves a single-step reactive extraction and is quicker and more convenient than protocols that involve separate extraction and esterification steps (Christie, 2005). Five replicates of each treatment for each genotype were analysed, taking 50 mg of leaf tissue and about 30 mg of anther/pollen (40 anthers), which were then placed in the Teflon-lined screw cap glass tubes and added 10 µl of internal standard fatty acids (C17:0 at 25 µL mL^{-1}). Then, added 1.0 mL of freshly prepared 2.5% H_2SO_4 in methanol and placed in an oven set at 70ºC for 1.5 hours (tubes were shaken few times). The tubes with the samples then cooled for about 5 min to room temperatures. To elicit phase separation, added 500 µL of hexane, then 1.5 mL 0.9% NaCl in water. The tubes were shaken vigorously then centrifuged at 500 g for 5 min. About 50 µL of the extract from the upper organic phase were collected to the glass vials with low volume insert and processed the GC-MS analysis (GCMS-QP2010 Plus).

Table 1 Pedigree of the wheat genotypes

Number	Pedigree of wheat genotypes
45	Sokoll/2/Sokoll/35888 M 500132
51	Sokoll/2/Sokoll/35888 M 500132
58	Sokoll/2/Sokoll/35888 M 500132
245	Ega Gregory///Croc1/224//Opta-
262	Suntop

Fatty acid methyl Ester (FAME) were identified and quantified using commercial FAME mixtures (37 component fatty acid methyl ester mix, and 27 component bacterial fatty acid methyl ester mix, both from Sigma-Aldrich) plus retention indices (based on even-numbered alkanes from C8 to C36) and EI mass spectra from the NIST library.

Pollen viability and grain formation

Pollen viability was determined on mature pollen collected at anthesis by harvesting one tagged spike per pot, for analysis by Amphasys cytometer (Ampha Z32), which was followed by the method described by Bokshi *et al*. (2020). In brief, two mature anthers were collected in an Eppendorf tube for dispersion of pollen in 2 mL of Ampha buffer #6 and filtered through a 70 µm sieving filter to a 5 mL sample holding tube. The pollen-buffer was immediately analysed in the cytometer which separates non-viable and viable pollen with the ready data. Percent grain formation and grain yield per spike were recorded at maturity after harvesting the tagged spikes from the heat treated or control plants.

Data analysis

Data were analysed with Genstat 18th edition software (VSNI, UK) to assess the main effects for breeding lines and heat treatment, and their interactions, for an ANOVA, and presented with standard error (SE) of means.

Results and Discussion

Leaf tissue from wheat was slightly affected by heat stress for most of the fatty acid contents. However, at various levels among the breeding lines. Saturated fatty acids such as C12:0, C14:0, C20:0, C22:0 and C24:0 were found at smaller amounts in leaf tissue that slightly increased from heat stress in most of the wheat genotypes (data not presented). The saturated fatty acid C16:0 (palmitic acid) was highest in content (about 10%) in wheat leaf and was at a consistent level at heat stress in genotypes 45 and 51 but had increased in 58

and 245, while slightly reduced in 262 (Fig 1A). This increase may have occurred from desaturation of C16:1 which was decreased by the heat stress (data not presented).The genotype 45 was earlier identified as heat-tolerant.

The leaf unsaturated fatty acids such as C16:1, C18:1 (data not presented) or C18:2 (linoleic acid) (Fig 1B) also did not vary by heat stress among the wheat genotypes. However, C18:3 (linolenic acid), a highly unsaturated leaf fatty acid (Fig 1C) that constituted the highest proportion (more than 60%) of the total fatty acids, did not vary significantly (P >0.05) among the genotypes or from a heat stress. Wheat leaf tissue by its physical nature can provide greater resilience to heat stress in order to maintain the integrity of lipid components (Muller and Rieu 2016).

Heat stress at meiosis significantly ($P \leq 0.001$) affected the fatty acids of anther/pollen tissue of wheat genotypes. The most prominent anther/pollen saturated fatty acid C16:0 (about 10%) was reduced by heat stress except in the genotype 45 (Fig 1D). Similar trend was observed in C18:0 (data not presented). Other minor-content saturated anther/pollen fatty acids such as C12:0, C14:0, C20:0, C22:0 or C24:0 were shown to have an increase or not affected from heat stress in genotype 45, but decreased in other genotypes (data not presented). Stability in the lipids of all contents in the genotype 45 may have helped maintain anther/pollen cell integrity and in the expression of resilience to heat stress.

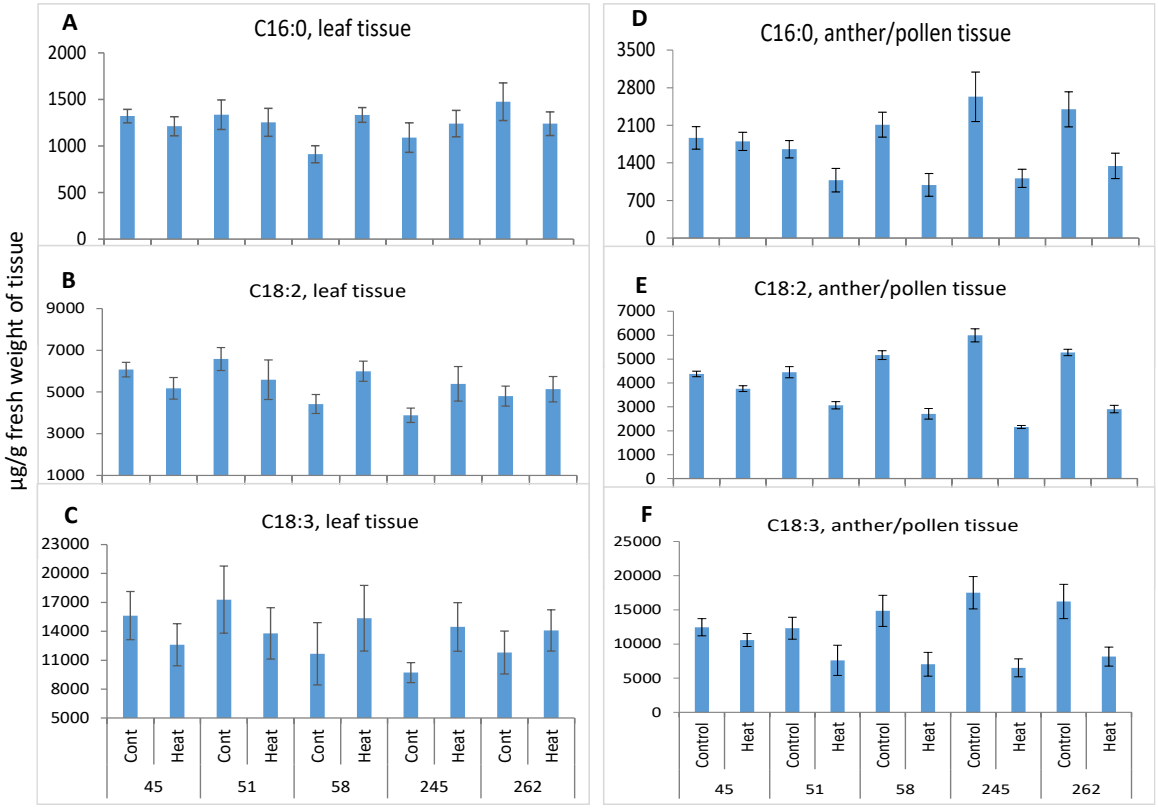

Figure 1 Saturated and unsaturated fatty acids in leaf and anther/pollen tissue after heat stress at meiosis. The leaf fatty acids C16:0 (A), C18:2 (B) or C18:3 (C) were assessed by analysing leaf tissue collected at the end of 3d heat-stress and the anther/poll fatty acids C16:0 (D), C18:2 (E) or C18:3 (F) collected at athesis. Bars on the column representing standard error (SE) of the mean.

The anther/pollen unsaturated fatty acids such as C16:1, C18:1 (data not presented) or C18:2 (Fig 1E), together constituted about 20% of the total fatty acids and were less affected by the heat stress in the genotype 45 than other wheat genotypes. The most prominent unsaturated pollen fatty acid C18:3 (more than 60%) reduced significantly from heat stress, however, least affected in the genotype 45 (Fig 1F). The exact role of the fatty acids in the cellular level of another/pollen is yet to be clarified, and suggested to have a strong association with heat-tolerance (Narayanan et al. 2018), which therefore could provide a useful tool for the breeding heat-tolerant wheat varieties.

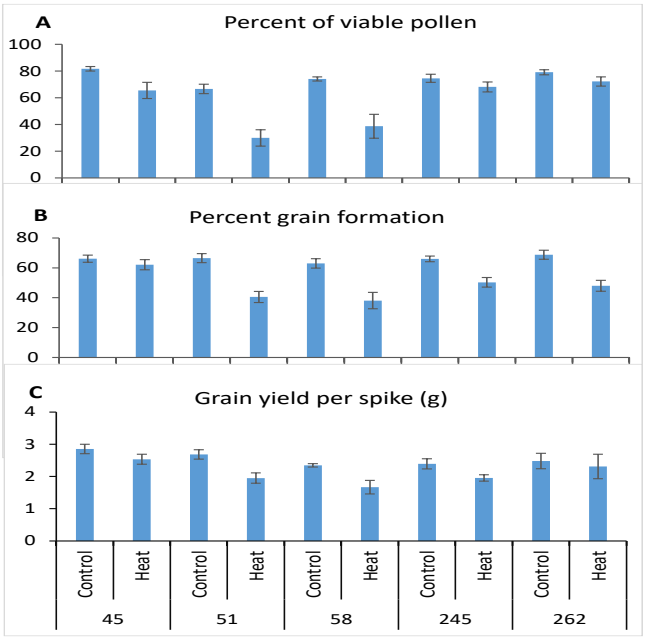

Figure 2 Effect of heat stress on pollen viability (A), percent grain formation (B) and grain yield per spike by the wheat genotypes. Bars representing standard error (SE) of the mean.

Percent pollen viability was affected (P<0.001) by the heat stress at meiosis, mostly in genotypes 51 and 58 but less in the genotypes 45, 245 or 262 (Fig 2A). Similarly, the percentage of grain formation was affected by heat stress, mostly in the genotypes 51 or 58 and least in the genotype 45 (Fig 2B). The genotypes 245 or 262 were also affected by the heat stress at meiosis for grain formation. The grain yield per spike reflected the trend of percent grain formation in different genotypes that resulted from the heat stress (Fig 2C). However, the yield loss from heat stress in genotype 262 was minimised because of increased grain size in the heat stressed plants (data not presented). Reduced grain yield by the genotype 51 or 58 were evident of an association to unstable pollen lipids which affected pollen viability of these genotypes under heat stress. A strong positive relationship between pollen viability and grain formation was observed previously (Bokshi et al. 2020).

Conclusion

Our data indicated a change in lipid metabolism, especially anther/pollen cells which affected pollen viability of wheat genotypes, and impacted grain formation and yield. Leaf tissue exhibited greater tolerance to heat stress in lipid metabolism. However, reduced levels of anther/pollen lipids may be due to a compromise in exporting respiration substrates from the leaf to the reproductive region of wheat plant during adaptation to heat stress. Further study on identifying the leaf and anther/pollen lipid components responsible for heat-tolerance may provide biochemical traits and help speed up the development of heat-tolerant wheat varieties.

Acknowledgments

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