High temperature effects on wheat yield, grain quality and pollen lipids

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A thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

Faculty of Science

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February 2025

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Abstract

Wheat (*Triticum aestivum* L.) provides roughly 20% of global dietary calories and 21% of daily protein intake, placing it at the core of global food security. Yet, warmer climates now threaten wheat yield and grain quality, by accelerating development and impairing reproductive success. High-temperature (HT) stress can reduce yield components, disrupt pollen viability, diminish starch accumulation, alter protein balance, and affect fibre formation, ultimately hindering end-use value. Although numerous studies have examined HT impacts on wheat, knowledge gaps remain regarding how HT influences multiple grain-quality traits across diverse environments and genotypes.

To address this, this thesis integrates both field and controlled-environment experiments to evaluate wheat cultivars differing in heat tolerance. Field experiments conducted in three distinct Australian locations (Narrabri, NSW; Horsham, VIC; and Merredin, WA) which form the basis of Chapters 4, 5 and 6, where late sowing (exposing plants to higher temperatures during grain filling) was compared with earlier sowing times. Chapter 7 focuses on a controlled-environment experiment with controlled day/night temperatures to more precisely capture HT effects on pollen lipid composition. The final chapter discusses overall findings and synthesises breeding and agronomic implications.

Field experiments were established in 2019 and 2020 using multiple cultivars, Berkut and Flanker (high heat tolerance), Sokoll and Suntop (moderate heat tolerance), and Cobra (heat-sensitive) using an alpha lattice design and two replicates. Soil properties (texture, pH, nutrient levels) were documented. Grain-quality analyses encompassed colour measurements (Konica Minolta Cr-400), crude protein (elemental analysis), protein fractions (bicinchoninic acid assays), starch and fibre (Megazyme methods), pasting properties (Rapid Visco-Analysis, RVA), mineral content (ICP-OES), and phytate (HPLC). ANOVA and Tukey's honestly significant difference (HSD) tests determined statistical significance.

Yield and Protein Traits (Chapter 4): Late sowing increased heat exposure, generally lowering yield and thousand kernel weight (TKW). Crude protein often rose in heat-stressed plants, but the glutenin-to-gliadin ratio declined in sensitive cultivars (e.g., Cobra). Heat-tolerant lines like Flanker showed more stable or improved protein fraction balances. Grain colour likewise shifted (higher L^* and lower b^*) under HT, and

there was a shorter grain filling period and altered grain composition.

Starch and Fibre (Chapter 5): Elevated temperatures led to lower starch content and altered pasting properties; Sokoll and Cobra, for instance, showed significant drops in peak and breakdown viscosities. At Merredin, extreme temperatures reduced grain filling and starch deposition, yet in some cases increased crude fibre through accelerated lignification. More heat-resilient cultivars like Berkut and Flanker maintained more stable starch profiles and fibre levels.

Minerals and Phytate (Chapter 6): HT often raised mineral concentrations due to reduced thousand kernel weight, especially at Merredin, where sandy soil exacerbated HT effects. Phytate tended to decrease under hotter conditions at Merredin, potentially improving mineral bioavailability but also reducing seed phosphorus stores. Berkut generally retained higher mineral content, while Sokoll showed notable declines in Zn and Fe. Soil factors (e.g., alkalinity at Horsham and Narrabri vs. sandy acidity at Merredin) strongly influenced nutrient uptake.

Reproductive processes in wheat, particularly pollen development, are highly sensitive to HT. Four cultivars with descending heat tolerance; Flanker (highest heat tolerance), Suntop, Sunchaser, and Cobra (heat sensitive) were tested under three temperature regimes (22/15°C, 35/22°C, and 40/22°C). Higher temperatures reduced total saturated and unsaturated fatty acids in pollen, but the extent of reduction differed between these two groups, resulting in a shift in the saturated/unsaturated fatty acid ratio. Cobra showed the steepest decline in total fatty acids and poorest pollen viability, consistent with its lower heat tolerance. In contrast, the lipid profiles of Flanker and Suntop were minimally affected by HT, reflecting higher thermotolerance. These data indicate that maintaining a constant lipid profile (i.e. lipid homeostasis) is crucial for heat resilience.

Both field and controlled-environment data confirm that HT shortens grain filling duration, reduces yield, and alters protein composition, starch structure, fibre deposition, and mineral uptake. While crude protein may increase under HT, functionality can decline if critical fractions (e.g., glutenins) decrease. Additionally, pollen viability and reproductive success hinge on lipid homeostasis. Collectively, these findings underscore the importance of integrating multiple strategies; including breeding heat-tolerant genotypes, adjusting sowing schedules to maintain wheat yield and grain quality in a warming world.

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ABBREVIATIONS

*a**: CIELAB colour data

AGT: Australian Grain Technologies

ANOVA: analysis of variance

*b**: CIELAB colour data

BCA: bicinchoninic acid

CIELAB: commission internationale de l'Eclairage

DGDG: digalactosyldiacylglycerol

FAME: fatty acid methyl esters

FAO: Food and Agriculture Organization of the United Nations.

FBPase: fructose-1,6-bisphosphatase

GDD: growing degree days

GFD: grain-filling days

GPT: glutamate pyruvate aminotransferase

GS: glutamine synthase

HMW: high-molecular-weight

HSD: honest significance difference

HT: high temperature

HPLC: high-performance liquid chromatography

IPCC: Intergovernmental Panel on Climate Change

ICP-OES: inductively coupled plasma optical emission spectrometry

*L**: CIELAB color data

LMW: low-molecular-weight

MDA: malondialdehyde

MGDG: monogalactosyldiacylglycerol

NADP-ME: nicotinamide adenine dinucleotide phosphate-malic enzyme

NS: no significant difference

NSW: New South Wales

OM: organic matter

PAWC: plant available water capacity

PBI: Plant Breeding Institute

PEPCase: phosphoenolpyruvate carboxylase

PPDK: pyruvate orthophosphate dikinase

PSI: photosystem I

PSII: photosystem II

ROS: Reactive Oxygen Species

RVA: rapid visco-analyser

SFA: total saturated fatty acids

SFA/UFA: saturated/unsaturated fatty acids ratio

TAGs: triacylglycerols

TCA: trichloroacetic acid

TKW: thousand-kernel weight

TOS1: normal sowing

TOS2: late sowing

UFA: total unsaturated fatty acids

VIC: Victoria

WA: Western Australia

WHO: World Health Organization

PUBLICATIONS ARISING FROM THIS THESIS

Journal publication

Bai, Y., Khoddami, A., Messina, V., Zhang, Z., & Tan, D. K. (2025). Response of Wheat Genotypes Stressed by High Temperature in Terms of Yield and Protein Composition Across Diverse Environments in Australia. *Agriculture*, *15*(5), 514 https://www.mdpi.com/2077-0472/15/5/514 (Chapter 4)

Conference publication

Bai Y, Khoddami A, Warren C, Tan D. K. Y. (2024) Variation in yield and grain protein components of two wheat genotypes grown under diverse environments. Australian Agronomy Conference – 21-24 October 2024, Albany, Western Australia (part of Chapter 4)

Conference oral presentations

Bai, Y., Khoddami, A., Messina, V., & Tan, D. K. Y. (2023) Variation in yield, quality of wheat genotypes grown under diverse environments. The 73rd Annual Australasian Grain Science Conference - 26-29 September 2023, Rockhampton, Australia (part of Chapter 4 and 5).

Bai Y, Khoddami A, Warren C, Tan D. K. Y. (2024) Variation in yield and grain protein components of two wheat genotypes grown under diverse environments. Australian Agronomy Conference - 21-24 October 2024, Albany, Western Australia (part of Chapter 4)

Poster presentation

Bai, Y., Tan, D. K. Y., Warren, C., & Bokshi, A. I. (2022) Pollen lipids in wheat (*Triticum aestivum*) varieties are affected by heat stress. Wheat Breeding Assembly (WBA) - 27-28 August 2022, Narrabri, NSW (part of Chapter 7)

ACKNOWLEDGEMENTS

This journey has been both long and fleeting, filled with moments of both pain and joy. Along the way, I have grown in many ways—not only in my learning ability but also in my analytical skills, writing proficiency, and capacity for self-discipline.

I am deeply grateful to my supervisors, Prof. Daniel Tan, Dr. Ali Khoddami, and Prof. Charles Warren, whose unwavering support, insightful guidance, and critical perspectives have propelled my growth over these years. I am especially thankful to Daniel, who has been a constant mentor throughout, offering continuous assistance particularly in refining my dissertation writing and enhancing my presentation skills at conferences.

I also extend my sincere thanks to Dr. Anowarul Bokshi, Dr. Valeria Messina, Dr. Zhao Zhang, Dr Lindsay Campbell and Ms. Yifeng Lu, among others, whose expertise in experimental techniques and data analysis has been invaluable to my research.

I feel profoundly honored to thank my parents, Ting Bai and Caihong Zhang, and my wife, Jiatong Li, for providing me with both financial and emotional support. Their unwavering encouragement helped me overcome challenges and achieve my goals. I would also like to acknowledge my friends—Guanglong, Zhenhong, and many others—who lifted my spirits during difficult times and shared in the joy of my successes.

We acknowledge the Grains Research and Development Corporation (GRDC Projects: US00057 and US00081) and the School of Life and Environmental Sciences (SOLES) Seeding Grant (Project 214478 Feeding chickens in the future—The potential of heat-tolerance wheat) for funding this project.

Finally, I offer my heartfelt thanks to myself for the persistence and continuous progress that made this accomplishment possible.

Chapter 1. General Introduction

1.1. Background

Wheat (*Triticum aestivum* L.) is one of the most important crops worldwide, providing approximately 20% of the global caloric intake and 21% of daily protein consumption, making it a cornerstone of food security and nutrition (Shewry, 2009a). Its versatility spans food products, livestock feed, and industrial applications, supported by its adaptability to diverse agro-climatic zones. Wheat proteins, particularly glutenins and gliadins, are essential for dough elasticity and extensibility, which are critical for producing bread, pasta, and other wheat-based staples (Belderok et al., 2000; Payne et al., 1984). Additionally, wheat is a major source of starch, crude fibre, and minerals, contributing to dietary energy, digestive health, and micronutrient intake (Copeland et al., 2009; Martínez-Ballesta et al., 2010).

However, climate change poses significant challenges to wheat production. Rising global temperatures, projected to increase by 1–6°Cby the end of the 21st Century, are expected to reduce wheat yields and quality (De Costa, 2011). Wheat, which thrives in an optimal temperature range of 12–22°C, is particularly vulnerable to HT. Each 1°C increase in temperature could reduce global wheat production by approximately 6%, with regions like Australia facing yield losses of up to 50% under a 2°C rise (Asseng et al., 2011; Zhao et al., 2017). These challenges highlight the urgent need to develop heat-tolerant wheat cultivars and adaptive strategies to sustain production in a warming world.

HT during flowering can reduce pollen viability, impair anther dehiscence, and hinder fertilisation success, thereby lowering the number of grains per spike (Bokshi et al., 2021; Kaur & Behl, 2010; Saini et al., 1984). Furthermore, HT during grain filling accelerates developmental processes, curtails the duration of assimilate translocation, and results in shriveled grains with lower thousand kernel weight (TKW) (Farooq et al., 2011; Yin et al., 2009). Consequently, both the reduced grain number and smaller grain size contribute to overall yield losses under high-temperature conditions.

HT in wheat triggers multiple compositional changes that collectively undermine both functional and nutritional quality. It disrupts lipid composition, a key factor for maintaining membrane integrity during pollen development by reducing polyunsaturated fatty acids and increasing lipid peroxidation, thereby impairing pollen viability and fertilization success (Bokshi et al., 2022; Narayanan et al., 2018; Djanaguiraman et al., 2018). In parallel, HT alters the glutenin-to-gliadin ratio, lowering dough elasticity and stability, even though total protein content may increase due to an elevated protein-to-starch ratio (Zhao et al., 2008; Blumenthal et al., 1993; Gupta et al., 2013). Starch, the primary carbohydrate, displays reduced accumulation and a higher amylose-to-amylopectin ratio, resulting in elevated gelatinisation temperatures and adversely affecting food texture and digestibility (Beckles & Thitisaksakul, 2014; Liu et al., 2011). Meanwhile, crude fibre, predominantly cellulose and lignin, rises as an adaptive response to accelerated crop maturity, improving thermal stability in food processing but diminishing digestibility in animal feed (Chaisawang and Suphantharika, 2006; Zhao et al., 2008). Essential minerals such as zinc and iron exhibit reduced bioavailability not only because of impaired nutrient uptake but also due to variable levels of phytate, an antinutrient that binds these minerals and significantly inhibits their absorption (Essemine et al., 2010; Velu et al., 2016; Peterson et al., 1986; Guttieri et al., 2006). Collectively, these heat-induced modifications to lipids, proteins, starch, fibre, and mineral profiles pose significant challenges for both end-use quality and nutritional outcomes.

1.2. Research Gaps and Study Objectives

Despite extensive research on HT, knowledge gaps remain in understanding its integrated effects on wheat physiology, yield, and quality across diverse genotypes and environments. Previous studies have largely focused on isolated traits or single locations, limiting their applicability to broader agricultural contexts. There is a need for comprehensive analyses that evaluate the combined impacts of HT on yield, protein composition, starch properties, fibre content, mineral accumulation, and pollen lipid profiles.

1.3. Central Research Question

What is the effect of high temperature on yield, grain quality (protein composition, starch properties, fibre content, mineral accumulation), and pollen lipids of wheat cultivars with different heat tolerance?

1.3.1 Sub-questions

1. What is the effect of cultivar and HT on the yield and protein content and composition of wheat? (Chapter 4)
2. What is the effect of cultivar and HT on the starch and crude fibre content, and starch gelatinisation properties of wheat? (Chapter 5)
3. What is the effect of cultivar and HT on the mineral accumulation and phytic acid content of wheat? (Chapter 6)
4. What is the effect of cultivar and HT on the lipid composition of wheat pollen? (Chapter 7)

1.3.2 Objectives

The primary objective of this study was to investigate the impact of high temperature on yield, quality, and pollen lipid composition in wheat cultivars with differing heat tolerance across three locations. To achieve this, two field experiments (Experiments 1 and 2) and one glasshouse experiment (Experiment 3) were conducted, each designed to address specific objectives as detailed below:

1.3.2.1 Yield, and Protein Content and Composition Analysis

To evaluate the effects of HT on yield, protein content, and protein composition in two wheat cultivars, Berkut and Sokoll, across three distinct locations, Horsham (VIC), Narrabri (NSW), and Merredin (WA), during a hot year (2019). The same parameters were assessed in three additional cultivars, Flanker, Cobra, and Suntop, grown in Narrabri under contrasting temperature conditions in 2019 (hot year) and 2020 (cool year) (Experiments 1 and 2, Chapter 4).

1.3.2.2 Starch and Fibre Analysis

To determine changes in starch content, crude fibre and starch gelatinisation properties in Berkut and Sokoll across the three locations, as well as in Flanker, Cobra, and Suntop grown in Narrabri under the contrasting temperature conditions of 2019 and 2020 (Experiments 1 and 2, Chapter 5).

1.3.2.3 Mineral Composition and Phytate Analysis

To examine the effects of HT on phytate content and mineral composition in Berkut and Sokoll across three locations and in Flanker, Cobra, and Suntop grown in Narrabri during the years of 2019 and 2020 with contrasting temperatures (Experiments 1 and 2, Chapter 6).

1.3.2.4 Pollen Lipid and Viability Analysis

To investigate the impact of HT on the lipid composition of wheat pollen and leaves, analyse the relationship between pollen viability and lipid profiles, and elucidate the mechanisms underlying heat tolerance in pollen lipids across four wheat cultivars (Experiment 3, Chapter 7).

Chapter 2. Literature Review

Abstract

Wheat (*Triticum aestivum*) is a cornerstone of global agriculture, providing essential nutrients and economic stability to billions worldwide. However, its productivity and quality are increasingly threatened by rising global temperatures, with high-temperature stress posing significant challenges to its physiological and biochemical processes. This review synthesises current knowledge on the impacts of HT on wheat growth, grain quality, and reproductive success. HT reduces yield by impairing flowering, grain filling, and photosynthesis, while disrupting pollen viability and fertilisation through changes in lipid composition and membrane stability. Furthermore, HTs alter wheat grain protein composition, starch functionality, crude fibre content, and mineral bioavailability, thereby affecting its nutritional and processing qualities. To mitigate these impacts, the development of heat-resilient wheat cultivars with stable biochemical traits is critical.

2.1. Introduction

Wheat (*Triticum aestivum*) is a foundational crop in global agriculture and food systems, providing a critical source of nourishment and economic stability to billions of people worldwide. As one of the earliest domesticated cereals, wheat originated in the Fertile Crescent of western Asia over 10,000 years ago and has since adapted to diverse regions and climates, evolving alongside human societies to meet their growing demand for energy-dense, nutrient-rich food (Bonjean, 2016). Wheat remains the world's second most cultivated cereal, valued for its versatility and high protein content, offering approximately 13% protein, which is substantially higher than other staple cereals such as maize and rice (Shewry, 2009b; FAO, 2017). Its diverse uses span from bread and pasta to livestock feed and industrial by-products, embedding wheat deeply in culinary traditions and global supply chains.

The scale of wheat production underscores its agricultural and economic significance. Global production is projected to reach 799 million tonnes in 2024 – 25, driven by contributions from key producers like China, India, and Russia, and stabilised by exporters such as Australia, Canada, and the European Union (Australian Bureau of Agricultural and Resource Economics and Sciences, April 5, 2022.). Wheat also holds particular importance in developing regions, where demand is expected to grow by over 77% by 2050, underscoring its critical role in addressing food security and economic stability (FAO, 2017). Furthermore, wheat by-products, including bran and gluten, have applications across industries such as bioplastics, bioenergy, and paper production, further enhancing its global value (Branlard & Chiron, 2016; Peña-Bautista et al., 2017).

Even though wheat plays a central role, its cultivation is increasingly threatened by the accelerating impacts of climate change. Rising global temperatures, driven by anthropogenic greenhouse gas emissions and land-use changes, have exacerbated the frequency and intensity of extreme weather events, including heatwaves, droughts, and floods, which directly impact agricultural productivity (Zandalinas et al., 2021). According to projections by the Intergovernmental Panel on Climate Change (IPCC), global temperatures are likely to rise by 1 – 6°C by the end of this century, posing severe risks to ecosystems and food production systems (De Costa, 2011). HT, in particular, poses a significant challenge for wheat, with a 1°C increase in global temperatures projected to reduce global wheat yields by approximately 6% (Zhao et al., 2017). Regions like Australia, characterised by already HTs, could experience yield reductions as severe as 50% under a 2°C rise (Asseng et al., 2011).

2.1.1. Objectives of this Review

This review systematically examines the impacts of HTs on wheat physiology and quality, addressing the critical challenges posed by HT to global food security. By integrating recent advancements and experimental evidence, this review aims to provide a comprehensive understanding of the sensitivity of wheat to HT and to underscore the urgency of breeding heat-tolerant cultivars. Adaptive strategies, such as selective breeding for heat resilience, are vital to ensuring wheat productivity under future climate scenarios and sustaining its essential role in global agriculture and nutrition.

2.2. Impacts of HT on Wheat Physiology and Reproductive Development

HT is a major abiotic factor affecting wheat growth, particularly during critical reproductive stages. The optimal temperature range for wheat flowering and grain filling is between 12°C and 22°C (Shewry, 2009b). Temperatures exceeding 24°C pose significant risks to reproductive success and overall yield. Numerous studies have demonstrated that exposure to temperatures above this range accelerates developmental processes, thereby reducing the time available for essential physiological activities such as pollen formation and grain filling, both of which are crucial for yield outcomes (Dias & Lidon, 2009a). The overview of physiological impacts of HT on wheat is summarised in Table 2.1.

2.2.1 Flowering and Grain Developmental Stages

The flowering stage is particularly vulnerable to HT, which can impair the functionality of both male and female reproductive organs. Temperatures exceeding 30°C during floret development adversely affect pollen viability, inhibit anther dehiscence, and can even induce male sterility in some wheat genotypes (Kaur & Behl, 2010). HTs can disrupt the formation of pollen mother cells and inhibit the dispersal of viable pollen, a critical step for successful fertilisation (Prasad et al., 2008). Furthermore, HT reduces the receptive window for pollen in wheat, significantly lowering fertilization rates and decreasing the number of grains per spike (Saini et al., 1984). Collectively, these effects severely compromise pollination efficiency, leading to substantial reproductive challenges under elevated temperatures.

The grain filling phase, essential for grain development and yield, is particularly sensitive to HT. Elevated temperatures accelerate the grain filling rate but simultaneously shorten its duration, limiting the translocation of assimilates into the developing grains (Farooq et al., 2011). A 5°C increase above the optimal grain filling temperature of 20°C can reduce the grain filling period by up to 12 days, resulting in smaller, shriveled grains with reduced weight (Yin et al., 2009). This accelerated grain filling diminishes grain size and yield potential. Additionally, HT during this phase disrupts photosynthesis and assimilate partitioning, further compromising the quality and quantity of the grains produced (Dias & Lidon, 2009a). These temperature-induced changes highlight the urgent need for effective temperature management strategies during reproductive stages to mitigate yield losses.

2.2.2 Photosynthetic Response

Photosynthesis in wheat leaves is highly sensitive to elevated temperatures, with HT significantly impairing photosynthetic efficiency and reducing yield potential (Wahid et al., 2007; Al-Khatib & Paulsen, 1990, 1999). Elevated temperatures reduce chlorophyll content and inactivate key chloroplast enzymes, both of which are essential for efficient photosynthesis. Furthermore, HT induces lipid peroxidation and oxidative damage, leading to membrane instability, enzyme inactivation, and protein degradation, all of which exacerbate photosynthetic decline (Sairam et al., 2000).

The enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) plays a central role in carbon fixation during photosynthesis (Ogren & Chollet, 1982). HTs alter Rubisco's kinetics and reduce the solubility of carbon dioxide and oxygen in leaf cells, ultimately lowering photosynthetic rates and increasing photorespiration (Long et al., 2004). This reduction in net photosynthesis is not solely due to increased respiration but is also influenced by changes in CO_2 and O_2 solubility and Rubisco kinetics, which promote photorespiration.

HT in wheat leaves can result in (1) reduced CO_2 solubility; (2) decreased activities of key photosynthetic enzymes, including nicotinamide adenine dinucleotide phosphate-malic enzyme (NADP-ME), fructose-1,6-bisphosphatase (FBPase), phosphoenolpyruvate carboxylase (PEPCase), pyruvate orthophosphate dikinase (PPDK), and Rubisco; (3) accelerated leaf senescence; (4) accumulation of reactive oxygen species (ROS); and (5) downregulation of non-cyclic electron transport (Marutani et al., 2012; Savicka & Skute, 2010; Huang et al., 2012). These effects collectively disrupt the photosynthetic balance, reducing carboxylation efficiency and ATP synthesis, further impairing photosynthetic capacity (Farooq et al., 2011).

At temperatures above 40°C, the photosynthetic apparatus, including photosystem II (PSII), photosystem I (PSI), and the Calvin cycle, sustains extensive damage (Sharkey, 2005). HT particularly destabilises PSII due to increased electron transport and enhanced thylakoid membrane fluidity, which disrupts membrane integrity and halts photophosphorylation (Prasad et al., 2008). This increase in fluidity not only destabilises

PSII active sites but also accelerates thylakoid degradation, leading to premature leaf senescence. Temperatures exceeding 42°C further inhibit chlorophyll synthesis, severely diminishing photosynthetic potential (Kumar & Charan, 1998). Additionally, Zhao et al. (2007) reported that substantial diurnal temperature fluctuations exacerbate flag leaf senescence under high-temperature conditions.

Table 2.1: Effects of HT on Wheat Flowering, Grain Filling, and Photosynthesis

Effect	Effects of HT	References
Flowering	- Reduced pollen viability	Kaur & Behl (2010)
	- Impaired anther dehiscence and pollen dispersal	Prasad et al. (2008)
	- Shortened receptive window for fertilisation	Saini et al. (1984)
	- Decreased grain number per spike	Prasad et al. (2008); Saini et al. (1984)
	- Possible male sterility in heat-sensitive genotypes	Kaur & Behl (2010)
Grain Filling	- Accelerated grain filling rate but reduced duration	Farooq et al. (2011); Yin et al. (2009)
	- Smaller, shrivelled grains with reduced weight	Yin et al. (2009)
	- Limited translocation of assimilates	Dias & Lidon (2009a)
	- Reduced grain size and yield	Dias & Lidon (2009a)
Photosynthesis	- Decreased chlorophyll content and Rubisco activity	Wahid et al. (2007)
	- Increased oxidative stress and photorespiration	Sairam et al. (2000); Long et al. (2004)
	- Damage to photosystem II (PSII) and thylakoid membranes	Sharkey (2005); Prasad et al. (2008)
	- Accelerated leaf senescence and reduced ATP synthesis	Kumar & Charan (1998); Zhao et al. (2007)

2.3. Wheat Protein and Protein Composition Response to High Temperature

2.3.1. Wheat Proteins: Classification and Functional Importance

Wheat is a vital global protein source, with proteins comprising 10 – 18% of its dry weight, playing essential roles in human nutrition and bread-making quality (Belderok et al., 2000). These proteins are primarily divided into gluten and non-gluten fractions. Gluten proteins, including glutenins and gliadins, account for approximately 75% of the total protein in wheat grains and are critical for the viscoelasticity and extensibility of dough. Glutenins which consist of high-molecular-weight (HMW) and low-molecular-weight (LMW) subunits, form disulfide-bonded polymers that provide dough strength and elasticity (Savill et al., 2018; Payne et al., 1984). Gliadins, monomeric proteins subdivided into α, ω, and γ isoforms, primarily contribute to dough extensibility and viscosity (Gianibelli et al., 2001). Non-gluten proteins, such as globulins and albumins, comprise the remaining 25% and are mainly involved in metabolic functions within the wheat seed coat.

2.3.2. Effects of HT on Total Protein Content

High-temperature stress, a growing concern under global climate change, significantly influences wheat protein content, composition, and functionality, with profound implications for grain quality (Gooding et al., 2003; Shew et al., 2020). Elevated temperatures during the grain filling period tend to increase the total protein content in wheat grains. However, this increase is often attributed to reduced starch accumulation, which alters the protein-to-starch ratio, rather than reflecting enhanced protein biosynthesis (Blumenthal et al., 1993; Zhao et al., 2008). For instance, wheat cultivated under HT in Australian studies exhibited higher protein percentages but weakened dough strength, indicating a compositional imbalance (Blumenthal et al., 1993).

2.3.3. Changes in Protein Composition

HT disproportionately affects the balance between glutenins and gliadins, increasing the gliadin-to-glutenin ratio. This shift reduces dough strength and elasticity, undermining bread-making quality (Zhao et al., 2008). Gliadins, which enhance dough extensibility, tend to accumulate under high-temperature conditions, while glutenins, responsible for elasticity and strength, decrease in relative proportion. In regions with frequent high-temperature events, such as tropical and low-latitude areas, these changes are often more pronounced, as seen in studies on wheat genotypes in China, where increased gliadin levels severely compromised dough properties (Zhao et al., 2008; Gupta et al., 2013).

The functional properties of wheat proteins, particularly gluten, are further impaired by HT-induced disruptions in lipid-protein interactions. Lipids play a key role in stabilising gluten proteins, and HT alters these interactions, reducing the stability of gluten networks and further diminishing dough elasticity and processing quality (Zhao et al., 2022). Such disruptions are particularly problematic for industrial applications, which require consistent gluten strength and stability.

2.3.4. Mechanisms of Protein Synthesis under High Temperature

The underlying mechanisms of protein synthesis under HT involve disruptions in nitrogen metabolism and related biosynthetic pathways. HT reduce the activity of glutamine synthase (GS) and glutamate pyruvate aminotransferase (GPT), enzymes essential for nitrogen assimilation and amino acid biosynthesis (Lea et al., 1990). Decreased activity of these enzymes limits nitrogen partitioning and protein synthesis, contributing to compositional changes. Additionally, HTs accelerate the grain filling rate while shortening its duration, further restricting nitrogen translocation and assimilation within the developing grain. This altered carbon-nitrogen balance often results in elevated protein content but with reduced quality, emphasising the multifactorial nature of HT impacts (Zhao et al., 2008).

Studies across different regions and genotypes illustrate the variable impacts of HT on wheat proteins. For example, research in temperate climates has identified genotypic differences in the resilience of wheat cultivars, with some heat-tolerant genotypes maintaining relatively stable glutenin-to-gliadin ratios under HTs (Gupta et al., 2013). Conversely, subtropical cultivars subjected to similar conditions often exhibit greater reductions in gluten strength and elasticity, reflecting the genetic and environmental interplay influencing protein composition. These findings underscore the critical need for breeding heat-tolerant wheat cultivars capable of maintaining both protein content and functionality under elevated temperatures (Table 2.2).

Table 2.2: Effect of High Temperature on Wheat Proteins

Aspect	Effects of HT	References
Total Protein Content	- Increased total protein content due to reduced starch accumulation (higher protein-to-starch ratio).	Blumenthal et al. (1993); Zhao et al. (2008)
	- Elevated protein percentage observed in Australian studies, but with weakened dough strength.	Blumenthal et al. (1993)
Protein Composition	- Increased gliadin-to-glutenin ratio under HT.	Zhao et al. (2008)
	- Gliadins (enhance extensibility) accumulate more, while glutenins (provide strength) decrease.	Zhao et al. (2008); Gupta et al. (2013)
	- More pronounced changes in tropical and low-latitude areas, severely compromising dough properties.	Gupta et al. (2013)
Gluten Functionality	- Reduced dough elasticity and stability due to disrupted lipid-protein interactions.	Zhao et al. (2022)
	- Altered lipid-gluten interactions destabilise gluten networks, impairing processing quality.	Zhao et al. (2022)
Mechanisms	- Decreased activity of enzymes involved in nitrogen assimilation (e.g., glutamine synthase [GS] and glutamate pyruvate aminotransferase [GPT]).	Lea et al. (1990)
	- Accelerated grain filling reduces nitrogen translocation and impacts protein synthesis.	Zhao et al. (2008)
	- Altered carbon-nitrogen balance elevates protein content but reduces protein quality.	Zhao et al. (2008)

2.4. Effects of HT on Wheat Starch and Crude Fibre

2.4.1. Change in Starch Content and Composition

Starch, the primary carbohydrate in wheat, constitutes 60 – 75% of its dry weight and plays a critical role in determining wheat quality. It is stored as two types of granules: large lenticular granules (25 – 40 μm) and small spherical granules (5 – 10 μm), formed at different stages post-pollination (Belderok et al., 2000). Wheat starch consists of two polysaccharides: amylose (20 – 30%) and amylopectin (70 – 80%), whose ratio is fundamental to starch physicochemical properties and its applications in food production (Konik-Rose et al., 2007).

HTs during the grain filling stage, exceeding the optimal range of 12 – 22°C, significantly reduce starch accumulation by shortening the grain filling duration. Each 1°C increase above 20°C reduces grain filling time by approximately 2.8 days, limiting the time available for starch synthesis (Zhao et al., 2008). HT impairs key enzymatic activities critical to starch biosynthesis. Enzymes such as sucrose synthase, soluble starch synthase, and granule-bound starch synthase exhibit reduced activity under HTs, leading to diminished starch synthesis rates and compromised granule structure (Morell & Myers, 2005; Macleod & Duffus, 1988).

HT also alters the amylose-to-amylopectin ratio, increasing amylose levels and reducing amylopectin. This shift affects starch gelatinisation properties, raising gelatinisation temperatures and modifying paste viscosity. These changes influence the texture, stability, and processing quality of wheat-based food products (Beckles & Thitisaksakul, 2014). Additionally, HTs activate enzymes that degrade amylopectin, further disrupting starch granule integrity and negatively impacting starch functionality and digestibility (Liu et al., 2011; Kumari et al., 2020).

2.4.2. Change in Starch Properties and Gelatinisation

The altered starch composition under HT has profound effects on its gelatinisation properties. Elevated amylose levels raise gelatinisation temperatures, while changes in paste viscosity affect the texture and stability of processed products. These modifications are critical for industries relying on consistent starch performance, such as baking and pasta manufacturing. Furthermore, HTs promote the breakdown of amylopectin, leading to structural instability in starch granules and reduced functionality in food applications (Beckles & Thitisaksakul, 2014).

2.4.3. Modifications in Crude Fibre

Crude fibre, primarily composed of cellulose and lignin, is an essential component of wheat, particularly for animal feed. It impacts digestibility, bulk, and nutritional value. HT typically increases crude fibre content, an adaptive response to accelerated crop maturity. This is associated with enhanced cellulose and lignin accumulation, which contributes to structural rigidity and resilience under stress conditions.

The increase in crude fibre content has dual effects. While it may reduce digestibility in animal feed, it also interacts with starch to enhance its thermal stability. Fibre acts as a physical barrier, limiting starch granule swelling and excessive breakdown during high-temperature processing. This interaction improves the resistance of starch pastes to shear and thermal degradation, yielding thicker and more stable gels. Such properties are advantageous for producing low-glycaemic index products and ensuring the texture and consistency of processed foods (Chaisawang & Suphantharika, 2006).

2.4.4. Mechanisms and Metabolic Implications

The compositional changes in starch and crude fibre under HT are closely linked to alterations in carbohydrate metabolism (Table 2.3). HT reduces photosynthetic efficiency, limiting the production of assimilates necessary for starch biosynthesis. The decline in sucrose availability and enzymatic activities further restricts carbon allocation to starch and fibre synthesis. These disruptions in metabolic pathways lead to lower starch accumulation and increased fibre deposition, reflecting the plant’s adaptive strategies to cope with heat-induced stress (Jenner & Hawker, 1993; Zhao et al., 2008).

2.4.5. Implications for Food and Feed Industries

The HT-induced changes in starch and crude fibre have significant implications for the food and feed industries. In food production, altered starch composition and gelatinisation properties affect product texture, consistency, and shelf life, requiring adaptations in processing methods. For animal feed, increased crude fibre content generally reduces digestibility, especially in monogastric species, yet it enhances thermal stability during high temperature processing methods such as pelleting and extrusion, which can improve the durability of feeds designed for ruminants. These insights emphasise the need for developing heat-resilient wheat cultivars and refining cultivation practices to mitigate HT impacts, ensuring the functional and nutritional quality of wheat in a changing climate.

Table 2.3: Effects of HT on Wheat Starch and Crude Fibre

Aspect	Effects of HT	References
Starch Content	-Decreased total starch content due to reduced grain filling duration.	Zhao et al. (2008); Morell & Myers (2005)
	- Reduced enzymatic activity (e.g., sucrose synthase, starch synthase) limits starch synthesis rates.	Macleod & Duffus (1988); Jenner & Hawker (1993)
Starch Composition	- Increased amylose-to-amylopectin ratio.	Beckles & Thitisaksakul (2014); Liu et al. (2011)
	- Amylopectin degradation disrupts granule integrity.	Kumari et al. (2020)
Starch Gelatinisation	- Higher gelatinisation temperatures due to increased amylose content.	Beckles & Thitisaksakul (2014)
	- Reduced paste viscosity affects texture and stability of food products.	Beckles & Thitisaksakul (2014)
Crude Fibre	- Increased crude fibre content (higher cellulose and lignin).	Zhao et al. (2008)
	- Fibre acts as a barrier, limiting starch swelling and enhancing thermal stability.	Chaisawang & Suphantharika (2006)
Mechanisms	- Reduced photosynthetic efficiency limits carbon availability for starch biosynthesis.	Zhao et al. (2008); Jenner & Hawker (1993)
	- Altered carbon-nitrogen balance affects assimilate partitioning between starch and fibre.	Morell & Myers (2005)

2.5. Effects of HT on Wheat Minerals and Phytate

2.5.1. Change in Mineral Content

Minerals such as zinc, iron, potassium, and sodium are essential for human health, supporting metabolic functions and enzymatic processes. Wheat grains, particularly the germ and aleurone layers, are rich in these nutrients (Martínez-Ballesta et al., 2010; Tekin et al., 2018). However, HTs during the grain filling stage significantly reduce the accumulation of these minerals. HT accelerates plant senescence, impairs root development, and limits nutrient uptake and transport (Essemine et al., 2010).

Zinc and iron, two critical micronutrients, are especially susceptible to HT. Elevated temperatures have been shown to reduce their concentrations in wheat grains, exacerbating deficiencies prevalent in many populations. For instance, studies on U.S. wheat cultivars observed declining zinc and iron levels over a century of breeding, attributed to yield increases diluting mineral content rather than changes in soil composition (Garvin et al., 2006; Fan et al., 2008). Similarly, potassium and sodium concentrations are adversely affected, impacting grain quality and storage properties (Velu et al., 2016).

While applying fertilisers enriched with minerals like zinc, magnesium, and potassium can partially offset these impacts, such measures are insufficient under prolonged or extreme HT. Addressing this issue requires an understanding of the underlying mechanisms limiting mineral accumulation under HT conditions.

2.5.2. Change in Phytate Content

Phytate, a phosphorus storage compound concentrated in the aleurone layer, strongly binds to essential minerals such as zinc, iron, and calcium, forming insoluble complexes that reduce their bioavailability. This anti-nutrient effect is particularly problematic in regions heavily reliant on wheat, where mineral deficiencies are widespread (Welch, 1986; Lönnerdal et al., 1989).

HT has variable effects on phytate content. Some studies report a reduction in phytate levels due to shortened grain filling periods and accelerated maturity, while others suggest stable or increased levels, potentially linked to disrupted phosphorus metabolism under stress (Peterson et al., 1986; Guttieri et al., 2006). Elevated phytate levels under certain conditions further hinder the bioavailability of minerals, compounding the nutritional challenges posed by HT.

Breeding low-phytate wheat cultivars has shown potential to enhance mineral bioavailability without compromising yield. These cultivars, combined with agronomic practices tailored to heat-stressed environments, can provide a sustainable solution to mitigate the adverse effects of HT on wheat nutritional quality (Raboy et al., 2000; Iqbal et al., 2024) (Table 2.4).

Table 2.4: Effects of HT on Wheat Minerals and Phytate

Aspect	Effects of HT	References
Mineral Content	- Decreased concentrations of zinc, iron, potassium, and sodium.	Garvin et al. (2006); Velu et al. (2016)
	- Reduced mineral accumulation due to impaired root development and nutrient transport.	Essemine et al. (2010)
	- Increased yield dilutes mineral content in grains.	Fan et al. (2008)
Phytate Content	- Variable effects: decreased phytate levels due to shorter grain filling periods or increased levels under disrupted metabolism.	Peterson et al. (1986); Guttieri et al. (2006)
	- High phytate levels reduce mineral bioavailability by forming insoluble complexes with zinc and iron.	Welch (1986); Lönnerdal et al. (1989)
	- Low-phytate wheat varieties enhance mineral absorption and bioavailability.	Raboy et al. (2000); Iqbal et al. (2024)
Mechanisms	- Photosynthesis decline: reduced assimilate production limits nutrient transport and storage.	Essemine et al. (2010)
	- Root impairment: HT inhibits root growth and mineral uptake from the soil.	Samineni et al. (2022)
	- Oxidative stress: Reactive oxygen species (ROS) damage cellular structures, disrupting nutrient transport pathways.	Velu et al. (2016)

	- Phosphorus metabolism: Altered phosphorus assimilation affects phytate synthesis and mineral storage.	Guttieri et al. (2006)

2.6. Effects of High-Temperature Stress on Lipid Composition in Wheat Pollen

2.6.1. Importance of Pollen Lipids

Pollen lipids play a crucial role in ensuring the reproductive success of wheat. They are essential for maintaining membrane stability, facilitating cellular signalling, and supporting nutrient transport during pollen development and fertilisation. The lipid composition of pollen membranes influences key physiological processes, such as pollen tube elongation and fertilisation capability, which are particularly vulnerable to environmental stressors like HTs (Krumova et al., 2010). Given their critical functions, understanding the impacts of HT on pollen lipids is vital for developing strategies to mitigate fertility losses in wheat under rising global temperatures.

2.6.2. Changes in Lipid Composition

HTs significantly alter the lipid composition of wheat pollen, leading to substantial changes in lipid unsaturation levels, lipid peroxidation, and metabolic remodelling. Under HT conditions, reductions in polyunsaturated fatty acids, such as linolenic acid (18:3), are frequently observed. Concurrently, levels of less unsaturated and saturated fatty acids, such as oleic acid (18:1), linoleic acid (18:2), and palmitic acid (16:0), increase (Narayanan et al., 2018). These compositional changes help stabilise membranes by preventing phase transitions to non-bilayer states under HTs, thereby preserving basic membrane functionality. However, the decrease in unsaturated fatty acids reduces membrane fluidity and flexibility, which are critical for processes like pollen tube growth and fertilisation (Narayanan et al., 2018).

2.6.7. Lipid Peroxidation and Oxidative Stress

In addition to compositional changes, HTs intensify oxidative stress in pollen cells, leading to lipid peroxidation. Chloroplast thylakoid membranes, which contain high concentrations of monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG), are particularly vulnerable. During HT, the levels of highly unsaturated MGDG and DGDG species, such as 36:6, are significantly reduced due to ROS-induced oxidative damage (Djanaguiraman et al., 2018). Lipid peroxidation releases malondialdehyde (MDA) and other byproducts, markers of severe oxidative damage that disrupt membrane integrity and functionality. Reduced activity of desaturase enzymes under HT further exacerbates the decline in polyunsaturated fatty acids, reflecting an adaptive response to limit ROS-mediated damage (Narayanan et al., 2018).

The effects of HT on lipid composition are particularly pronounced during critical stages of pollen development, such as microsporogenesis and microgametogenesis (Masoomi-Aladizgeh et al., 2024). During microsporogenesis, the tapetum, a nutrient-rich layer essential for pollen development, plays a key role in supplying lipids and their precursors. HTs accelerate the degeneration of tapetal cells, disrupting the supply of fatty acids and sterols necessary for pollen maturation (Kurusu & Kuchitsu, 2017; Mittler, 2017). Premature tapetal cell death also leads to excessive ROS accumulation, further impairing lipid metabolism and membrane stability. During microgametogenesis, lipid remodelling is crucial for maintaining membrane integrity and supporting the asymmetric cell division and maturation of pollen grains. HT interferes with these processes, resulting in defective pollen grains with reduced viability and germination capacity (Rezaei et al., 2010).

Mechanistically, HT-induced changes in lipid composition reflect the interplay of adaptive and stress-induced responses. Reduced desaturase activity limits the production of polyunsaturated fatty acids, stabilising membranes at the cost of flexibility (Djanaguiraman et al., 2018). Additionally, the accumulation of triacylglycerols (TAGs) in plastoglobules represents a protective mechanism. TAG synthesis sequesters destabilized acyl chains from peroxidized membranes, reducing oxidative damage while maintaining cellular homeostasis (Narayanan et al., 2016b). Lipid remodelling under stress also involves the production of oxidised lipid species, which act as signalling molecules to activate stress-responsive pathways. These metabolic adaptations highlight the complex regulatory networks underlying lipid metabolism in wheat pollen during HT.

Understanding lipid composition and metabolism under HT could inform the development of heat-tolerant wheat cultivars. For instance, maintaining higher levels of polyunsaturated fatty acids or enhancing TAG accumulation could improve membrane resilience, supporting pollen viability and reproductive success under elevated temperatures (Narayanan et al., 2016a; Djanaguiraman et al., 2018). These findings underscore the potential of lipid-based markers in breeding programs aimed at mitigating the adverse effects of HT on wheat productivity (Table 2.5).

Table 2.5: Effects of High-Temperature Stress on Wheat Pollen and Pollen Lipids

Aspect	Effects of HT	References
Pollen Development	- Reduced pollen viability and germination capacity.	Rezaei et al. (2010); Mittler (2017)
	- Premature degeneration of tapetal cells disrupts lipid supply and impairs pollen maturation.	Kurusu & Kuchitsu (2017); Mittler (2017)
	- Accelerated ROS accumulation causes oxidative damage during key development stages (microsporogenesis, microgametogenesis).	Djanaguiraman et al. (2018); Narayanan et al. (2018)
Pollen Lipid Composition	- Decrease in polyunsaturated fatty acids (e.g., linolenic acid, 18:3).	Narayanan et al. (2018); Djanaguiraman et al. (2018)
	- Increase in less unsaturated and saturated fatty acids (e.g., oleic acid, linoleic acid, palmitic acid).	Narayanan et al. (2018)
	- Lipid remodelling involves reduced desaturase activity and increased triacylglycerol (TAG) synthesis.	Narayanan et al. (2016b); Djanaguiraman et al. (2018)
Oxidative Stress on Lipids	- ROS-induced lipid peroxidation damages thylakoid membranes rich in MGDG and DGDG.	Djanaguiraman et al. (2018)
	- Release of malondialdehyde (MDA) and byproducts signals severe oxidative damage.	Narayanan et al. (2018)
	- Oxidised lipids act as signalling molecules, activating stress response pathways.	Narayanan et al. (2016a)
Impact on Functionality	- Stabilised membranes under HT prevent phase transitions but reduce fluidity and flexibility.	Narayanan et al. (2018)

	- Impaired lipid metabolism affects pollen tube growth and fertilisation success.	Kurusu & Kuchitsu (2017); Narayanan et al. (2018)

2.7. Conclusion

Wheat plays a pivotal role in sustaining food security worldwide, delivering essential nutrients to billions of people. However, its production is increasingly threatened by climate change, particularly the adverse effects of high-temperature stress. This review highlights the multifaceted impacts of HT on wheat's physiological processes, grain quality, and reproductive success.

HT significantly compromises wheat yield by impairing flowering, grain filling, and photosynthesis. Pollen viability and fertilisation success are reduced due to disruptions in lipid composition and membrane stability, with pronounced effects during critical reproductive stages. Similarly, HT alters protein composition, starch functionality, crude fibre content, and mineral bioavailability of wheat grains, undermining its nutritional and processing quality. The mechanisms underlying these changes, including oxidative stress, enzymatic inhibition, and metabolic remodelling, emphasize the need for targeted adaptive strategies.

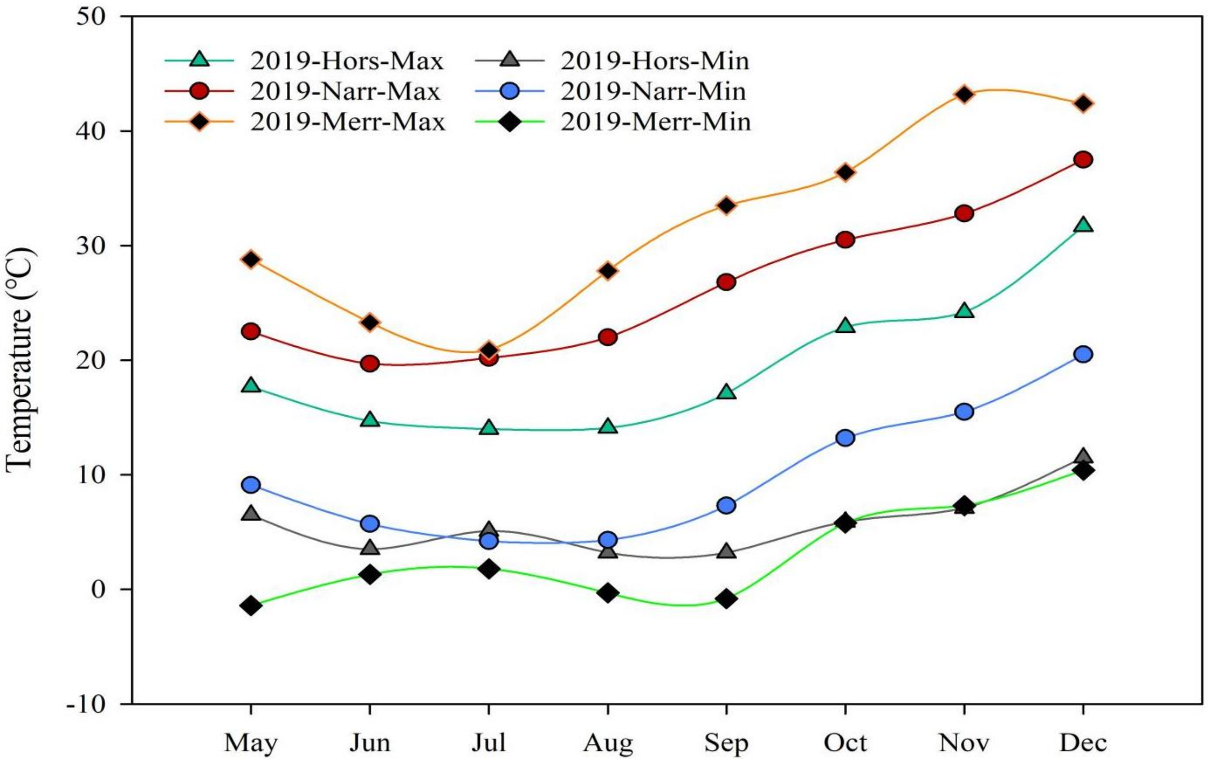
Efforts to mitigate these impacts must prioritise the development of heat-resilient wheat cultivars through advanced breeding techniques. Such cultivars must maintain stable protein composition, optimised starch properties, enhanced mineral concentrations, and robust pollen lipid profiles under elevated temperatures. Additionally, sustainable agronomic practices and precision agriculture can help buffer wheat against environmental stressors, safeguarding productivity and quality in a warming climate.

Chapter 3. General Materials and Methods

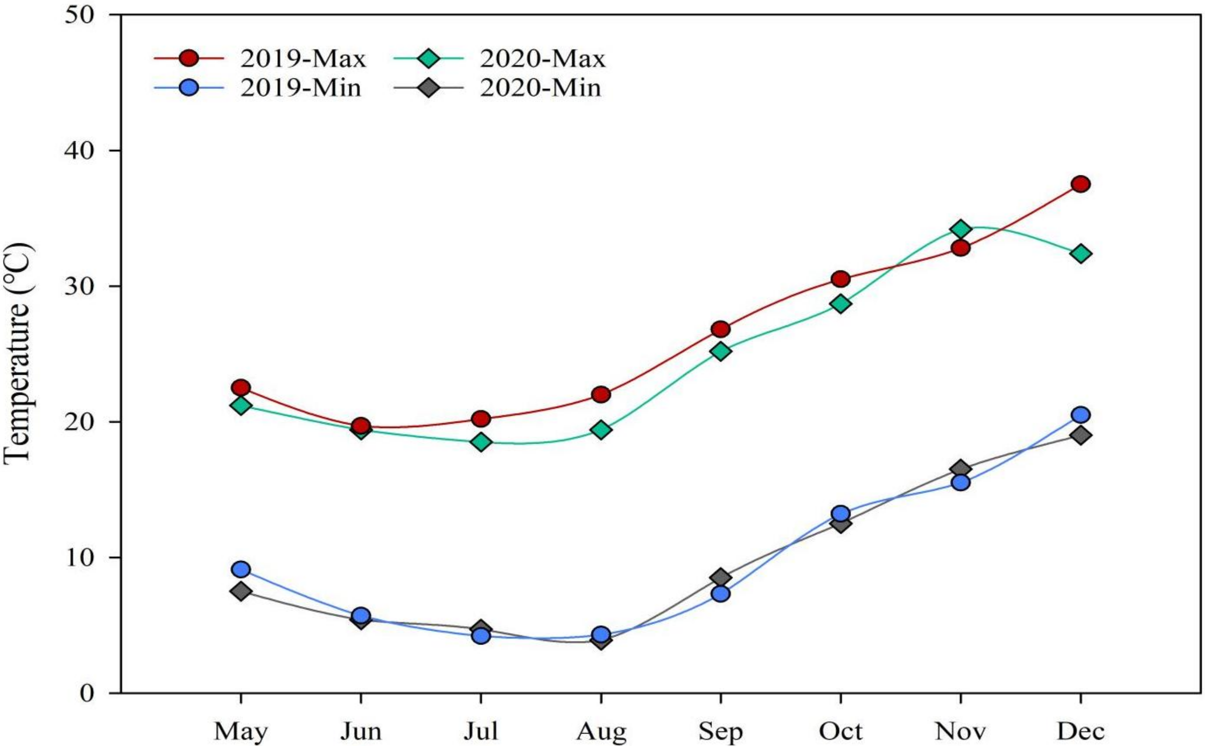
3.1. Field Experiment: Locations- Climate and Soil

Temperature and rain data for 2019 in Narrabri, Horsham, and Merredin are illustrated in Figure 3.1 (a) (c), and Figure 3.1 (b) displays the temperature variations for Narrabri across 2019 and 2020. In the first experiment conducted in 2019, Berkut and Sokoll were sown in Narrabri (northwest NSW), Horsham (Victoria), and Merredin (Western Australia), following both normal (TOS1) and late (TOS2) sowing schedules. The second experiment involved planting Flanker, Suntop, and Cobra in Narrabri in 2019 and 2020, again adhering to both TOS1 and TOS2 sowing schedules. Table 3.1 provides an overview of the soil properties at the three locations.

a

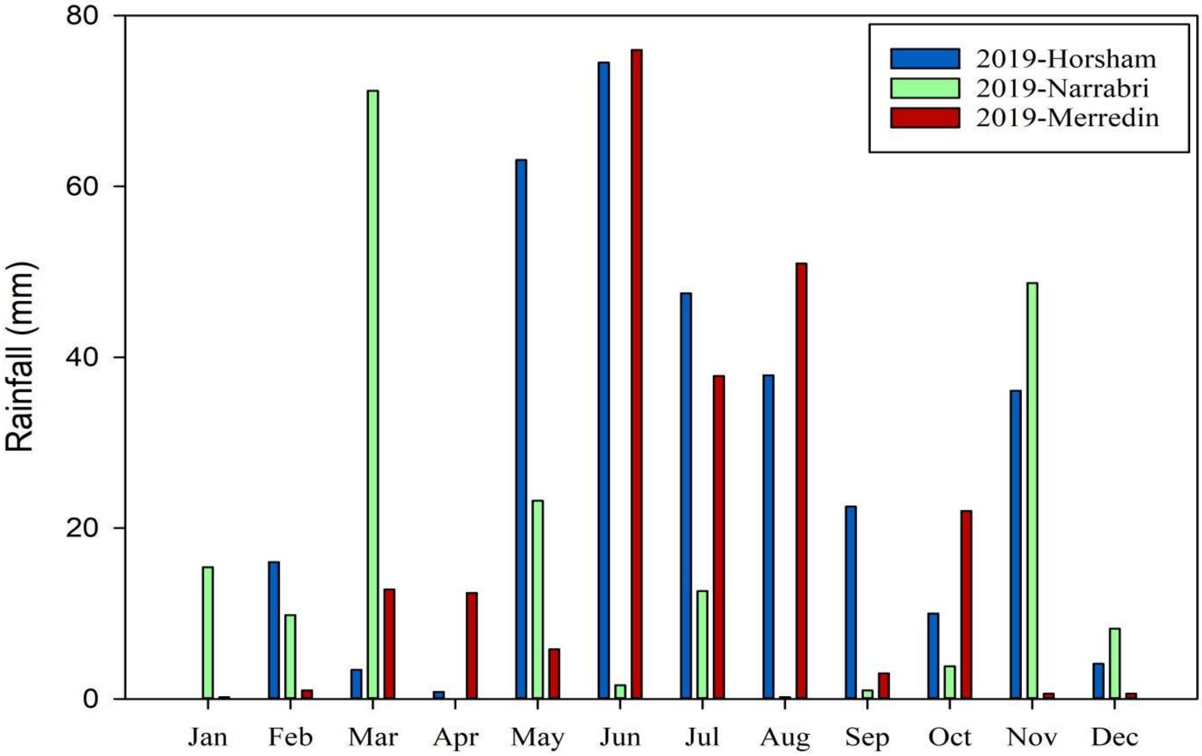


b



50
40
30
20
10
0
Temperature (°C)
2019-Max
2019-Min
2020-Max
2020-Min
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec

c



80
60
40
20
0
Rainfall (mm)
2019-Horsham
2019-Narrabri
2019-Merredin
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec

d

Figure 3.1 Maximum and minimum temperatures and sowing times for Narrabri (NSW), Horsham (VIC) and Merredin (WA) in 2019 (Australian Bureau of Meteorology, April 5, 2022) (a). Maximum and minimum temperatures and sowing times for Narrabri in 2019 and 2020 (Australian Bureau of Meteorology, April 5, 2022) (b). Monthly rainfall (mm) for the year 2019 in three locations (c). Field locations in Australia's main wheat growing area for three sowing experiments (d).

Table 3.1 Physical and chemical characteristics of soil at different locations in 2019.

Soil Characteristics	**Horsham**	**Narrabri**	**Merredin**
Soil Type	Grey Vertosol	Grey Vertosol	Orthic Tenosol
Texture	Clay	Clay	Sandy Loam
PAWC (Plant Available Water Capacity) (mm/m) (Padarian Campusano, 2014)	160.6	158.7	132.9
Organic Carbon (%)	1.13	0.63	0.96
Nitrate Nitrogen (mg/kg) (soil extract)	19.0	11.5	20.0

Phosphorus - Colwell (mg/kg) (soil extract)	70.5	66.5	42.0
Organic Matter (OM) %	1.95	1.05	1.65
Total Ca (%)	0.74	0.46	0.18
Total Mg (%)	0.70	0.63	0.20
Total Na (%)	0.075	0.039	0.025
Total K (%)	0.76	0.36	0.30
pH (1:5 Water)	8.65	8.65	6.00
pH (1:5 $CaCl_2$)	7.9	7.6	5.5

3.2. Field Experiment Design

In the first experiment, two cultivars (Berkut (pedigree: Irena/Babax/Pastor) and Sokoll (pedigree: Pastor/Altar84/OpataM85)) were sown at two times (normal sowing and late sowing) in three locations (Horsham, Narrabri, and Merredin) in 2019 (Experiment 1). Three cultivars (Cobra (pedigree: Westonia/W29), Flanker (pedigree: EGA Gregory//EGA Gregory/Lang), and Suntop (pedigree: SUNCO/2*PASTOR//SUN436E)) were sown at two times (normal sowing and late sowing) in the Narrabri in 2019 and 2020 (Experiment 2). Suntop was bred by Australian Grain Technologies (AGT) and Cobra and Flanker were bred by LongReach Plant Breeders. In all experiments, two sowing times were implemented, with the late sown wheat exposed to higher temperatures during the grain filling and maturation periods.

The experimental design involved two sowing dates for all wheat cultivars: normal sowing (Time of Sowing, TOS1) in May and late sowing (TOS2) in July. The sowing rate was 42 kg/ha for all experiments. Supplementary irrigation was implemented to eliminate the effects of water stress, thereby ensuring that only the HT treatment was the primary abiotic stress. To preserve soil structure, minimum tillage practices were applied. The experimental site was fallowed during the summer and rotated with legume crops (e.g., chickpea) in alternate years to reduce disease incidence and maintain soil health. The field experiment utilised an alpha lattice design, which was a randomised complete block layout with two replicates.

In 2019, the time required from sowing to maturity for TOS1-sown wheat was approximately 202 days (2280 Growing Degree Days (GDD)) in Horsham (Cool), Victoria; 152 days (2260 GDD) in Narrabri (Warm), New South Wales; and 150 days (2332 GDD) in Merredin (Hot), Western Australia. The grain filling to maturity period was approximately 59 days (878 GDD) in Horsham, 48 days (914 GDD) in Narrabri, and 44 days (883 GDD) in Merredin.

For TOS2-sown wheat in 2019, the time required from sowing to maturity was approximately 164 days (2236 GDD) in Horsham, 126 days (2202 GDD) in Narrabri, and 119 days (2312 GDD) in Merredin. The grain filling to maturity period was approximately 45 days (872 GDD) in Horsham, 37 days (913 GDD) in Narrabri, and 38 days (878 GDD) in Merredin.

In 2020 in Narrabri, the time required from sowing to maturity was approximately 159 days (2275 GDD) for TOS1-sown wheat and 117 days (2314 GDD) for TOS2-sown wheat.

3.3. Heat Tolerance of Wheat Cultivars

The present study utilised five wheat cultivars grown in Australia, namely Berkut, Cobra, Flanker, Sokoll, and Suntop, provided by the Plant Breeding Institute, University of Sydney. These cultivars are categorised based on their heat tolerance based on previous field studies (Table 3.2).

Table 3.2 The summary of wheat cultivar and heat tolerance rating (based on previous field studies (Bokshi et al., 2022))

Cultivar	Flanker	Berkut	Suntop	Sokoll	Cobra
Rating	Highly tolerant	High	Medium	Medium	Sensitive

3.4. Statistical Analyses

The experimental data were subjected to two-way and three-way analysis of variance (ANOVA) using

Statistix 8.1 and Genstat 22nd Edition. Where there were significant differences, post hoc Tukey's Honestly Significance Difference (HSD) tests were used (and assessed at $p < 0.05$). Correlations between each component were also determined.

Chapter 4. Response of wheat genotypes stressed by HT in yield and protein composition across diverse environments

Abstract

Global climate change poses a significant threat to wheat (*Triticum aestivum* L.) production due to rising temperatures. This study aimed to investigate the impact of HTs on wheat yield, thousand kernel weight (TKW), colour, and protein composition to inform breeding strategies for heat tolerance. Two field experiments were conducted: one at three locations in Australia (Horsham, (Vic) Narrabri, (NSW) and Merredin, (WA)) in 2019, involving two wheat cultivars (Berkut (high heat tolerant) and Sokoll (medium heat tolerant)) sown at normal (TOS1) and late (TOS2) sowing times and a second experiment at Narrabri in 2019 and 2020, involving three wheat cultivars (Cobra (heat sensitive), Flanker (high heat tolerant) and Suntop (medium heat tolerant)) sown at normal (TOS1) and late (TOS2) sowing times. A reduction in yield and TKW occurred under HTs ($p<0.05$), particularly in late sowing conditions. The glutenin/gliadin ratio decreased, affecting dough strength and elasticity, especially at Merredin. Heat-tolerant cultivars like Flanker and Suntop maintained protein quality with increased the glutenin/gliadin ratio under HT. These findings highlight the necessity for breeding heat-tolerant wheat cultivars that can sustain both yield and quality. Future research should focus on genetic traits for heat tolerance, advanced molecular techniques, and interdisciplinary approaches to ensure sustainable wheat production in a changing climate.

Keywords: High-temperature, Yield, Thousand kernel weight, Protein composition, Glutenins/gliadins ratio

4.1. Introduction

As global climate change intensifies, high-temperature stress impacting plant growth and development is becoming a focus of concern. Among these stresses, rising global temperatures present a formidable challenge to agriculture worldwide. Wheat (*Triticum aestivum* L.), the most extensively cultivated cereal and a major contributor to global grain markets, is particularly vulnerable to these changing conditions. In fact, similar to the findings of Rivelli et al. (2024) in South America, high-temperature stress has been shown to reduce both wheat yield and quality. Wheat now accounts for approximately 20% of global human calorific intake and 20% of daily protein consumption, making its sustainability critical for global food security (Shi et al., 2024; FAO, 2021). Wheat's sensitivity to heat stress is especially pronounced in low-latitude regions, where approximately 100 million ha are cultivated (Braun et al., 2010; Gupta et al., 2013). Each 1°C increase in temperature is associated with an approximate 6% reduction in global wheat production on an annual basis, with regions like Australia facing yield losses of up to 50% under a 2°C rise (Zhao et al., 2017; Asseng et al., 2011).

Wheat is a crucial source of protein for human consumption, containing approximately 10-18% protein by dry weight. Wheat proteins are categorised into gluten and non-gluten proteins. Gluten, which include glutenins and gliadins, account for approximately 75% of wheat protein content. These proteins are essential for the viscoelastic properties of dough. Glutenins play a crucial role in dough elasticity and strength, as they are composed of high-molecular-weight (HMW) and low-molecular-weight (LMW) subunits linked by disulfide bonds (Wieser et al., 2020). Recent studies show that the protein content of wheat grains is heavily influenced by genetic factors as well as environmental stresses, such as heat and drought, which can affect the proportion of HMW and LMW glutenins (Savill, et al., 2018; Zhao et al., 2022). Non-gluten proteins, such as globulin and albumin, make up the remaining 25% of wheat proteins. These proteins are involved in various metabolic processes and are primarily found in the testa.

High temperatures adversely affect wheat protein synthesis, impacting both the quality and quantity of protein within the grain (Zhao et al., 2008). Heat stress shortens the grain filling period, crucial for protein and starch deposition, thereby reducing overall protein content and altering its composition (Gooding et al., 2003). High temperatures may indirectly affect wheat protein synthesis and quality by affecting nitrogen absorption and utilisation, especially by reducing the glutenin/alcohol-soluble protein ratio, thereby reducing dough strength and stability (Wan et al., 2022). This is in line with studies showing that high temperatures increase total protein content but the composition of wheat’s primary protein fraction, gluten is altered, notably with a shift in the ratio of glutenin to gliadin (Zhao et al., 2022; Shewry et al., 2024). Under heat stress, the synthesis rate of both gliadins and glutenins increases; however, gliadins are synthesised at a faster rate than glutenins. This results in a lower glutenins/gliadins ratio, negatively affecting the dough's viscoelastic properties and stability (Blumenthal et al., 1993). In particular, elevated temperatures during the grain filling stage can increase the proportion of gliadins and reduce the overall quality of gluten, thus impacting the bread-making properties of wheat (Tabbita, et al., 2024). Given these challenges, there is a critical need to develop heat-tolerant wheat cultivars through targeted breeding programs, to ensure stability in wheat production and protein quality in response to the projected future climate. These efforts are essential for sustaining the growing global demand and for the adaptation of wheat production systems to the changing environmental conditions. Currently, there is a lack of research on the effects of heat stress on the yield, protein content, and composition of different heat-tolerant wheat cultivars commonly grown in major wheat-growing regions of Australia.

The hypothesis is that the harvest year, sowing time, location, and wheat cultivars affect wheat yield and protein content as influenced by temperature. Therefore, the main objective of this study is to assess the impact of heat stress on yield, protein content, and composition on two wheat cultivars, Berkut and Sokoll, in three locations (Horsham (VIC), Narrabri (NSW), and Merredin (WA)) during a hot year (2019) (Experiment 1), and the same for three other wheat cultivars, Flanker, Cobra, and Suntop, grown in Narrabri over 2019 (hot year) and 2020 (cool year) (Experiment 2).

4.2. Materials and Methods

The experimental design adhered to the protocols in Chapter 3 (General Materials and Methods) of the field experiments, ensuring consistent sample collection, preparation, and analysis (see Sections 3.1, 3.2 and 3.3 for details). All wheat grains intended for analysis were finely ground into flour utilising Laboratory mill 3100 (Perten Instrument, Perkin Elmenr, Hagersten, Sweden,) and subsequently passed through a 0.5 mm sieve.

4.2.1. Wheat Flour Colour Analyses

The colour of wheat flour was evaluated using the Konica Minolta Cr-400 Chromameter. The data were reported in terms of the Commission Internationale de l'Eclairage (CIELAB) $L*$, $a*$, and $b*$ values, which describe the transition of flour colour from black to white ($L*$), green to red ($a*$), and blue to yellow ($b*$), respectively (Hughes et al., 2020). Each colour testing was conducted five times to ensure the repeatability of the measurements.

4.2.2. Protein Content

The protein content in the wheat flour samples was ascertained using the Vario MACrO cube organic elemental analyser. For this analysis, 40 mg of wheat flour underwent rapid oxidation in an ultra-high-purity oxygen environment at a temperature of 950°C, leading to the formation of nitrogen dioxide, nitrogen monoxide and water. The oxidised compounds were subsequently passed through a secondary furnace to eliminate any residual particulates. The resulting nitrogen content was quantified using a thermal conductivity detector and was utilised to determine the total protein content in the samples. Each sample was analysed twice to improve precision and reliability of the findings (Hwang et al., 2020). A conversion factor of 6.05 was used for protein estimation.

4.2.3. Protein Fraction

The bicinchoninic acid (BCA) protein assay method was employed in this study to determine the quantities of albumin, globulin, gliadins, and glutenins in the samples. This method leverages the principle of protein-induced reduction of Cu^{2+} to Cu^{+} in an alkaline medium, coupled with the sensitive, specific, and colorimetric detection of the copper (I) cation (Cu^{+}) by bicinchoninic acid (Smith et al., 1985). For albumin extraction, 1 g of flour was mixed with 10 mL of water, stirred at 200 rpm for 30 minutes, and then centrifuged at 3,500 rpm for 20 minutes. One milliliter of the supernatant was collected and stored at -5°C, while the remainder was discarded. This extraction process was repeated four times to obtain four separate 1 mL supernatant samples. The residual flour was then extracted four times with 10 mL of 0.4 M NaCl solution to isolate globulin, followed by four subsequent extractions using 10 mL of 70% ethanol for gliadin, and finally, four extractions with 10 mL of 0.1 M NaOH for glutenin. The collected supernatants were combined with an indicator and incubated at 37°C for 30 minutes, and absorbance was measured at 562 nm using a Shimadzu UV-1900 spectrophotometer.

4.3. Results

4.3.1. Experiment 1

The interactions occurred between location, time of sowing, and cultivar for yield, thousand kernel weight (TKW), crude protein content, the glutenins/gliadins ratio, gliadins, L^*, a^* and b^* values (Table 4.1) ($p<0.05$). Sokoll had lower yield at all three locations for TOS2 compared with TOS1 (Figure 4.1 (A)). There was a reduction in yield for Berkut at Merredin for TOS2 compared with TOS1 ($p<0.001$). TKW of Berkut was consistently higher than Sokoll across all three locations and both sowing dates, particularly at Merredin (Figure 4.1 (B)). Crude protein content was higher for TOS2 compared with TOS1 for both cultivars ($p=0.012$), except for Berkut at Horsham (Figure 4.1 (C)). At all three locations, glutenins/gliadins ratio of Berkut in TOS1 were higher than TOS2, whereas Sokoll showed higher glutenins/gliadins in TOS1 grown in Narrabri ($p=0.002$) (Figure 4.1 (D)). In comparison to TOS1, the gliadins content in TOS2 was consistently higher across the two cultivars and three regions. Specifically, there were no differences between TOS2 and TOS1 for Berkut in the Horsham and Narrabri regions (Figure 4.1 (E)). Compared with TOS1, the L^* values for Berkut and Sokoll increased in TOS2, except for Berkut in Narrabri and Sokoll in Merredin (Figure 4.1 (F)). The highest a^* value for Berkut during TOS1 was in Horsham, whereas the lowest for Sokoll in TOS2 occurred in Merredin (Figure 4.1 (G)). Regarding the b^* values, Sokoll consistently exhibited higher values than Berkut (Figure 4.1 (H)). The grain filling to maturity stage of wheat varied by locations and TOS, and the sowing to maturity stage also differed notably by locations and TOS. In general, wheat required the shortest time to grow in Merredin (warmest location) and the longest time in Horsham (coolest location). The growth time required in TOS2 was shorter than that in TOS1.

TKW was negatively correlated with crude protein ($r=-0.665$, $p<0.001$), gliadins ($r=-0.418$, $p=0.042$), glutenins ($r=-0.433$, $p=0.035$) and b^* value ($r=-0.724$, $p<0.001$). Additionally, crude protein showed a negative correlation with L^* value ($r=-0.507$, $p=0.011$) and a positive correlation with b^* value ($r=0.574$, $p=0.003$). Glutenins/gliadins ratio was positively correlated with globulin ($r=0.639$, $p<0.001$). Gliadins and glutenins were both negatively correlated with globulin ($r=-0.709$, $p<0.001$ and $r=-0.683$, $p=0.001$). Albumin was positively correlated with L^* value ($r=0.463$, $p=0.023$) and negatively with a^* value ($r=-0.436$, $p=0.033$). L^* value was negatively correlated with a^* value ($r=-0.77$, $p<0.001$). The number of days from grain filling to maturity and from sowing to maturity were both negatively correlated with the L^*

value (r=-0.473, p=0.019; r=-0.534, p=0.117, respectively) and the b^* value (r=-0.601, p=0.002; r=-0.432, p=0.035, respectively), and positively correlated with the a^* value (r=0.745, p<0.001; r=0.719, p<0.001, respectively) (Table 4.3).

4.3.2. Experiment 2

The interactions occurred between sowing period and variety for crude protein content, glutenins/gliadins, gliadins, glutenins, globulin, and a^* value for Cobra, Flanker and Suntop at Narrabri in 2019 (Table 4.2, (p<0.05)). The wheat yield ((4.46 t/ha) p<0.001) and TKW (36.72 g p=0.011) were higher for TOS1 than those under TOS2 (3.48 t/ha and 27.18 g) in 2019 (Figure 4.2).

Compared to TOS1, the crude protein content (p<0.001) of three wheat cultivars (Cobra, Flanker, and Suntop) for TOS2 had increased, with Cobra and Flanker exhibiting the highest crude protein (Figure 4.3 (a)). Furthermore, the glutenins/gliadins ratio (p=0.006) of Cobra and Suntop decreased in TOS2 compared with TOS1, while that of Flanker increased in TOS2 (Figure 4.3 (b)). The gliadins (p=0.024) of Cobra, Flanker and Suntop increased in TOS2 (Figure 4.3 (c)). The glutenins (p=0.024) of Cobra and Suntop increased in TOS2, while only Flanker decreased in TOS2 (Figure 4.3 (d)). Cobra had the highest globulin content (p<0.001) in TOS2, while Suntop had the lowest globulin content in TOS1 (Figure 4.3 (e)). Compared with TOS1, the a^* value (p=0.01) of the three wheat cultivars increased in TOS2 (Figure 4.3 (f)).

The wheat yield had positive correlations with TKW (r=0.753, p=0.005), glutenins (r=0.621, p=0.031), grain filling to maturity (r=0.699, p =0.012) and sowing to maturity (r=0.656, p=0.021). TKW was positively correlated with glutenins (r=0.597, p=0.04), grain filling to maturity ((r=0.798, p=0.002) and sowing to maturity (r=0.801, p=0.002). Glutenins/gliadins ratio was positively correlated with L^* value (r=0.629, p=0.029), and negatively correlated with gliadins (r=-0.898, p<0.001), a^* value (r=-0.598, p=0.04) and sowing to maturity (r=-0.787, p=0.002), respectively. Gliadins was positively correlated with glutenins (r=0.747, p=0.005), grain filling to maturity ((r=0.65, p=0.022) and sowing to maturity (r=0.82, p=0.001), and negatively correlated with L^* value (r=-0.703, p=0.011). Glutenins had positive correlations with grain filling to maturity ((r=0.716, p=0.009) and sowing to maturity (r=0.657, p=0.02). The L^* value had negative correlations with *a** value (*r*=-0.591, *p*=0.043), *b** (*r*=-0.924, *p*<0.001), grain filling to maturity (*r*=-0.736, *p*=0.006) and sowing to maturity (*r*=-0.689, *p*=0.013). The *a** value was positively correlated with *b** value (*r*=0.761, *p*=0.004). The *b** value had positive correlations with grain filling to maturity (*r*=0.637, *p*=0.026) and sowing to maturity (*r*=0.613, *p*=0.034). Grain filling to maturity was positively correlated with sowing to maturity (*r*=0.91, *p*<0.001) (Table 4.4).

The year 2020 was characterised by lower than average temperatures, which resulted in minimal differences in wheat performance between the two sowing times, TOS1 and TOS2.

Table 4.1. The main effects and interactions of locations (Horsham, Narrabri and Merredin), time of sowing (TOS1 and TOS2) and cultivar (Berkut and Sokoll) on wheat yield, protein, protein fractions, kernel colour and maturity days in 2019 were analysed, with corresponding p-values.

Effect	**Loc (Location)**	**TOS**	**Cul (Cultivar)**	**Loc×TOS**	**Loc×Cul**	**TOS×Cul**	**Loc×TOS×Cul**
Yield	<.001	<.001	<.001	<.001	<.001	<.001	<.001
Thousand kernel weight (TKW)	<.001	<.001	<.001	<.001	NS	<.001	0.011
Crude protein	<.001	<.001	<.001	<.001	<.001	0.027	0.012
Glutenins/gliadins	NS	<.001	<.001	0.01	<.001	<.001	0.002
Gliadins	<.001	<.001	<.001	0.041	<.001	NS	<.001
Glutenins	<.001	NS	<.001	NS	<.001	0.002	NS
Albumin	0.016	<.001	NS	NS	NS	0.001	NS
Globulin	NS	NS	<.001	NS	NS	NS	NS
***L** Value**	<.001	<.001	NS	<.001	<.001	NS	<.001
***a** Value**	<.001	<.001	<.001	<.001	<.001	NS	<.001
***b** Value**	<.001	0.003	<.001	<.001	<.001	<.001	<.001
Grain filling to maturity	<.001	<.001	NS	<.001	NS	NS	NS
Sowing to maturity	<.001	<.001	NS	<.001	0.019	NS	NS

12 Treatments =3 locations × 2 cultivars × 2 sowings; number of replications = 4; NS = $p>0.05$.

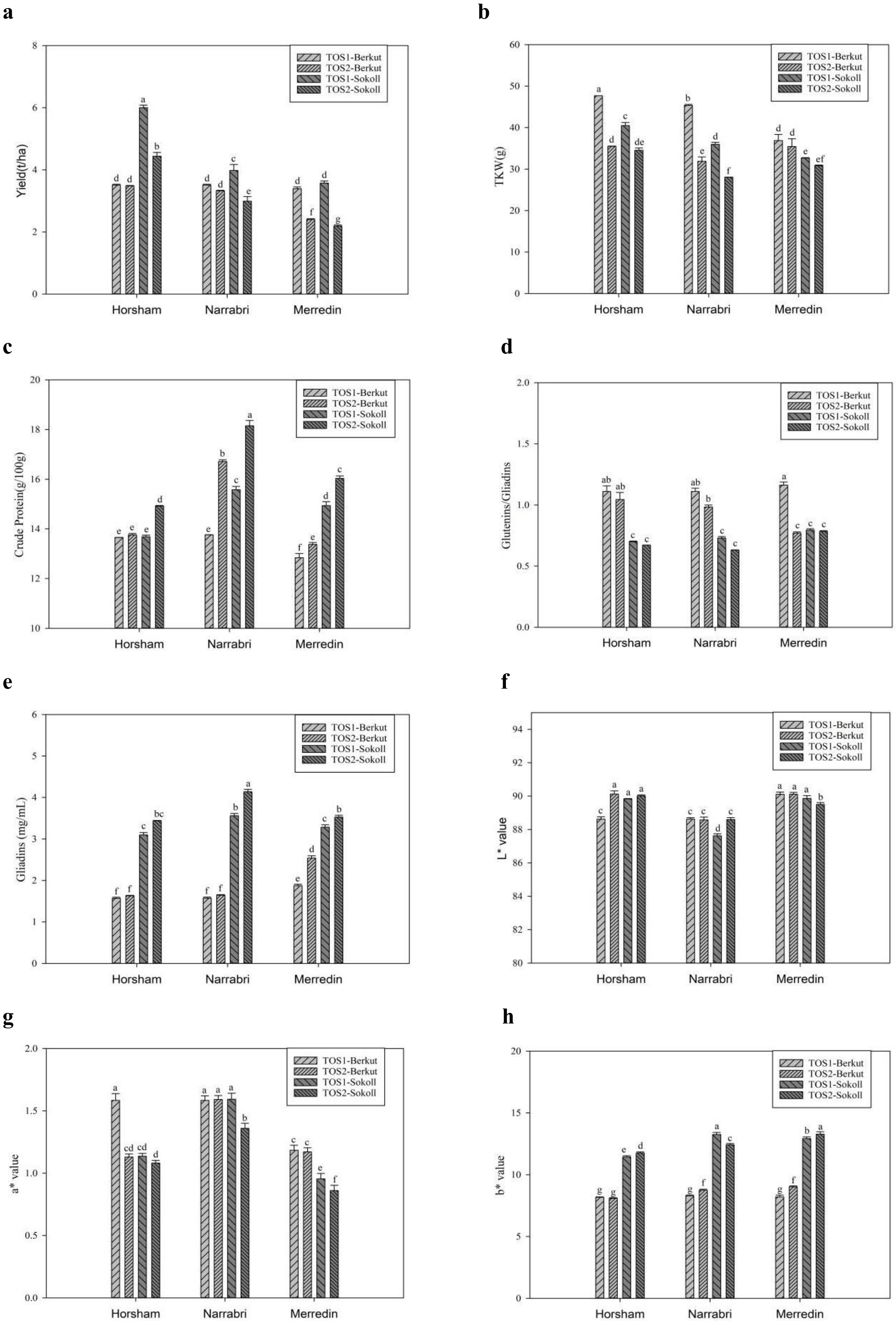


Figure 4.1. Effect of locations (Horsham, Narrabri and Merredin), sowing dates (TOS1 and TOS2) and cultivars (Berkut and Sokoll) on (a) yield (t/ha), (b) TKW (g), (c) crude protein (g/100g), (d) glutenins/gliadins, (e) gliadins (mg/mL), (f) *L value, (g) *a** value and (h) *b** value in 2019. Means followed by the same letters are not different at $p<0.05$ for location×sowing period×cultivar**

interaction. Error bars represent standard error of the mean (n=4).

Table 4.2. The main effects and interactions of year, time of sowing and cultivar on wheat yield, protein, protein fractions, kernel colour and maturity days by Cobra, Flanker and Suntop in Narrabri in 2019 were analysed, with corresponding p value.

Effect	**TOS**	**Cultivar**	**TOS×Cultivar**
Yield	0.027	NS	NS
TKW	0.001	NS	NS
Crude protein	<.001	<.001	<.001
Glutenins/gliadins	NS	0.001	0.006
Gliadins	<.001	<.001	0.024
Glutenins	<.001	0.002	0.024
Albumin	0.024	<.001	NS
Globulin	0.045	<.001	<.001
L* **Value**	<.001	<.001	NS
a* **Value**	<.001	<.001	0.01
b* **Value**	<.001	<.001	NS
Grain filling to maturity	<.001	0.02	NS
Sowing to maturity	<.001	NS	NS

6 Treatments =3cultivars × 2 sowings; number of replications = 4; NS = p>0.05

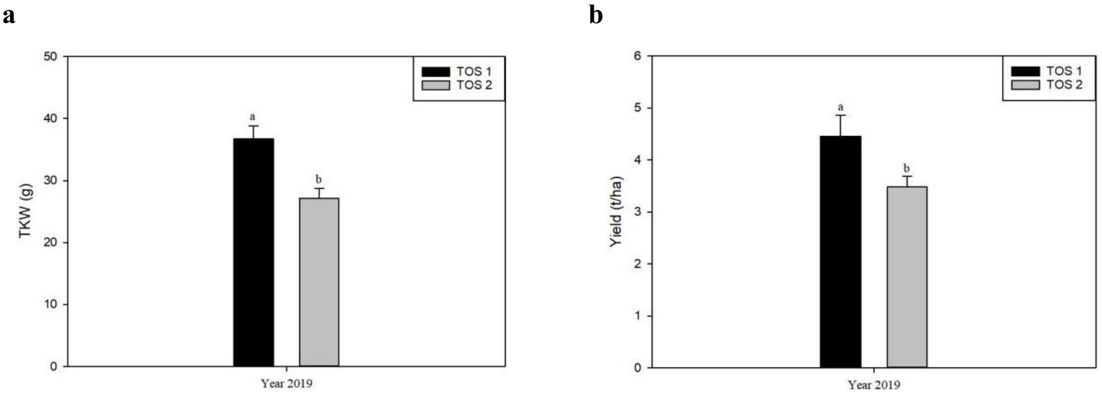


Figure 4.2. Effect of sowing dates (TOS1 and TOS2) on (a) yield (t/ha) and (b) TKW (g) in Narrabri in 2019. Means followed by the same letters are not significantly different at p<0.05 for sowing date. Error bars represent standard error of the mean (n=4).

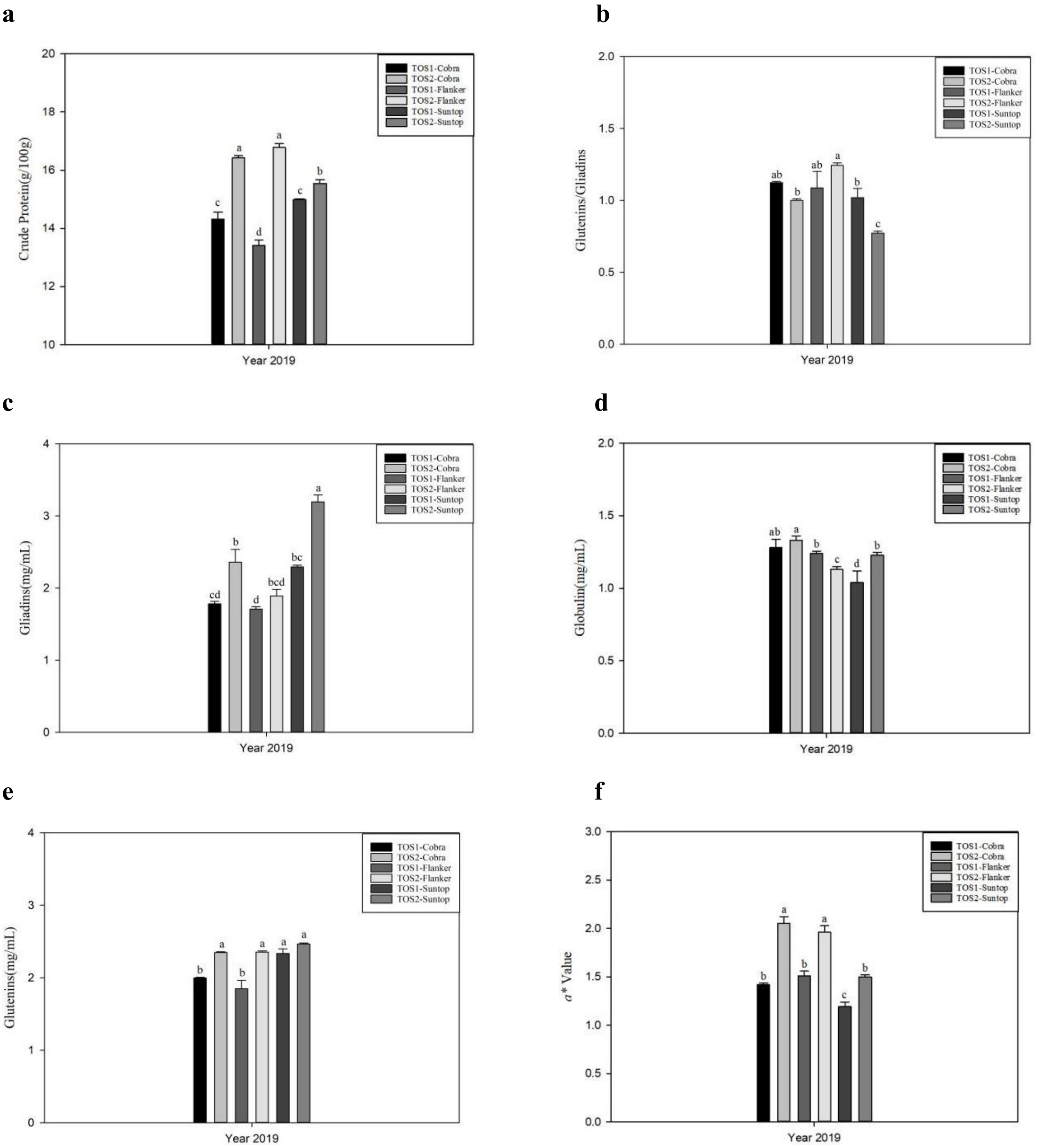


Figure 4.3. Effect of sowing dates (TOS1 and TOS2) and cultivars (Cobra, Flanker and Suntop) on (a)

crude protein (g/100g), (b) glutenins/gliadins, (c) gliadins (mg/mL), (d) glutenins (mg/mL), (e) globulin (mg/mL) and (f) *a value in Narrabri in 2019. Means followed by the same letters are not significantly different at $p<0.05$ for sowing period×cultivar interaction. Error bars represent standard error of the mean (n=4).**

Table 4.3. The pairwise correlations between wheat yield, protein, protein fractions, kernel colour and maturity days by Berkut and Sokoll in three locations in 2019.

		Yield	**TKW**	**Crude protein**	**Glutenins/ Gliadins**	**Gliadins**	**Glutenins**	**Albumin**	**Globulin**	**L***	**a***	**b***	**Grain filling to maturity**	**Sowing to maturity**
Yield	*r* =	1												
	p =													
TKW	*r* =	NS	1											
	p =													
Crude protein	*r* =	NS	-0.665	1										
	p =		<0.001											
Glutenins/ Gliadins	*r* =	NS	NS	NS	1									
	p =													
Gliadins	*r* =	NS	-0.418	NS	-0.909	1								
	p =		0.042		<0.001									
Glutenins	*r* =	NS	-0.433	NS	-0.6604	0.899	1							
	p =		0.035		<0.001	<0.001								
Albumin	*r* =	NS	NS	NS	NS	NS	NS	1						
	p =													
Globulin	*r* =	NS	NS	NS	0.639	-0.709	-0.683	NS	1					
	p =				<0.001	<0.001	0.001							

L*	*r* =	NS	NS	-0.507	NS	NS	NS	0.463	NS	1				
	p =			0.011				0.023						
a*	*r* =	NS	NS	NS	NS	NS	NS	-0.436	NS	-0.77	1			
	p =							0.033		<0.001				
b*	*r* =	NS	-0.724	0.574	NS	NS	NS	NS	NS	NS	NS	1		
	p =		<0.001	0.003										
Grain filling to maturity	*r* =	NS	NS	NS	NS	NS	NS	NS	NS	-0.473	0.745	-0.601	1	
	p =									0.019	<0.001	0.002		
Sowing to maturity	*r* =	NS	NS	NS	NS	NS	NS	NS	NS	-0.534	0.719	-0.432	0.935	1
	p =									0.007	<0.001	0.035	<0.001	

6 Treatments =3 cultivars × 2 sowings; number of replications = 4; NS = non-significant

Table 4.4. The pairwise correlations between wheat yield, protein, protein fractions, kernel colour and maturity days by Cobra, Flanker and Suntop in Narrabri in 2019.

		Yield	**TKW**	**Crude protein**	**Glutenins/ Gliadins**	**Gliadins**	**Glutenins**	**Albumin**	**Globulin**	***L****	***a****	***b****	**Grain filling to maturity**	**Sowing to maturity**
Yield	*r* =	1												
	p =													

TKW	*r* =	0.753	1										
	p =	0.005											
Crude protein	*r* =	NS	NS	1									
	p =												
Glutenins/ Gliadins	*r* =	NS	NS	NS	1								
	p =												
Gliadins	*r* =	NS	NS	NS	-0.898	1							
	p =				<0.001								
Glutenins	*r* =	0.621	0.597	NS	NS	0.747	1						
	p =	0.031	0.04			0.005							
Albumin	*r* =	NS	NS	NS	NS	NS	NS	1					
	p =												
Globulin	*r* =	NS	NS	NS	NS	NS	NS	0.58	1				
	p =							0.048					
L*	*r* =	NS	NS	NS	0.629	-0.703	NS	NS	NS	1			
	p =				0.029	0.011							
a*	*r* =	NS	NS	NS	-0.598	NS	NS	NS	NS	-0.591	1		
	p =				0.04					0.043			
b*	*r* =	NS	NS	NS	NS	0.621	NS	NS	NS	-0.924	0.761	1	
	p =					0.031				<0.001	0.004		
Grain	*r*=	0.656	0.801	NS	NS	0.65	0.716	NS	NS	-0.736	NS	0.637	1

filling to maturity	*p*=	0.021	0.002			0.022	0.009			0.006		0.026		
Sowing to maturity	*r*=	0.699	0.798	NS	-0.787	0.82	0.657	NS	NS	-0.689	NS	0.613	0.91	1
	p=	0.012	0.002		0.002	0.001	0.02			0.013		0.034	<0.001	

6 Treatments =3 cultivars × 2 sowings; number of replications = 4; NS = non-significant

4.4. Discussion

4.4.1. Impact of HTs on Wheat Yield and Thousand Kernel Weight (TKW)

HTs reduced wheat yield and TKW, particularly under late sowing conditions (TOS2) compared with normal sowing (TOS1). This reduction was most pronounced in Merredin, the hottest location in the study, aligning with previous research indicating that elevated temperatures accelerate phenological development, reducing the grain filling period essential for kernel development (Shew et al., 2020; Wieser et al., 2020). The reduction in yield and TKW observed in 2019 for both Berkut and Sokoll, especially under TOS2, highlights the adverse effects of HTs on wheat growth and productivity. The shorter grain filling days (GFD) under HTs reduced TKW, as shorter GFD provided less time for biomass accumulation and grain filling. This was particularly evident in Merredin, where grain filling times were shortest across all cultivars. Shortened GFD due to HT impairs nutrient translocation to the grain, contributing to smaller kernels and lower yields. There was little difference between the early and late sowing times within a location for growing degree days (GDD) from sowing to maturity and from anthesis to maturity.

HTs not only reduce the grain filling period but also impair pollen tube formation and promote pollen sterility, further contributing to yield losses (Ullah et al., 2022). The negative correlations found between TKW and crude protein, gliadins, and the $b*$ value, along with the positive correlation with the glutenins/gliadins ratio, suggest a complex interplay between yield components and protein composition under HT. These correlations indicate that HTs not only reduce yield and TKW but also alter the protein balance within the grain, affecting both quantity and quality. These findings are critical as they underscore the need for breeding heat-tolerant wheat cultivars that can maintain yield and grain quality.

4.4.2. Impact of HT on Wheat Colour

Flour colour is an important quality attribute, with high $L*$ values indicating lighter flour and high $b*$ values indicating more yellow flour, both of which are desirable traits in many wheat products (Hughes et al., 2020). TOS2 generally resulted in higher $L*$ values compared with TOS1, except in specific instances such as Berkut in Narrabri and Sokoll in Merredin. This increase in $L*$ values under late sowing may be due to the accelerated maturation process induced by HTs, which affects the pigment composition and distribution within the grain (Dias & Lidon, 2009a). The correlation between GFD and colour traits further supports these findings, as shorter grain filling times resulted in lighter (higher $L*$) and less yellow (lower $b*$) flour. This is likely due to accelerated grain maturation under HT, which alters pigment biosynthesis.

The correlations between anthesis to maturity duration and flour colour suggest that the shortened grain filling period under HT not only affects yield but also impacts the visual quality of the flour. The negative correlations between $L*$ and $b*$ values with the duration from anthesis to maturity imply that shorter grain filling periods result in lighter and less yellow flour. This finding aligns with previous studies that have shown HT can accelerate grain maturation, leading to lighter and less yellow flour due to changes in pigment concentration and composition (Shirdelmoghanloo et al., 2016).

4.4.3. Impact of HTs on Wheat Protein Content and Composition

The data analyses demonstrate interactions between location, year, sowing time, and cultivar, influencing the crude protein content and the glutenins/gliadins ratio, which are essential determinants of wheat quality. Late sowing times (TOS2) generally resulted in higher crude protein content compared to normal sowing times (TOS1) across all cultivars, locations and years. For instance, in experiment 1, Berkut and Sokoll exhibited higher crude protein content in TOS2 in Narrabri and Merredin in 2019, except for Berkut at Horsham; in experiment 2, Cobra, Flanker and Suntop had significantly higher crude protein content in Narrabri in TOS2 in 2019. This increase in protein content under late sowing conditions aligns with previous studies indicating that HT can lead to an increase in protein concentration due to the reduced grain filling period (Zhao et al., 2022). The grain filling period is critical for protein accumulation. Under HTs, the reduced GFD contributed to smaller grains with a higher concentration of protein, as starch deposition is more sensitive to heat than protein synthesis. This is evident from the negative correlation between TKW and crude protein content observed (r = -0.6652, p<0.001). The shorter GFD also affected the protein composition, leading to higher proportions of gliadins relative to glutenins, which weakens dough quality.

Gliadins and glutenins are the two major types of proteins in wheat that contribute to its unique baking qualities. HT alters the composition of wheat proteins, particularly the ratio of gliadins to glutenins. Gliadins confer extensibility, while glutenins provide dough strength and elasticity (Tabbita et al., 2024; Blumenthal et al., 1993). This study also showed that the glutenins/gliadins ratio varied with sowing time, location, and cultivar. For experiment 1, the glutenins/gliadins ratio of Berkut and Sokoll were higher in TOS1 compared to TOS2 across all three locations, while Berkut showed a higher ratio in Merredin. Under HT, there was an increase in the proportion of gliadins relative to glutenins, particularly in TOS2, which can adversely affect the viscoelastic properties of the dough (Zhao et al., 2008; Tabbita et al., 2024). This decrease in the gliadins/glutenins ratio leads to weaker dough, which is less suitable for bread-making. The increase in crude protein content under HT was accompanied by changes in the protein fractions. The wheat cultivars used in this experiment are more characteristic of high protein wheats; therefore, despite the reduction in the glutenins/gliadins ratio under HTs in TOS2, most varieties still exhibited a relatively high glutenins/gliadins ratio. Only the dough strength and elasticity of the flour will decrease, and the baking quality will be affected to a certain extent. Dough strength and elasticity are key characteristics of bread and other fermented products (Dhaka & Khatkar, 2015).

Furthermore, in Experiment 1, HTs can alter the molecular structure of gluten proteins, thereby affecting their functionality. At Merredin, under TOS2, the glutenins/gliadins ratio showed a greater decrease in Berkut and a smaller decrease in Sokoll, indicating that dough quality varied between cultivars under higher HT. This finding is corroborated by previous research, which showed that HTs disrupt the synthesis of glutenins more than that of gliadins, resulting in a disproportionate increase in gliadins (Zhao et al., 2022). However, in Experiment 2, the glutenins/gliadins ratio of Flanker under TOS2 at Narrabri in 2019 was higher than that under TOS1. This suggests that, for some heat-tolerant wheat cultivars, the glutenins/gliadins ratio may be resilient and not decrease even if the crude protein content increases under HT. It appears that the glutenins/gliadins ratio in wheat is influenced by genotype. HT increased the extractable gliadins and decreased the unextractable glutenins, which affects the formation of strong gluten networks. This alteration is critical for the baking industry, as it directly impacts the quality of baked goods. HTs during grain filling increase the proportion of LMW glutenins and reduce HMW glutenins, further weakening the gluten network (Shirdelmoghanloo et al., 2016). These changes in protein composition under HT underscore the importance of selecting and breeding wheat cultivars that can maintain a balanced glutenin/gliadin ratio and high protein quality under HTs. The development of heat-tolerant wheat cultivars should focus not only on yield and TKW but also on maintaining or improving protein quality to ensure the production of high-quality wheat flour suitable for baking.

This research is novel as it was conducted across three distinct locations in Australia, Narrabri, Merredin, and Horsham, representing a diverse range of growing conditions on a continent larger than Western Europe. These locations encompass a broad spectrum of environments that are likely to be affected by global warming, making the findings relevant to many regions of Australia, China, India, USA and around the world. Specifically, it highlights the critical glutenins/gliadins ratio under HT. These findings offer important implications for breeding strategies aimed at improving heat tolerance in wheat cultivars.

4.4.4 Future prospects for wheat research

This study provides several important directions for future wheat research. The impact of HTs on yield, grain quality, and protein composition emphasises the urgent need for breeding programs to develop heat-tolerant wheat cultivars. Future research should focus on identifying and incorporating genetic traits that confer heat tolerance, such as enhanced pollen viability, efficient stem reserve mobilisation, and robust carbohydrate metabolism under stress (Ullah et al., 2022). Moreover, advances in molecular biology and genomics offer new opportunities to understand the genetic basis of heat tolerance in wheat. Techniques such as marker-assisted selection and genomic selection can accelerate the breeding process by allowing for the precise incorporation of heat-tolerance traits into high-yielding cultivars. Additionally, exploring the role of epigenetics in HT responses could provide insights into how wheat plants can be better prepared to cope with rising temperatures.

Another important area for future research is the detailed study of protein composition changes under HT. Using advanced techniques like size-exclusion high-performance liquid chromatography and mass spectrometry, researchers can gain a deeper understanding of how HT affects the structure and functionality of gluten proteins. This knowledge will be crucial for developing wheat cultivars with superior baking quality even under adverse climatic conditions.

4.5. Conclusions

This study underscores the impact of HTs on wheat yield, TKW, colour, and protein composition. Elevated temperatures reduce yield and TKW, particularly under late sowing conditions, and modify wheat colour and protein balance, thereby affecting flour quality. The protein quality of highly heat-tolerant cultivars, such as Flanker, sown under TOS2 did not decline and even increased in the TOS2 hot conditions of 2019. These findings highlight the necessity of breeding heat-tolerant wheat cultivars that can maintain both yield and quality under increasing temperatures. Future research should focus on identifying genetic traits associated with heat tolerance, employing advanced molecular techniques, and adopting interdisciplinary approaches to ensure sustainable wheat production in the face of a changing climate.

Chapter 5 Response of wheat genotypes in starch and total dietary fibre under HT across diverse environments

Abstract

High-temperature stress significantly impacts wheat yield, starch content, total dietary fibre, and starch gelatinisation properties, with variations influenced by genotype, environmental conditions, and sowing time. This study investigated five wheat cultivars (Berkut, Sokoll, Flanker, Cobra, and Suntop) across three Australian regions (Horsham, Narrabri, and Merredin) during 2019 and 2020. Late sowing (TOS2) and higher temperatures in Merredin led to reduced starch content and significant alterations in starch gelatinisation behaviour, particularly peak and breakdown viscosities, attributed to changes in amylose-amylopectin ratios and starch granule structure. Total dietary fibre responses were complex, with increases linked to enhanced lignin and cellulose synthesis under moderate stress, while extreme heat accelerated leaf senescence and reduced fiber deposition. Heat-tolerant cultivars, such as Berkut and Flanker, demonstrated greater resilience in maintaining starch stability and fibre content compared with heat-sensitive cultivars like Sokoll and Cobra. Notably, this study is the first to evaluate the interactive effects of genotype, sowing time, and diverse environmental conditions on wheat starch and total dietary fibre under high-temperature stress. Furthermore, innovative correlation analyses revealed a trade-off between starch accumulation and structural carbohydrate synthesis under thermal stress, providing novel mechanistic insights for breeding climate-resilient wheat cultivars. These findings highlight the importance of selecting heat-tolerant genotypes, optimising sowing strategies, and developing wheat cultivars with improved thermostability to mitigate the adverse effects of climate change. This research provides a foundation for adaptive agricultural practices and breeding strategies to ensure wheat quality and food security in a warming climate.

Keywords： Wheat genotypes, High-temperature stress, Starch gelatinisation, Total dietary fibre

5.1. Introduction

Starch and total dietary fibre are essential components of wheat that play distinct but complementary roles in human nutrition and health. Starch, the primary carbohydrate in wheat, contributes 50-70% of total energy intake, serving as a fundamental source of dietary calories (Copeland et al., 2009). Beyond its nutritional value, starch is widely used in the food industry as a thickener, stabiliser, and texture modifier. During gelatinisation, starch granules absorb water and swell upon heating, disrupting their crystalline structure and forming a viscous paste, which significantly influences the texture, consistency, and digestibility of food products (Blazek et al., 2011). This process is particularly relevant for glycemic control and metabolic health, as it determines the rate of starch digestion and glucose release in the body (Wang et al., 2017). Total dietary fibre, primarily composed of cellulose and lignin, complements starch by providing structural support to wheat grains and offering significant health benefits. It promotes gut motility, enhances digestive health, and helps prevent chronic diseases such as obesity, type 2 diabetes, and cardiovascular disorders (Du et al., 2010). Additionally, total dietary fibre binds bile acids and toxins, reducing their reabsorption and aiding in detoxification (Papandreou et al., 2015). The balance between starch and total dietary fibre is crucial for optimising wheat's nutritional value, with starch delivering readily available energy and total dietary fibre ensuring slower digestion, enhanced satiety, and improved metabolic regulation.

HT has significant effects on both starch and total dietary fibre in wheat, resulting in significant changes in their composition, function and nutritional properties. During the grain filling period, HT accelerates starch biosynthesis at the expense of total dietary fibre, resulting in changes in carbohydrate composition (Shewry, 2009b). The reduction in total dietary fibre content is attributed to the shortened grain development period and impaired lignin deposition in the cell wall, which affects the structural integrity and nutritional value of the grain (Brestic et al., 2018). HT also changes the physicochemical properties of starch, increasing its gelatinisation temperature and affecting gelatinisation characteristics such as peak viscosity and decomposition during heating (Blazek et al., 2011). These changes affect the texture, stability and digestibility of starch-based foods, and may reduce their suitability as low-glycemic index products. However, dietary fibres such as arabinoxylan interact with starch during gelatinisation, forming a physical barrier that limits particle expansion and excessive decomposition. This interaction enhances the thermal stability of starch pastes and improves resistance to shear and thermal degradation, which can help in the development of functional foods with enhanced texture stability and glycemic control (Chaisawang & Suphantharika, 2006). Understanding these heat-induced changes is essential for optimising wheat processing and improving the quality of starch- and fibre-based products.

There is a notable gap in research concerning the impact of HT on the yield, starch content, total dietary fibre, and starch gelatinisation properties of heat-tolerant wheat cultivars cultivated in Australia's primary wheat-growing regions. Therefore, the main objective of this study is to assess the changes in yield, starch content, total dietary fibre, and starch gelatinisation properties of two wheat cultivars, Berkut and Sokoll, in three regions (Horsham (VIC), Narrabri (NSW), and Merredin (WA)) during a hot year (2019) (Experiment 1), and three other wheat cultivars, Flanker, Cobra, and Suntop, in Narrabri over two years (2019 and 2020) (Experiment 2). In all experiments, two sowing times were implemented, with the late sowing wheat exposed to higher temperatures during the grain filling and maturation periods. The primary hypothesis posits that the hotter conditions in Merredin will have a more pronounced effect on the yield and quality of less heat-tolerant wheat cultivars, particularly in late sowing scenarios. Moreover, it is expected that the starch content of wheat grown in Merredin will be higher than that in Horsham and Narrabri, while the total dietary fibre content and starch stability will be lower in Merredin compared to the other regions. Additionally, TOS2 wheat is hypothesised to have a higher starch content but lower total dietary fibre content and starch stability compared with TOS1 wheat.

5.2. Materials and Methods

Experimental procedures followed the guidelines of Chapter 3 (General Materials and Methods) from the field experiments, providing a standardised framework for sample handling and analysis. See Sections 3.1, 3.2 and 3.3 for detailed descriptions of the field experiments.

5.2.1. Total starch and total dietary fibre

The total starch content of the samples was analysed using a method adapted from Megazyme (Megazyme International Ireland Ltd., Wicklow, Ireland), as detailed in Mahasukhonthachat et al. (2010). The total fibre content was measured using a Megazyme test kit, following the procedure described by Gebreegziabher and Tsegay (2020).

5.2.2. Starch pasting properties

Starch pasting properties were determined by Rapid Visco-Analysis (RVA) using an RVA-4 analyser (Newport Scientific, Warriewood, Australia), following a method similar to that described by Beta and Corke (2004). Ground wheat grain (4.2 g) was mixed with deionised water (23.8 g) and subjected to a programmed heating and cooling cycle lasting 13 minutes. The slurry was maintained at 50°C for 1 minute, then heated to 95°C and held for 2.5 minutes before cooling to 50°C and holding for an additional 2 minutes. The mixing paddle operated at 960 rpm for the initial 10 seconds and then at 160 rpm for the remainder of the cycle. Parameters recorded included peak viscosity, holding viscosity, final viscosity, breakdown viscosity (peak – holding), and setback viscosity (final – peak), as well as peak time and pasting temperature.

5.3. Results

5.3.1. Experiment 1

The interactions of location, sowing period, and cultivar were significant for yield, thousand kernel weight (TKW), starch, total dietary fibre, RVA starch pasting properties, $L*$, $a*$ and $b*$ values (Table 5.1).

Table 5.1. The main effects and interactions of locations (Horsham, Narrabri and Merredin), sowing period (TOS1 and TOS2) and cultivar (Berkut and Sokoll) on wheat yield, starch, total dietary fibre, RVA starch pasting properties, kernel colour and maturity days in 2019.

Effect	**Loc(Location)**	**TOS**	**Cul(Cultivar)**	**Loc*TOS**	**Loc*Cul**	**TOS*Cul**	**Loc*TOS*Cul**
Yield	<.001	<.001	<.001	<.001	<.001	<.001	<.001
TKW	<.001	<.001	<.001	<.001	NS	<.001	0.011
Starch	<.001	<.001	NS	0.026	<.001	NS	0.006
Fibre	<.001	<.001	<.001	<.001	<.001	<.001	<.001
Pasting Temp.	NS	NS	NS	NS	NS	NS	NS
Peak viscosity	<.001	<.001	<.001	<.001	<.001	NS	0.002
Trough	<.001	<.001	<.001	<.001	<.001	<.001	NS
Breakdown	0.005	<.001	<.001	<.001	<.001	NS	0.003
Final	<.001	<.001	0.003	<.001	<.001	<.001	NS
Setback	<.001	<.001	NS	0.04	0.013	<.001	NS
Peak_time	<.001	<.001	<.001	NS	0.001	NS	NS
$L*$ Value	<.001	<.001	NS	<.001	<.001	NS	<.001

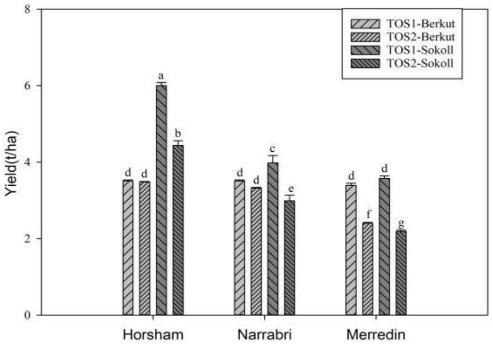
a* **Value**	<.001	<.001	<.001	<.001	<.001	NS	<.001
b* **Value**	<.001	0.003	<.001	<.001	<.001	<.001	<.001
Days from anthesis to maturity	<.001	<.001	NS	<.001	NS	NS	NS
Days from sowing to maturity	<.001	<.001	NS	<.001	0.019	NS	NS

12 Treatments =3 locations × 2 cultivars × 2 sowings; number of replications = 4; NS = non-significant.

Sokoll had lower yield at all three locations for TOS2 compared with TOS1 (Figure 5.1 (a)). There was a significant reduction in yield for Berkut at Merredin for TOS2 compared with TOS1(p<0.001). TKW of Berkut was consistently higher than Sokoll across all three locations and both sowing dates, particularly at Merredin (Figure 5.1 (b)). The contents of starch, fibre, and certain starch viscosity parameters (peak viscosity and breakdown) exhibited significant differences across the interactions of location, time of sowing (TOS), and variety (Loc*TOS*Var). In Merredin, the highest starch content (63.91 g/100 g) was in Berkut sown at TOS1, while the lowest starch content (56.03 g/100 g) was in Berkut sown at TOS2 in Narrabri (Figure 5.1 (c)). The highest total dietary fibre content (1.64 g) was in Berkut sown at TOS2 in Horsham, whereas the lowest total dietary fibre content (1.33 g) was in Sokoll sown at TOS1 in Merredin (Figure 5.1 (d)). For breakdown viscosity, Berkut sown at TOS1 in Merredin exhibited the highest value (686 cP), while Sokoll sown at TOS2 in Merredin showed the lowest value (199 cP) (Figure 5.1 (e)). The peak viscosity was highest in Berkut sown at TOS2 in Merredin (1632 cP) and lowest in Sokoll sown at TOS2 in Narrabri (897 cP) (Figure 5.1 (f)). Pasting temperature showed no significant differences across the conditions, while peak time exhibited significant differences only under the interaction of location and cultivar (Loc*Var).

Yield exhibited a negative correlation with both setback and peak time (*r*=-0.047, *p*=0.049 and *r*=-0.069, *p*<0.001, respectively). Thousand kernel weight (TKW) was negatively correlated with the *b** value (*r*=-0.724, *p*<0.001). Starch content showed a negative correlation with total dietary fibre (*r*=-0.479, *p*=0.018), while anthesis-to-maturity days and sowing-to-maturity days were also negatively correlated with starch content (*r*=-0.452, *p*=0.027 and *r*=-0.426, *p*=0.038, respectively). Total dietary fibre demonstrated a positive correlation with breakdown, anthesis-to-maturity days, and sowing-to-maturity days (*r*=0.635, *p*<0.001; *r*=0.458, *p*=0.024; and *r*=0.595, *p*=0.002, respectively) but a negative correlation with the *L** value (*r*=-0.427, *p*=0.038). Peak viscosity was positively correlated with trough, breakdown, final viscosity, *a** value, anthesis-to-maturity days, and sowing-to-maturity days (*r*=0.645, *p*<0.001; *r*=0.959, *p*<0.001; *r*=0.529, *p*=0.011; *r*=0.521, *p*<0.001; *r*=0.418, *p*=0.042; and *r*=0.595, *p*=0.002, respectively) but negatively correlated with the *L** value (*r*=-0.503, *p*=0.012). Breakdown showed a positive correlation with peak time, *a** value, anthesis-to-maturity days, and sowing-to-maturity days (*r*=0.435, *p*=0.034; *r*=0.669, *p*<0.001; *r*=0.578, *p*=0.003; and *r*=0.669, *p*<0.001, respectively) but a negative correlation with the *L** value (*r*=-0.636, *p*<0.001) (Table 5.2).

a



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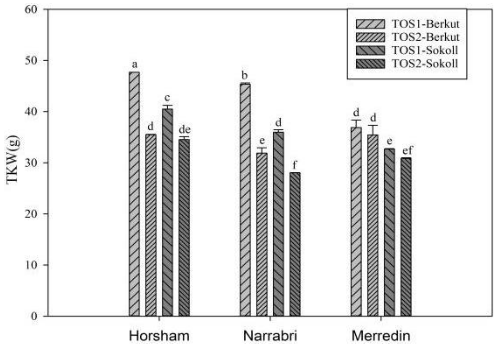

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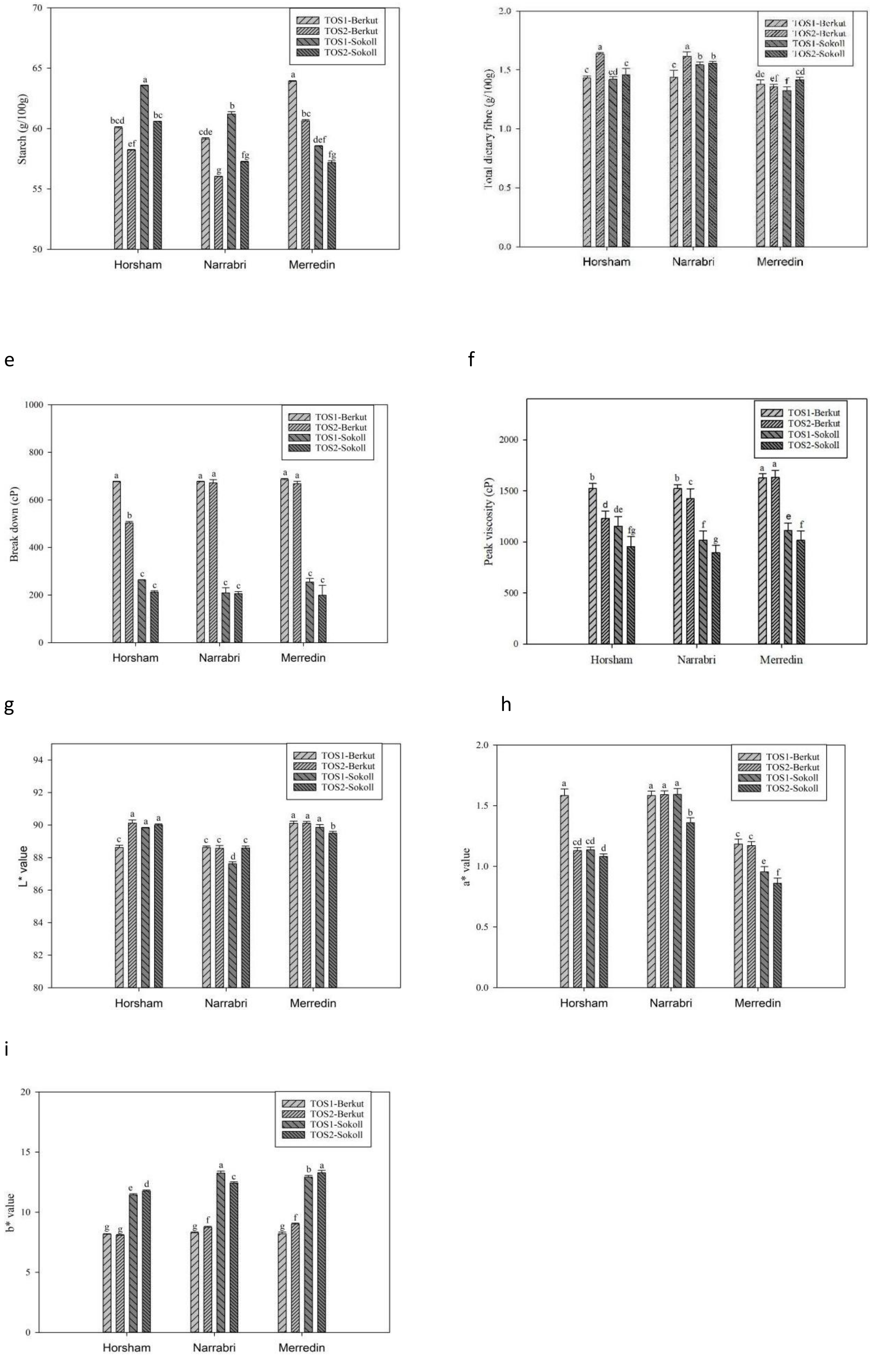


Figure 5.1. Effect of locations (Horsham, Narrabri and Merredin), sowing dates

(TOS1 and TOS2) and cultivars (Berkut and Sokoll) on (a) yield (t/ha), (b) TKW (g), (c) starch (g/100 g), (d) total dietary fibre (g/100g), (e) break down (cP), (f) peak viscosity (cP), (g) *L value, (h) *a** value and (i) *b** value in 2019. Means followed by the same letters are not significantly different at p<0.05 for location*sowing period*cultivar interaction. Error bars represent standard error of the mean (n=4).**

Table 5.2. The pairwise correlations between wheat yield, starch, total dietary fibre, RVA starch pasting properties, kernel colour and maturity days by Berkut and Sokoll in three locations in 2019.

		Yield	**TKW**	**Starch**	**Crude fiber**	**Pasting Temp.**	**Peak Viscosity**	**Trough**	**Break down**	**Final**	**Set back**	**Peak time**	***L****	***a****	***b****	**Anthesis to maturity**	**Sowing to maturity**
Yield	*r* =	1															
	p =																
TKW	*r* =	NS	1														
	p =																
Starch	*r* =	NS	NS	1													
	p =																
Fibre	*r* =	NS	NS	-0.479	1												
	p =			0.018													
Pasting Temp.	*r* =	NS	NS	NS	NS	1											
	p =																
Peak	*r* =	NS	NS	NS	NS	NS	1										
	p =																
Trough	*r* =	NS	NS	NS	NS	NS	0.645	1									
	p =						<0.001										
Break down	*r* =	NS	NS	NS	0.635	NS	0.959	NS	1								
	p =				<0.001		<0.001										

Final	*r* =	NS	NS	NS	NS	NS	0.529	0.961	NS	1							
	p =						0.011	<0.001									
Set back	*r* =	-0.407	NS	NS	NS	NS	NS	0.772	NS	0.918	1						
	p =	0.049						<0.001		<0.001							
Peak time	*r* =	-0.691	NS	NS	NS	NS	NS	NS	0.435	NS	NS	1					
	p =	<0.001							0.034								
L*	*r* =	NS	NS	NS	-0.427	NS	-0.503	NS	-0.636	NS	NS	-0.512	1				
	p =				0.038		0.012		<0.001			0.011					
a*	*r* =	NS	NS	NS	0.449	0.443	0.521	NS	0.669	NS	-0.436	0.556	-0.769	1			
	p =				0.028	0.031	<0.001		<0.001		0.033	0.005	<0.001				
b*	*r* =	NS	-0.724	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	1		
	p =		<0.001														
Anthesis to maturity	*r*=	NS	NS	-0.452	0.458	NS	0.418	NS	0.578	NS	NS	NS	-0.534	0.719	-0.432	1	
	p=			0.027	0.024		0.042		0.003				0.007	<0.001	0.035		
Sowing to maturity	*r*=	NS	NS	-0.426	0.595	NS	0.545	NS	0.669	NS	NS	NS	-0.473	0.745	-0.601	0.935	1
	p=			0.038	0.002		0.006		<0.001				0.019	<0.001	0.002	<0.001	

6 Treatments =3 cultivars × 2 sowings; number of replications = 4; NS = non-significan

5.3.2. Experiment 2

The interactions for Cobra, Flanker and Suntop of sowing period, and variety were significant for starch, total dietary fibre, RVA starch pasting properties, L^*, a^* and b^* values in Narrabri in 2019 (Table 5.3).

Table 5.3. The main effects and interactions of year, sowing period and cultivar on wheat yield, starch, total dietary fibre, RVA starch pasting properties, kernel colour and maturity days of Cobra, Flanker and Suntop grown at Narrabri in 2019.

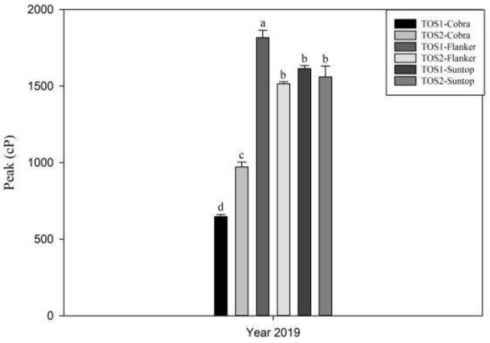
Effect	**TOS**	**Cultivar**	**TOS.Cultivar**
Yield	0.027	NS	NS
Thousand kernel weight (TKW)	0.001	NS	NS
Starch	0.011	0.333	NS
Fibre	<.001	<.001	NS
Pasting Temp.	0.037	0.215	NS
Peak Viscosity	0.749	<.001	<.001
Trough	0.012	<.001	<.001
Breakdown	0.002	<.001	NS
Final	0.008	<.001	<.001
Setback	0.008	<.001	<.001
Peak_time	0.044	<.001	0.023
L^* Value	<.001	<.001	NS
a^* Value	<.001	<.001	0.01
b^* Value	<.001	<.001	NS
Days from anthesis to maturity	<.001	0.02	NS
Days from sowing to maturity	<.001	NS	NS

6 Treatments =3 cultivars × 2 sowings; number of replications = 4; NS = $p > 0.05$

The wheat yield and TKW were significantly different under different sowing periods, with both yield (4.46 t/ha) and TKW (36.72 g) of wheat for TOS1 significantly higher than those under TOS2 (3.48 t/ha and 27.18 g) in 2019. The content of starch viscosity (peak, trough, final, and setback) and peak time showed significant differences in TOS*Variety. In TOS1, Flanker exhibited the highest values for peak, trough, final, and setback (1816, 971.5, 1921, and 949.5 cP, respectively), whereas Cobra had the lowest values for these parameters (647, 370.2, 944.0, and 574 cP, respectively) (Figure 5.2). The longest peak viscosity time was observed for Flanker in TOS1 (5.45 mins), while Cobra in TOS1 had the shortest peak time (5.20 mins). Starch, fibre, and pasting temperature were significantly different under TOS. The starch content in TOS1 (60.86 g/100g) was higher than in TOS2 (58.29 g/100g), the fibre content in TOS2 (1.42 g) was higher than in TOS1 (1.33 g), and the pasting temperature in TOS1 (74.0℃) was higher than in TOS2 (64.4℃).

The year 2020 was characterised by lower-than-average temperatures, which resulted in minimal differences in wheat performance between the two sowing times, TOS1 and TOS2.

a



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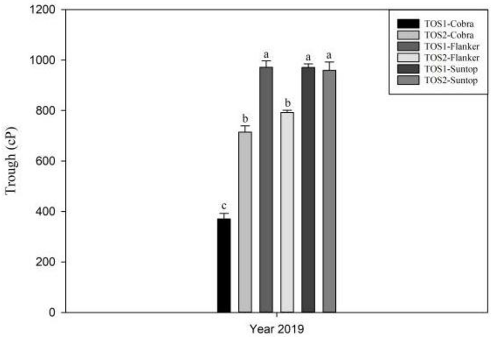

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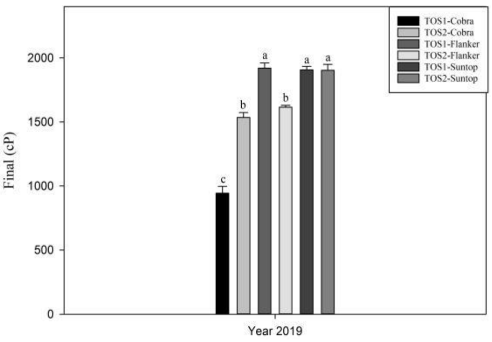

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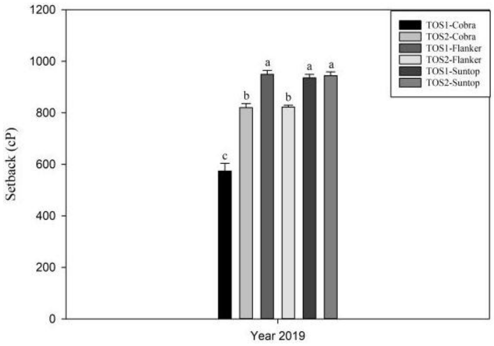

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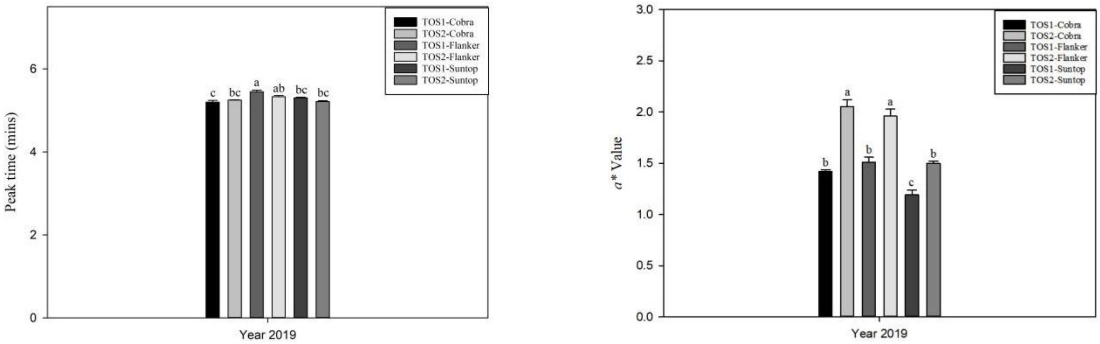


Figure 5.2. Effect of sowing dates (TOS1 and TOS2) and cultivars (Cobra, Flanker and Suntop) on (a) Peak Viscosity(cp), (b) Tough (cp), (c) Final (cp), (d) Setback (cp), (e) Peak time (mins) and (f) *a value in Narrabri in 2019. Means followed by the same letters are not significantly different at p<0.05 for sowing period × cultivar interaction. Error bars represent standard error of the mean (n=4).**

5.4 Discussion

5.4.1. Effects of HT on Starch and Total dietary fibre

HTs significantly influenced starch content across the three regions and sowing times observed in this study. There was a notable reduction in starch content, particularly under late sowing conditions (TOS2) in Merredin, where temperatures were highest among the three locations. For instance, the starch content of Berkut sown in Merredin during TOS2 dropped to 56.03 g/100 g compared with 63.91 g/100 g in TOS1. These findings align with previous research by Dwivedi et al. (2017), which demonstrated that increased temperatures during the grain filling period limit the accumulation of photosynthetic assimilates, thereby decreasing starch storage and total biomass.

HTs significantly affected total dietary fibre content in this study, with variations across regions, sowing times, and wheat varieties. Total dietary fibre content increased under HT in certain conditions but showed decreases in others, indicating a complex response influenced by both genotype and environmental interactions. In Horsham, late-sown Berkut exhibited the highest total dietary fibre content (1.64 g), which may be attributed to accelerated lignin and cellulose accumulation under high-temperature stress, as suggested by Chaisawang and Suphantharika (2005). Conversely, early-sown Sokoll in Merredin had the lowest total dietary fibre content (1.33 g), likely due to extreme HT and accelerated leaf senescence, which diminished structural carbohydrate deposition (Farooq et al., 2011; Gupta et al., 2015). This dual pattern aligns with findings from Gupta et al. (2015), which highlighted that HT can either increase fibre content through induced cellulose and lignin synthesis or decrease it by accelerating leaf degradation and reducing carbohydrate deposition in vegetative tissues.

The increase in total dietary fibre in certain genotypes and environments may also serve as a protective mechanism against heat-induced oxidative damage, as fibre components like cellulose contribute to structural stability. However, excessive HT, as experienced in Merredin, appears to surpass the plant's compensatory mechanisms, leading to reduced fibre content. This observation is consistent with the significant negative correlation between starch and total dietary fibre content ($r = -0.479$, $p = 0.018$), as seen in this study, suggesting a trade-off between carbohydrate storage and structural component synthesis under HT conditions.

These findings highlight the complexity of plant responses to HT, where total dietary fibre content may either increase or decrease depending on the severity and duration of heat exposure, as well as the genotype's inherent heat tolerance. For example, Berkut, categorised as a heat-tolerant cultivar, maintained higher total dietary fibre levels under late sowing conditions in Horsham compared to Sokoll, which exhibited lower fibre content in the hotter Merredin location. This suggests that heat-tolerant genotypes are better equipped to manage structural carbohydrate synthesis under thermal stress, potentially contributing to their overall resilience.

5.4.2. Effect of HT on Starch Gelatinisation Properties

RVA analysis revealed significant differences in starch gelatinisation properties, especially in peak viscosity and breakdown viscosities, across varying temperatures and sowing conditions. For example, Berkut sown at Merredin during TOS1 displayed the highest breakdown viscosity (686 cP), whereas Sokoll sown at Merredin during TOS2 exhibited the lowest (199 cP). Previous studies have suggested that HTs can reduce amylopectin content in wheat starch and alter its chain length and crystallinity, which are critical factors influencing gelatinisation behaviour. These structural changes may explain the significant differences observed in RVA parameters, such as the lower breakdown viscosity of Sokoll during TOS2 at Merredin, where HT was more severe (Zhao et al., 2022).

Moreover, Liu et al. (2017) proposed that HTs negatively impact starch content due to the disruption of endosperm cell division, leading to fewer starch granules and compromised structure. This observation is consistent with the reduced peak viscosity found in Sokoll at Narrabri under TOS2, suggesting that starch granule structure may degrade at elevated temperatures. The amylose-amylopectin ratio and crystalline structure are also critical in determining starch water absorption and gelatinisation capabilities under thermal conditions (Bao et al., 2020). Higher breakdown viscosity at elevated temperatures may indicate increased sensitivity of starch to mechanical and thermal stresses, thus affecting paste stability and the texture of final products.

The presence of waxy starch mutants, where amylose is nearly entirely replaced by amylopectin, suggests potential for faster gelatinisation at lower temperatures (Hurkman et al., 2003). In the non-waxy wheat varieties studied here, HTs led to reduced gelatinisation stability and increased breakdown, indicating that the amylopectin-dominant structure was affected. Thus, the effect of HTs on starch gelatinisation properties underscores the variability in gelatinisation and viscosity profiles across different wheat genotypes, with important implications for processing under diverse climatic conditions.

5.4.3. Overall Impact and Future Directions

This study underscores the importance of understanding climate change impacts on wheat quality, particularly in regions with significant temperature variability. By leveraging data from three geographically diverse locations across two years (2019 as a hot year and 2020 as a cooler year), this research provides a unique perspective on the influence of environmental and sowing variables on wheat starch, total dietary fibre, and gelatinisation properties. These findings highlight both the resilience and adaptability of wheat cultivars like Berkut and Flanker under high-temperature conditions, as well as the potential challenges of maintaining wheat quality as global temperatures rise.

This study is the first to evaluate the interactive effects of genotype, sowing time, and diverse environmental conditions on wheat starch and total dietary fibre properties under high-temperature stress, offering a novel perspective not previously reported in the literature. Furthermore, the correlation analysis revealed a trade-off between starch deposition and structural carbohydrate synthesis under thermal stress which provides fresh insights to guide the breeding direction of climate-resilient wheat cultivars.

Future research should prioritise the development of wheat cultivars with improved thermostability in starch composition and fibre content. Additionally, genetic modifications, such as the integration of waxy starch genes, could enhance wheat functionality in warmer climates. Adjustments to agricultural practices, including optimised sowing times and the selection of heat-tolerant varieties, may help mitigate the adverse effects of elevated temperatures on wheat quality. Overall, these insights provide a valuable foundation for enhancing wheat resilience to HT, with broader implications for food security and processing under a warming climate.

5.5 Conclusion

This study highlights the significant effects of HT on wheat yield, starch content, total dietary fibre, and starch gelatinisation properties, revealing variations influenced by genotype and environmental conditions. Total dietary fibre content showed a complex response, increasing under some conditions due to enhanced cellulose and lignin synthesis, while decreasing under others due to accelerated leaf senescence and reduced carbohydrate deposition. HTs also altered starch gelatinisation properties, particularly breakdown and peak viscosity, reflecting changes in the amylose-amylopectin ratio, starch crystallinity, and granule structure, which compromised starch stability and processing quality. Heat-tolerant cultivars such as Berkut and Flanker showed greater resilience in maintaining quality parameters compared to heat-sensitive cultivars such as Sokoll and Cobra. Importantly, this work represents the first analysis across diverse environments that combines yield performance with detailed starch and total dietary fibre profiling under high-temperature conditions. The novel insights obtained from the correlation analyses highlight a trade-off between starch deposition and structural carbohydrate synthesis and provide a mechanistic basis for developing climate-resilient wheat cultivars capable of sustaining both yield and quality under high temperature conditions. Future efforts should focus on breeding strategies to improve wheat starch and fibre stability under high temperatures, while exploring genetic modifications to improve wheat functionality under rising global temperatures. This study provides valuable insights into the impacts of climate change on wheat quality, laying the foundation for adaptive strategies to ensure food security under global warming conditions.

Chapter 6 Response of wheat genotypes under HT in minerals and phytate across diverse environments

Abstract

Minerals are vital nutrients essential for human health, yet their bioavailability in wheat, a staple crop consumed by 2.5 billion people globally, is constrained by genetic, environmental, and soil factors. This study evaluates the impact of HT, sowing time, and location on wheat yield, phytate content, and mineral composition across two experiments. Experiment 1 examined Berkut and Sokoll grown in three Australian locations (Horsham, Narrabri, and Merredin) under two sowing times (normal and late) in 2019, while Experiment 2 focused on Cobra, Flanker, and Suntop grown in Narrabri during 2019 and 2020. The analyses revealed significant genotype-environment interactions influencing yield and mineral content. Late sowing (TOS2) under high-temperature conditions reduced yield but enhanced mineral concentrations such as zinc (Zn) and iron (Fe) due to stress-induced nutrient remobilization. Merredin, characterised by HTs and sandy soils, exhibited the lowest phytate content, improving mineral bioavailability but at the cost of reduced yield and nutrient storage. Genotypic variation was evident, with heat-tolerant Berkut outperforming Sokoll in yield and mineral stability under stress. Soil properties, particularly pH and organic matter (OM), significantly influenced nutrient retention, with alkaline soils limiting Zn and Fe bioavailability. Notably, this study is the first to integrate multi-environmental data with detailed correlation analyses to elucidate the combined effects of HT, sowing time, and soil characteristics on wheat mineral composition and phytate levels. The novel mechanistic insights gained here provide a foundation for breeding climate resilient wheat cultivars that balance yield and nutritional quality, offering a sustainable pathway to combat hidden hunger.

Keywords: Wheat genotypes, High-temperature, Mineral, Phytate content, Genotype-environment interaction

6.1. Introduction

Minerals are important dietary components essential for human health, supporting a variety of metabolic functions and enzyme activation processes. They are divided into macrominerals (e.g., calcium, magnesium, potassium) that are required in large quantities and trace minerals or trace elements (e.g., zinc, iron, copper, selenium) that are required in small quantities (Martínez-Ballesta et al., 2010, Tekin et al., 2018). Deficiencies in these nutrients are associated with chronic diseases, and common problems such as iron and zinc deficiency affect more than one-third of the world's population, especially in developing regions (WHO (World Health Organisation), 2009; Alloway, 2008). Wheat, a staple crop consumed by 2.5 billion people worldwide, is an important source of minerals and is a key target for addressing mineral deficiencies through improved agricultural practices and biofortification (Listman & Ordóez, 2019; Pfeiffer & McClafferty, 2007). Grain mineral concentrations are influenced by genetic factors (e.g., variety selection) and environmental factors (including soil type, climate, and management practices) (Lopez et al., 2003). Modern wheat varieties are generally lower in mineral and vitamin content compared to ancient varieties such as Einkorn and Emmer, which are rich in micronutrients such as zinc, iron, and B vitamins (Watanabe, 2017; Abdel-Aal & Hucl, 2014). Efforts to increase mineral content, such as biofortification, offer a sustainable solution to combat hidden hunger” and improve food security (Krishnappa et al., 2022). However, the bioavailability of these minerals is severely limited by phytic acid, a phosphorus storage compound found primarily in the aleurone layer of the wheat kernel (Welch, 1986). Phytic acid binds tightly to essential minerals such as calcium, zinc, and iron, forming insoluble complexes that are not easily absorbed by the human digestive system (Lönnerdal et al., 1989). This anti-nutritional effect is particularly problematic in regions where wheat is a major dietary component and mineral deficiencies are prevalent (WHO, 2009). Breeding strategies aimed at reducing phytic acid content while maintaining high mineral density have emerged as a promising approach to address these challenges (Raboy et al., 2000; Iqbal et al., 2024).

HTs during key growth stages, such as grain filling, significantly affect wheat yield and its mineral content by shortening the grain filling period, reducing starch accumulation, and decreasing grain size (Zahra et al., 2021). The combination of rising global temperatures and extreme heat waves, especially in key wheat-growing regions, exacerbates these effects, leading to reduced wheat yield and lower mineral concentrations (Velu et al., 2016). Environmental stress, especially HTs, affects the genetic regulation of mineral accumulation in wheat, highlighting the importance of genotype-environment interactions for maintaining a stable nutritional status under such conditions (Samineni et al., 2022).

Phytate, the major phosphorus storage compound in wheat, plays a key role in regulating mineral bioavailability and its accumulation in the grain. Higher phytate levels can compete with minerals such as zinc, thereby reducing their bioavailability (Sinclair & Krämer, 2012). Environmental factors further complicate this relationship, as phytate concentrations and mineral accumulation vary significantly across genotypes and environmental conditions (Guttieri et al., 2006; Peterson et al., 1986). Understanding how genotype-environment interactions affect mineral content and phytate levels under HT is essential for breeding wheat cultivars with higher nutritional quality. Strategies that address this balance, such as breeding cultivars with reduced phytate levels while maintaining mineral stability, could provide avenues to mitigate the adverse effects of HTs on wheat grain nutrition (Velu et al., 2014; Samineni et al., 2022).

Comparative studies on the mineral content of wheat grown under different temperatures, in varying locations with distinct soil types, and across diverse genotypes remain limited. The hypothesis is that harvest year, sowing time, planting location, and wheat genotype would influence wheat yield, phytate content, and mineral composition. It was further hypothesised that the hot conditions in Merredin, combined with its soil characteristics (predominantly sandy and stony), would exert a more pronounced impact on yield, mineral content, and phytate levels, particularly in wheat cultivars with lower heat tolerance and when sown later in the season.

The objective of this study is to evaluate the changes in yield, phytate content, and mineral composition of two wheat cultivars, Berkut and Sokoll, grown in three regions, Horsham (VIC), Narrabri (NSW), and Merredin (WA), during a hot year (2019) (Experiment 1). Additionally, the study investigates three other wheat cultivars, Flanker, Cobra, and Suntop, cultivated in Narrabri over two years (2019 and 2020) (Experiment 2).

6.2. Materials and Methods

We based our experimental design on the field experiments as described on Chapter 3 (General Materials and Methods), thereby ensuring uniformity in sample collection, preparation, and analysis. Detailed descriptions of the field experiments can be found in Sections 3.1, 3.2 and 3.3.

6.2.1. Mineral

Mineral analysis was conducted using inductively coupled plasma optical emission spectrometry (ICP-OES). Approximately 0.5 g of the sample was precisely measured and placed into a clean Teflon microwave digestion vessel. To this, 5 mL of concentrated analytical-grade nitric acid was added, and the mixture was left to react for 10 minutes. The vessel was sealed, and digestion was performed using a MAR S6 microwave digestion system (CEM Co., Charlotte, NC, USA) by heating the sample to 180℃ for 20 minutes. Once cooled to room temperature, the vessel was carefully opened, and the contents were transferred to a 50 mL volumetric flask, where the solution was diluted to the required volume with deionised water. The prepared solution was subsequently analysed using ICP-OES (Khoddami et al., 2024).

6.2.2. Phytate

Phytate was extracted from 1 g of milled wheat flour by placing the sample in a labeled SARSTEDT screw-cap tube, adding 20 mL of 3% trichloroacetic acid (TCA), and shaking at medium speed for 1.5 h. The resulting suspension was centrifuged at 12,000 rpm for 15 min at room temperature, and the supernatant was transferred to a separate tube, where chelex was introduced and allowed to sit for 30 min with occasional stirring. An aliquot (2 mL) of this mixture was then filtered through a 0.2 μm membrane filter, discarding the first 1 mL and collected in a labeled vial. Phytate levels were determined by ion-exchange high-performance liquid chromatography (HPLC) using a Hamilton PRP-100 column with a mobile phase consisting of 0.20 M $NaNO_3$ that was adjusted to pH 3.0 with nitric acid, and detection at 500 nm with ferric-sulphosalicylic acid. The final phytate concentration was reported as grams of phytic acid phosphorus per 100 g of whole grain flour (g/100 g) (Khoddami et al., 2024).

6.3. Results

6.3.1. Experiment 1

Interactions were observed between location, time of sowing, and cultivar for yield, thousand kernel weight (TKW), phytate, Ca, Fe, K, Mg, Mn, Na, Zn, Cu, and kernel color values ($L*$, $a*$, $b*$) ($p < 0.05$; Table 6.1). Sokoll exhibited lower yields at all three locations for TOS2 compared to TOS1 (Figure 6.1a). Similarly, Berkut showed a significant yield reduction at Merredin under TOS2 compared to TOS1 ($p < 0.001$). TKW of Berkut was consistently higher than Sokoll across all locations and sowing dates, with the largest differences observed in Merredin (Figure 6.1(b)). Phytate content was reduced under TOS2 ($p = 0.002$) compared with TOS1, with the highest levels recorded in Narrabri and the lowest in Merredin. Berkut in TOS1 at Narrabri exhibited the highest phytate content (0.38 g/100 g), whereas Berkut in TOS2 at Merredin had the lowest (0.14 g/100 g; Figure 6.1(c)).

In general, the contents of Ca, Fe, K, Mg, Mn, Na, Zn, and Cu were higher in TOS2 than in TOS1. Ca levels peaked in Narrabri and were lowest in Merredin, with Sokoll in TOS2 at Narrabri recording the highest Ca content (606.9 mg/kg) and Sokoll in TOS1 at Merredin the lowest (379.9 mg/kg; $p < 0.001$; Figure 6.1(d)). Fe concentrations were highest in Narrabri and lowest in Horsham, with Sokoll in TOS2 at Narrabri showing the highest Fe content (40.6 mg/kg) and Berkut in TOS2 at Horsham the lowest (24.1 mg/kg; $p < 0.001$; Figure 6.1(e)). K content was highest in Horsham and lowest in Merredin, with Berkut in TOS1 at Horsham recording the maximum (3351 mg/kg) and Sokoll in TOS1 at Merredin the minimum (2087 mg/kg; $p = 0.021$; Figure 6(f)). Mg content was highest in Narrabri and lowest in Merredin, with Sokoll in TOS2 at Narrabri showing the maximum (1206 mg/kg) and Berkut in TOS2 at Merredin the minimum (787 mg/kg; $p = 0.006$; Figure 6.1(g)). Mn levels were highest in Narrabri and lowest in Horsham, with Berkut in TOS2 at Narrabri recording the maximum (56.68 mg/kg) and Sokoll in TOS2 at Horsham the minimum (30.30 mg/kg; $p < 0.001$; Figure 6.1(h)). Na concentrations peaked in Horsham, with Berkut in TOS2 at Horsham showing the highest levels (220.20 mg/kg), while Sokoll in TOS1 at Narrabri recorded the lowest (88.3 mg/kg; $p < 0.001$; Figure 6.1(i)). Zn content was highest in Narrabri and lowest in Merredin. The highest Zn content was in Berkut in TOS2 at Narrabri (28.40 mg/kg), whereas Sokoll in TOS1 at Merredin had the lowest (11.85 mg/kg; $p < 0.001$; Figure 6.1(j)). Cu levels were highest in Merredin and lowest in Horsham, with Berkut in TOS2 at Merredin exhibiting the maximum (8.55 mg/kg) and Berkut in TOS1 at Merredin the minimum (4.70 mg/kg; $p = 0.026$; Figure 6.1k). Kernel colour values also varied across treatments. Compared with TOS1, the $L*$ values for both Berkut and Sokoll increased in TOS2, except for Berkut in Narrabri and Sokoll in Merredin (Figure 6.1(l)). The highest $a*$ value for Berkut during TOS1 was recorded in Horsham, whereas the lowest for Sokoll in TOS2 occurred in Merredin (Figure 6.1(m)). Sokoll consistently exhibited higher $b*$ values than Berkut across all treatments (Figure 6.1(n)).

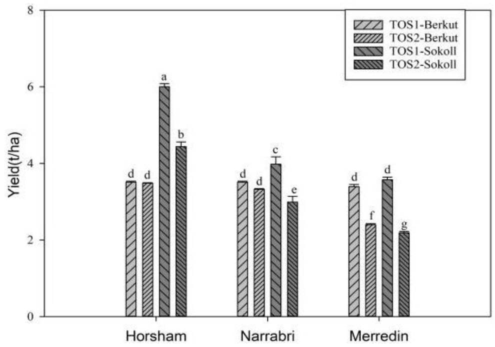
Correlations among variables provided additional insights (Table 6.2). Yield was positively correlated with TKW ($r = 0.290$, $p = 0.045$) and negatively correlated with Ca ($r = -0.468$, $p = 0.001$) and Cu ($r = -0.341$, $p = 0.018$). TKW showed negatively correlated with Fe ($r = -0.308$, $p = 0.033$), Cu ($r = -0.353$, $p = 0.014$), and $b*$ values ($r = -0.724$, $p < 0.001$). Ca and K were positively correlated with Cu ($r = 0.357$, $p = 0.013$; $r = 0.361$, $p = 0.012$). Fe showed positive correlations with K ($r = 0.597$, $p < 0.001$), Mn ($r = 0.725$, $p < 0.001$), Na ($r = 0.319$, $p = 0.027$), and Zn ($r = 0.733$, $p < 0.001$). Mn was positively correlated with Na ($r = 0.379$, $p = 0.008$), Zn ($r = 0.848$, $p < 0.001$), and Cu ($r = 0.884$, $p < 0.001$), while negatively correlated with P ($r = -0.552$, $p < 0.001$). Zn showed positive correlations with Cu ($r = 0.847$, $p < 0.001$).

6.3.2. Experiment 2

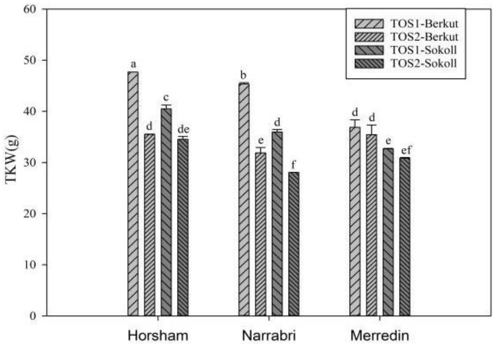
Interactions between sowing period and cultivar were significant for K, Mg, Zn and $a*$ value for Cobra, Flanker, and Suntop at Narrabri in 2019 ($p < 0.05$; Table 6.3).

In 2019, wheat yield (4.46 t/ha; $p < 0.001$) and TKW (36.72 g; $p = 0.011$) were significantly higher under TOS1 compared with TOS2 (3.48 t/ha and 27.18 g, respectively; Figure 6.2). TOS2 exhibited higher levels of K ($p = 0.001$), Mg ($p = 0.036$), and Zn ($p = 0.001$) than TOS1, with significant differences driven by the interaction of cultivar and TOS. Cobra under TOS2 had the highest K and Mg contents (3163 and 1379 mg/kg), while Suntop under TOS1 had the lowest (2181 and 988 mg/kg; Figures 6.3(a), (b)). Similarly, Cobra under TOS2 exhibited the highest Zn content (31.23 mg/kg), whereas Flanker under TOS1 had the lowest (19.55 mg/kg; Figure 6.3(c)). The $a*$ values of all three wheat cultivars increased in TOS2 compared to TOS1 ($p = 0.01$; Figure 6.3(d)).

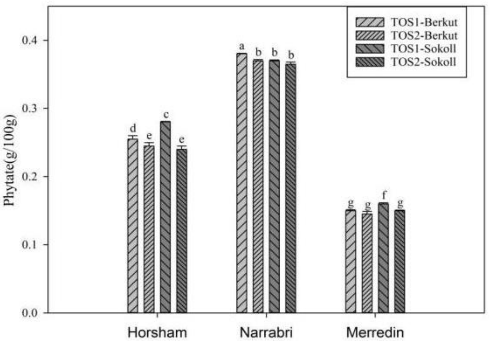
a


Yield(t/ha)
0
2
4
6
8
TOS1-Berkut
TOS2-Berkut
TOS1-Sokoll
TOS2-Sokoll
d
d
a
b
d
d
c
e
d
f
d
g
Horsham
Narrabri
Merredin

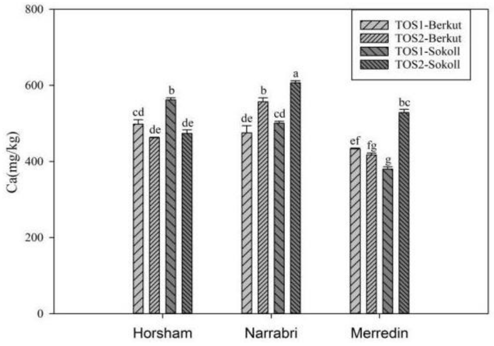
b


TKW(g)
0
10
20
30
40
50
60
TOS1-Berkut
TOS2-Berkut
TOS1-Sokoll
TOS2-Sokoll
a
d
c
de
b
e
d
f
d
d
e
ef
Horsham
Narrabri
Merredin

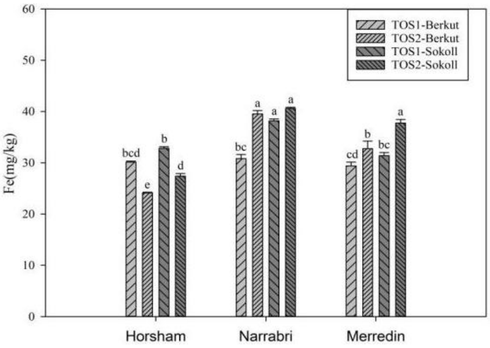
c


Phytate(g/100g)
0.0
0.1
0.2
0.3
0.4
TOS1-Berkut
TOS2-Berkut
TOS1-Sokoll
TOS2-Sokoll
d
e
c
e
a
b
b
b
g
g
f
g
Horsham
Narrabri
Merredin

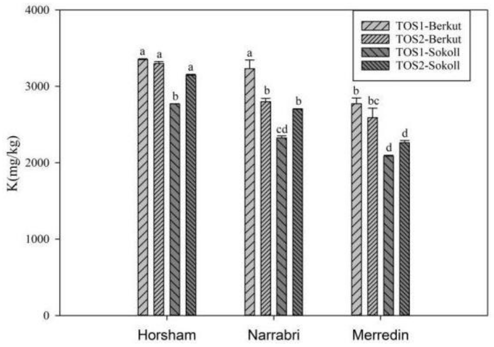
d


Ca(mg/kg)
0
200
400
600
800
TOS1-Berkut
TOS2-Berkut
TOS1-Sokoll
TOS2-Sokoll
cd
de
b
de
de
b
cd
a
ef
fg
g
bc
Horsham
Narrabri
Merredin

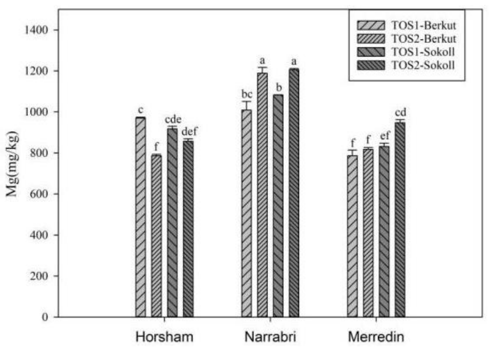
e


Fe(mg/kg)
0
10
20
30
40
50
60
TOS1-Berkut
TOS2-Berkut
TOS1-Sokoll
TOS2-Sokoll
bcd
e
b
d
bc
a
a
a
cd
b
bc
a
Horsham
Narrabri
Merredin

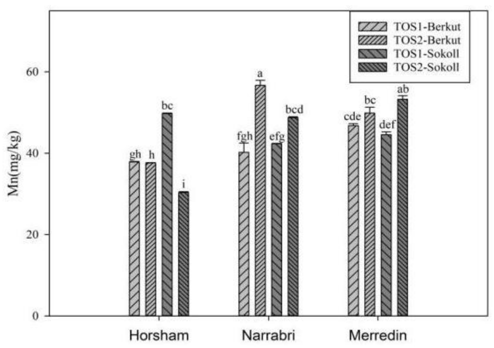
f


K(mg/kg)
0
1000
2000
3000
4000
TOS1-Berkut
TOS2-Berkut
TOS1-Sokoll
TOS2-Sokoll
a
a
b
a
a
b
cd
b
b
bc
d
d
Horsham
Narrabri
Merredin

g


Mg(mg/kg)
0
200
400
600
800
1000
1200
1400
TOS1-Berkut
TOS2-Berkut
TOS1-Sokoll
TOS2-Sokoll
c
f
cde
def
bc
a
b
a
f
f
ef
cd
Horsham
Narrabri
Merredin

h


Mn(mg/kg)
0
20
40
60
TOS1-Berkut
TOS2-Berkut
TOS1-Sokoll
TOS2-Sokoll
gh
h
bc
i
fgh
a
efg
bcd
cde
bc
def
ab
Horsham
Narrabri
Merredin

i

j

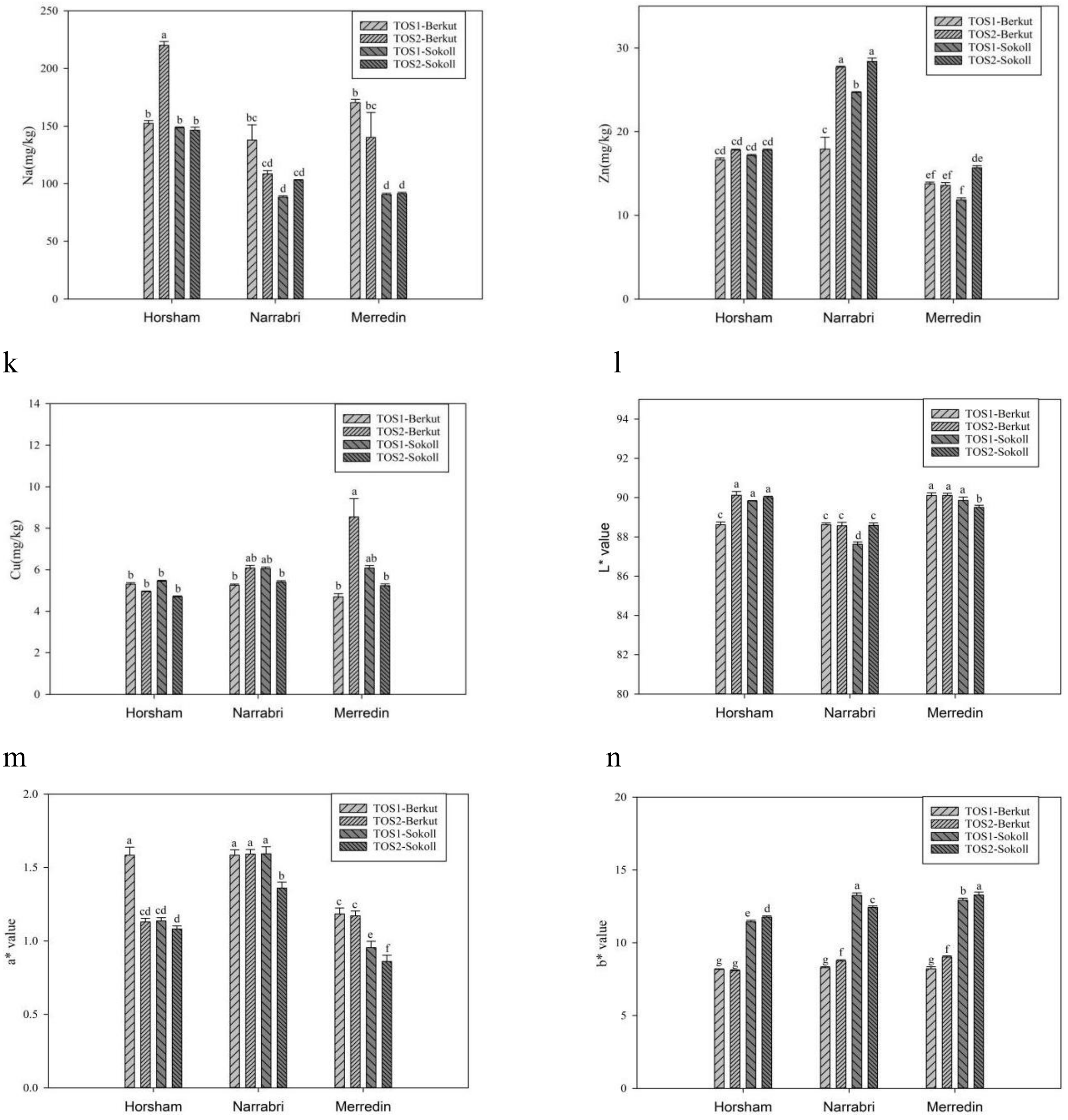


Figure 6.1. Effect of locations (Horsham, Narrabri and Merredin), sowing dates (TOS1 and TOS2) and cultivars (Berkut and Sokoll) on (a) yield (t/ha), (b) TKW (g), (c) phytate (g/100g), (d) Ca (mg/kg), (e) Fe (mg/kg), (f) K (mg/kg), (g) Mg (mg/kg), (h) Mn (mg/kg), (i) Na (mg/kg), (j) Zn (mg/kg), (k) Cu (mg/kg), (l) *L value, (m) *a** value and (n) *b** value in 2019. Means followed by the same letters are not different at $p<0.05$ for location×sowing period×cultivar interaction. Error bars represent standard error of the mean (n=4).**

Table 6.1. The main effects and interactions of locations (Horsham, Narrabri, and Merredin), time of sowing (TOS1 and TOS2) and cultivar (Berkut and Sokoll) on wheat yield, minerals, phytate, kernel colour and maturity days in 2019, with corresponding p-values.

Effect	**Loc (Location)**	**TOS**	**Cul (Cultivar)**	**Loc×TOS**	**Loc×Cul**	**TOS×Cul**	**Loc×TOS×Cul**
Yield	<.001	<.001	<.001	<.001	<.001	<.001	<.001
TKW	<.001	<.001	<.001	<.001	NS	<.001	0.011
Phytate	<.001	<.001	NS	0.001	0.002	0.01	0.002
Ca	<.001	<.001	<.001	<.001	NS	<.001	<.001
Fe	<.001	<.001	<.001	<.001	NS	0.231	<.001
K	<.001	NS	<.001	NS	NS	<.001	0.021
Mg	<.001	0.004	<.001	<.001	0.025	0.029	0.006
Mn	<.001	<.001	NS	<.001	0.001	<.001	<.001
Na	<.001	NS	<.001	<.001	0.005	NS	<.001
P	<.001	NS	NS	<.001	NS	0.024	NS
Zn	<.001	<.001	<.001	<.001	<.001	0.105	<.001
Cu	0.04	NS	NS	0.038	NS	0.002	0.026
L* **Value**	<.001	<.001	NS	<.001	<.001	NS	<.001
a* **Value**	<.001	<.001	<.001	<.001	<.001	NS	<.001
b* **Value**	<.001	0.003	<.001	<.001	<.001	<.001	<.001
Days from filling to maturity	<.001	<.001	NS	<.001	NS	NS	NS
Days from sowing to maturity	<.001	<.001	NS	<.001	0.019	NS	NS

12 Treatments =3 locations × 2 cultivars × 2 sowings; number of replications = 4; NS = p>0.05.

Table 6.2. The pairwise correlations between wheat yield, minerals, phytate, kernel colour and maturity days by Berkut and Sokoll in three locations in 2019.

		Yield	**TKW**	**Ca**	**Fe**	**K**	**Mg**	**Mn**	**Na**	**P**	**Zn**	**Phytate**	**Cu**	L*	a*	b*	**Grain filling to maturity**	**Sowing to maturity**
Yield	*r*=	1																
	p=																	
TKW	*r*=	0.290	1															
	p=	0.045																
Ca	*r*=	-0.468	NS	1														
	p=	0.001																
Fe	*r*=	NS	-0.308	NS	1													
	p=		0.033															
K	*r*=	NS	NS	0.357	0.597	1												
	p=			0.013	<0.001													
Mg	*r*=	NS	NS	NS	NS	-0.560	1											
	p=					<0.001												
Mn	*r*=	NS	NS	NS	0.725	0.806	NS	1										
	p=				<0.001	<0.001												

Na	*r*=	NS	NS	NS	0.319	0.708	-0.568	0.379	1								
	p=				0.027	<0.001	<0.001	0.008									
P	*r*=	NS	NS	NS	NS	-0.801	0.750	-0.552	-0.458	1							
	p=					<0.001	<0.001	<0.001	0.001								
Zn	*r*=	NS	NS	NS	0.733	0.443	0.348	0.848	NS	NS	1						
	p=				<0.001	0.002	0.015	<0.001									
Cu	*r*=	-0.341	-0.353	0.361	0.725	0.632	NS	0.884	NS	-0.307	0.847	1					
	p=	0.018	0.014	0.012	<0.001	<0.001		<0.001		0.034	<0.001						
Phytate	*r*=	NS	NS	NS	NS	0.306	-0.365	NS	0.346	-0.367	NS	NS	1				
	p=					0.034	0.011		0.016	0.010							
L*	*r*=	NS	NS	NS	NS	NS	NS	NS	NS	NS	-0.377	NS	NS	1			
	p=										0.008						
a*	*r*=	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.387	NS	NS	-0.769	1		
	p=										0.007			<0.001			
b*	*r*=	NS	-0.724	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-0.373	1	
	p=		<0.001												0.009		
Grain filling to maturity	*r*=	NS	0.334	NS	NS	NS	0.287	NS	NS	NS	0.303	NS	NS	-0.534	0.719	-0.432	1
	p=		0.020				0.048				0.036			<0.001	<0.001	0.002	

Sowing to maturity	*r*=		0.335											-0.473	0.745	-0.601	0.935	1
		NS		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS					
	p=		0.020											<0.001	<0.001	<0.001	<0.001	

6 Treatments =3 cultivars × 2 sowings; number of replications = 4; NS = non-significant

Table 6.3. The main effects and interactions of year, time of sowing and cultivar on wheat yield, minerals, phytate, kernel colour and maturity days by Cobra, Flanker and Suntop in Narrabri in 2019, with corresponding p value.

Effect	**TOS**	**Cultivar**	**TOS×Cultivar**
Yield	0.027	NS	NS
TKW	0.001	NS	NS
Phytate	0.037	0.006	NS
Ca	<.001	0.001	NS
Fe	<.001	<.001	NS
K	<.001	<.001	0.001
Mg	<.001	<.001	0.036
Mn	<.001	<.001	NS
Na	<.001	<.001	NS
P	<.001	<.001	NS
Zn	<.001	<.001	0.001
Cu	0.002	<.001	NS
***L** Value**	<.001	<.001	NS
***a** Value**	<.001	<.001	0.01
***b** Value**	<.001	<.001	NS
Days from filling to maturity	<.001	0.02	NS
Days from sowing to maturity	<.001	NS	NS

6 Treatments =3 cultivars × 2 sowings; number of replications = 4; NS = p>0.05

a b

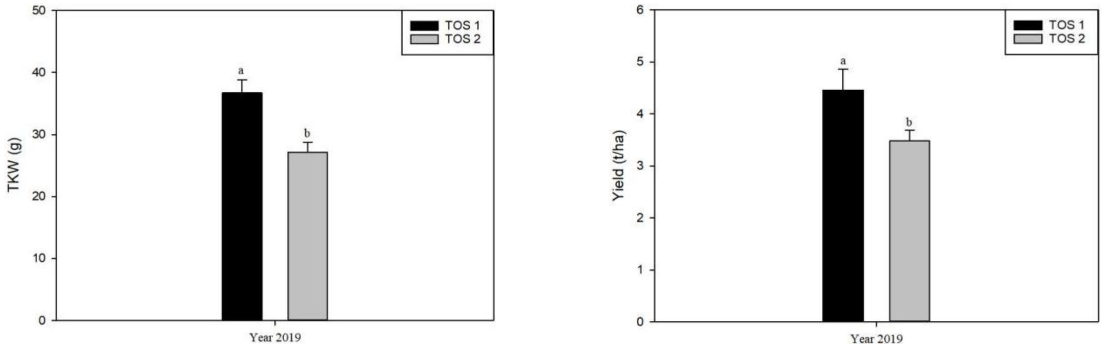


Figure 6.2. Effect of sowing dates (TOS1 and TOS2) on (a) yield (t/ha) and (b) TKW (g) in Narrabri in 2019. Means followed by the same letters are not significantly different at p<0.05 for sowing date. Error bars represent standard error of the mean (n=4).

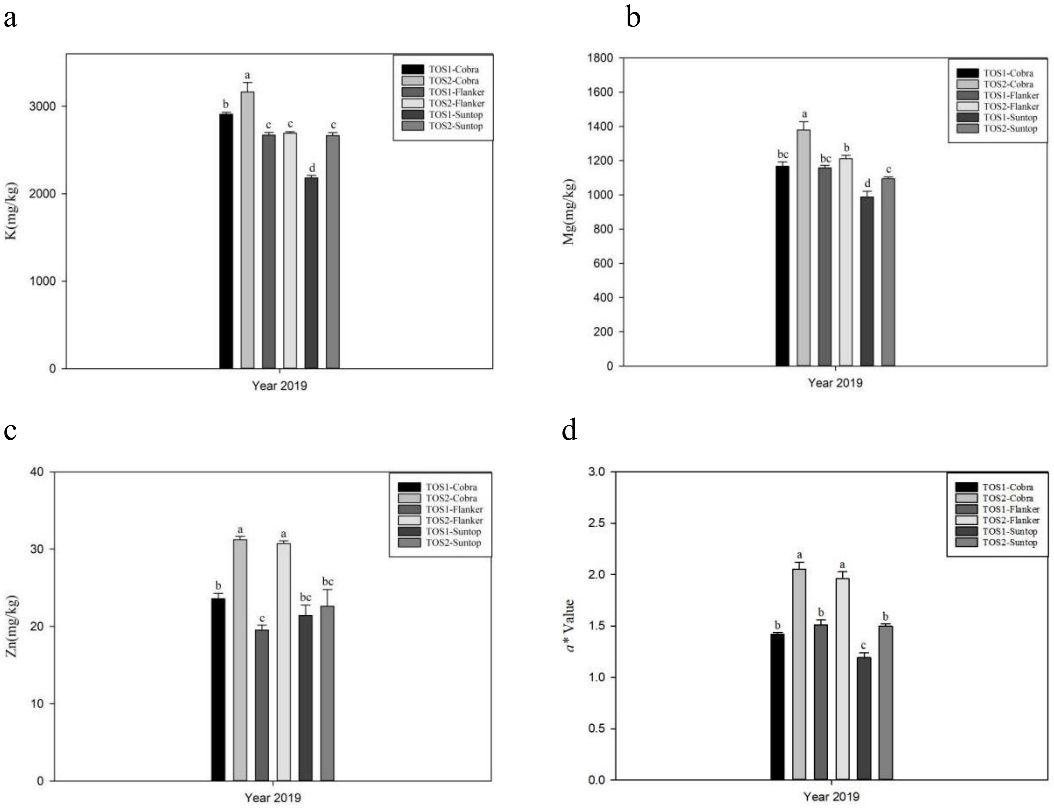


Figure 6.3. Effect of sowing dates (TOS1 and TOS2) and cultivar (Cobra, Flanker and Suntop) on (a) K (mg/kg), (b) Mg (mg/kg), (c) Zn (mg/kg) and (d) *a value in Narrabri in 2019. Means followed by the same letters are not significantly different at p<0.05 for sowing period × cultivar interaction. Error bars represent the standard error of the mean (n=4)**

6.4. Discussion

6.4.1. Impact of Sowing Time and Temperature on Yield and Phytate Content

The findings of this study highlight the significant influence of environmental conditions, particularly sowing time and HTs, on wheat yield and phytate content. Across both experiments, yields were consistently higher in TOS1 compared with TOS2, with the most pronounced reductions in Merredin, a location characterised by its hot climate and sandy soils. This yield reduction aligns with previous studies suggesting that elevated temperatures during critical growth stages disrupt nutrient uptake, shorten the grain filling period, and impair starch accumulation (Dias & Lidon, 2009b; Farooq et al., 2011). For instance, Sokoll exhibited a notable yield decline under TOS2 in Merredin, indicating a greater susceptibility to HT compared to Berkut, which displayed higher yields across all locations. These data support the hypothesis that genotype-specific heat tolerance plays a pivotal role in mitigating yield losses under adverse conditions.

The observed reduction in phytate content under TOS2, particularly in Merredin, is consistent with reports that HTs impair phosphorus metabolism and enzymatic pathways regulating phytate synthesis (Prasad & Djanaguiraman, 2014). Phytate levels in this study were highest in Narrabri and lowest in Merredin, with Berkut in TOS1 at Narrabri showing the maximum phytate content (0.38 g/100 g). This variation highlights the dual role of environmental factors, such as soil phosphorus availability and temperature, in modulating phytate accumulation. Notably, lower phytate levels under high-temperature conditions may enhance mineral bioavailability, but they also coincide with yield reductions, posing a trade-off between productivity and nutritional quality (Velu et al., 2014). These findings emphasise the importance of optimising sowing times to balance yield and nutritional outcomes, particularly in heat-prone regions.

6.4.2. Genotypic Variation in Mineral Composition and Phytate Content

Genotypic differences significantly affected mineral composition and phytate content across locations and sowing times, highlighting the importance of genetic factors in determining the nutritional profile of wheat. For example, Berkut consistently had higher Ca, K, and Mg contents compared with Sokoll, which showed higher Zn and Fe concentrations under TOS2 in Narrabri. These data confirm earlier findings that genotype-environment interactions are key determinants of mineral accumulation, with high Zn genotypes maintaining their ranking across environments due to their moderately high heritability (Velu et al., 2017).

The increase in Zn concentrations in TOS2, especially in Narrabri, may be attributed to heat-induced remobilization of Zn from shoots to grains (Liu et al., 2011). For example, in Narrabri, Cobra had the highest Zn content (31.23 mg/kg) under TOS2, indicating that the response to HTs is genotype-specific. However, yields decreased simultaneously, highlighting the complex interactions between nutrient transport and productivity under HT. These findings are consistent with the hypothesis that HTs disrupt nutrient transport processes, particularly in sandy soils with low cation exchange capacity, as observed in Merredin (Peck & McDonald, 2010).

Phytate levels showed clear genotype-specific differences, with Berkut having higher phytate levels than Sokoll at most locations and sowing times. This variation may be related to differences in P metabolism, as genotypes with higher phytate levels tend to show lower Zn bioavailability due to competitive binding (Sinclair & Krämer, 2012). The interaction between phytate and mineral content highlights the need for breeding strategies that reduce phytate levels while maintaining or increasing mineral concentrations to improve nutritional outcomes. For example, breeding low-phytate wheat cultivars while stabilising Zn and Fe content could address “hidden hunger” by improving mineral bioavailability, particularly in regions where wheat is the main crop (Velu et al., 2014).

6.4.3. Influence of Soil Properties and Location

Soil properties, especially pH, OM and texture, have a significant impact on the mineral composition and phytic acid content of wheat kernels. The differences in mineral content observed at the three experimental sites (Horsham, Narrabri and Meridian) can be attributed to the unique physical and chemical properties of their soils, as well as environmental conditions such as temperature and genotype-specific responses.

Soil pH is a key factor in determining mineral solubility and availability. Alkaline soils (pH 8.65) at Horsham and Narrabri reduce the bioavailability of key micronutrients such as Zn, Fe and Mn due to the formation of insoluble hydroxides and carbonates (Kabata-Pendias, 2004; Brallier et al., 1996). In contrast, the slightly acidic soils (pH 5.45) at Merredin enhanced the solubility of these minerals but also presented challenges associated with aluminum (Al) toxicity, which impairs root elongation and nutrient uptake, ultimately reducing plant uptake (Foy, 1984; Zheng, 2010). This dual effect of soil pH was evident in the mineral profile of wheat kernels, with Zn and Fe concentrations lower in Horsham-grown wheat than in Merredin-grown wheat despite HT at Merredin (Figure 6.1(e) (j)).

Soil texture and OM content play a crucial role in determining cation exchange capacity (CEC), which influences nutrient retention and availability. Clay soils at Horsham have a higher OM content (1.95%) and a higher CEC, which favours better retention and uptake of potassium K, Mg and Ca, resulting in higher mineral content in grain compared to sandy and stony soils at Merredin (Eriksson Jan et al., 2017; Manzeke et al., 2019). Low OM content (1.65%) and limited plant available water capacity (PAWC) (132.9 mm/m) at Merredin may have exacerbated nutrient leaching and reduced mineral mobilisation to grain, particularly Mn, Zn and Mg (Figure 6.1 (h) (j)(g)). Clay soils at Narrabri show moderate mineral retention, with Mg and Zn concentrations peaking under late sowing conditions due to enhanced remobilisation under stress (Fernando et al., 2014; Yasmeen et al., 2017).

Phytic acid is the major form of organic phosphorus (Po) in wheat grains and is strongly influenced by soil properties, including pH, clay content, and OM. The high phytic acid levels observed in Narrabri wheat can be attributed to soil P availability (66.5 mg/kg) and phytic acid adsorption onto clay and OM, which is enhanced under alkaline conditions (Yan et al., 2014; Turner et al., 2007). In contrast, the lowest phytic acid levels were found in Merredin wheat, reflecting the combined effects of low P availability (42 mg/kg) and heat-induced downregulation of phosphate transporters and biosynthetic enzymes, as previously reported under HT conditions (Högy et al., 2009; Hamilton et al., 2008). While reduced phytic acid content in wheat grown in Merredin may improve the bioavailability of the mineral, it also poses challenges for P storage and seedling development during subsequent growth cycles (Celi & Barberis, 2005; Gerke, 2010).

Genotype responses to soil properties were evident at all sites. Heat-tolerant cultivars such as Berkut maintained high mineral concentrations under the harsh conditions of Merredin, whereas Sokoll showed significant decreases under the same conditions, especially for Zn and Fe (Figure 6.1(e) (j)). This highlights the importance of selecting genotypes that can adapt to specific soil and environmental conditions to optimise yield and nutritional quality. For example, Berkut's adaptability to low P availability and HT in Merredin highlights its potential for cultivation in marginal soils with limited nutrient retention (Velu et al., 2017; Sinclair & Krämer, 2012).

6.4.4. Implications for Agronomic and Nutritional Outcomes

The interactions between soil properties, environmental stress, and genotype underscore the need for integrated management strategies to enhance wheat nutritional quality under diverse conditions. In regions like Merredin, improving soil OM through amendments such as compost or biochar could enhance nutrient retention and mitigate the adverse effects of sandy soils. Additionally, breeding programs should prioritise genotypes with stable mineral accumulation and reduced phytate levels to address the dual challenges of mineral deficiency and HT (Rakszegi et al., 2019; Tricker et al., 2018).

These findings also emphasise the importance of location-specific soil management practices and genotype selection to optimise wheat productivity and nutritional quality. Future research should focus on molecular mechanisms of nutrient uptake and remobilisation under varying soil and climatic conditions to inform breeding and agronomic strategies for climate-resilient wheat production (Tyler & Olsson, 2001; Samineni et al., 2022).

Notably, this study is the first to integrate a multi-environment approach with correlation analyses to elucidate the synergistic effects of high-temperature stress, sowing time, and soil properties on wheat mineral composition and phytate content. By systematically linking genotype-specific responses with nutrient remobilisation under high temperature stress, this work provides novel mechanistic insights that can inform breeding strategies for climate-resilient wheat, addressing both yield and nutritional quality.

6.5. Conclusion

This study highlights the intricate interactions between genotype, environmental conditions, and soil properties in shaping wheat yield, phytate content, and mineral composition. HTs and late sowing (TOS2) reduced yield but increased the concentrations of essential minerals such as zinc (Zn) and iron (Fe), with heat-tolerant genotypes like Berkut and Cobra demonstrating superior adaptability under stress. Soil characteristics, including pH, OM, and texture, significantly influenced mineral bioavailability and phytate accumulation, with alkaline soils limiting Zn and Fe availability and sandy soils exacerbating nutrient leaching. Merredin, characterised by HTs and poor soil conditions, exhibited lower phytate levels, improving mineral bioavailability at the cost of reduced yield and phosphorus storage. These findings emphasise the importance of breeding strategies that focus on developing low-phytate wheat genotypes with stable mineral profiles to enhance bioavailability and mitigate mineral deficiencies. Additionally, targeted soil management practices, such as the application of organic amendments, are crucial for optimising nutrient retention and improving wheat nutritional quality under diverse environmental conditions. Importantly, this study represents the first analysis to integrate multi-environment experiments with correlation analyses, revealing the synergistic impacts of HT, sowing time, and soil properties on wheat nutrient dynamics. The novel insights derived from this work provide a mechanistic basis for developing climate resilient wheat cultivars that can improve both yield and nutritional quality, thereby contributing to enhanced food security and nutritional outcomes in a warming climate.

Chapter 7. Changes in lipids and pollen viability under HT in wheat

Abstract

This study evaluated the effects of high temperature (HT) stress on pollen viability and lipid composition in wheat (*Triticum aestivum*), focusing on genotype-specific responses across four cultivars (Cobra, Flanker, Sunchaser, and Suntop). Wheat plants were exposed to temperature treatments of 22/15℃ (control), 35/22℃, and 40/22℃ (day/night) during critical reproductive stages. There were significant reductions in pollen viability and changes in lipid composition under high temperatures. The heat-sensitive cultivar Cobra exhibited pronounced decreases in both saturated and unsaturated fatty acids, resulting in compromised lipid composition and reduced pollen viability. In contrast, Suntop demonstrated enhanced thermotolerance, maintaining relatively stable levels of unsaturated fatty acids (UFA) and a balanced saturated-to-unsaturated fatty acid (SFA/UFA) ratio. Correlation analyses revealed strong associations among individual fatty acids, indicating that wheat thermotolerance depends primarily on coordinated changes in lipids rather than isolated fatty acid changes. The stability of lipid composition, particularly higher levels of UFAs under HT, was linked to reduced lipid peroxidation, improved membrane integrity, and higher pollen viability. These findings highlight the importance of coordinated changes in lipids in response to heat stress.

Keywords: Wheat cultivars, Pollen viability, Coordinated changes in lipids

7.1. Introduction

The global increase in carbon dioxide emissions, driven by the rising use of fossil fuels, deforestation, and burning, has led to a steady rise in global temperatures (Abrahamson, 1989). This warming not only causes the melting of polar ice caps and rising sea levels but also threatens agricultural productivity, particularly in key crops such as wheat. Projections indicate that by the end of the 21st Century, global temperatures could increase by 1–6°C (De Costa, 2011), significantly affecting crop yields and quality. Wheat, a staple food and one of the most widely cultivated cereals worldwide, is particularly vulnerable to HT. Since 1960, global cereal production has more than tripled, with wheat seeing substantial growth. However, HT, especially during critical developmental stages, can severely reduce yields. For every 1°C increase in global temperatures, wheat production could decrease by approximately 6.0 ± 2.9% (Zhao et al., 2017).

HT adversely impacts wheat growth by accelerating leaf senescence, reducing photosynthetic efficiency, and promoting the premature degradation of cellular components (Kosova et al., 2011). Wheat seed germination, plant growth, and maturation can also be severely affected by elevated temperatures. For instance, temperatures exceeding 45°C hinder seed germination and emergence, while temperatures between 28–30°C shorten the germination and maturation periods (Essemine et al., 2010; Yamamoto et al., 2008). In addition, HTs exacerbate the respiration rate in wheat, impair mitochondrial function, reduce ATP production, and consequently, compromise seed vigour and quality (Balla et al., 2021).

Among the most heat-sensitive components of wheat are the developing microspores and pollen. HT can lead to significant disruptions in pollen development, with both short-term and long-term high-temperature events negatively affecting pollen viability and function. During meiosis, the earliest stages of heat-induced damage occur, leading to abnormal cell division and the production of diploid and triploid gametes, which can result in male sterility (Pecrix et al., 2011; Rezaei et al., 2010). Furthermore, HTs promote oxidative damage in pollen cells, with reactive oxygen species (ROS) accumulation playing a crucial role in the premature degeneration of tapetum cells. This degeneration disrupts the formation of viable pollen and impairs fertilisation processes, ultimately reducing wheat yield (Mittler, 2017; Sharma et al., 2012). The optimal temperature for wheat pollen and grain filling ranges from 12 to 22°C, and temperatures exceeding 24°C during the reproductive phase can lead to significant yield reductions (Shewry, 2009b).

In addition to the effects on cellular structures, HTs also influence lipid metabolism in wheat. Lipids, particularly membrane lipids, are essential for maintaining cellular integrity under stress conditions. HTs induce changes in lipid composition, increasing the interaction between proteins and lipids, leading to the production of cytotoxic compounds that damage cellular membranes (Krumova et al., 2010). To mitigate the effects of HT, wheat plants adjust their lipid profiles, reducing the unsaturation levels of fatty acids and inhibiting lipid peroxidation (Narayanan et al., 2016a). These adaptive mechanisms, including the modification of fatty acid composition in pollen, help maintain membrane stability during prolonged heat exposure. However, persistent HTs (>30°C) exacerbate lipid peroxidation, causing the breakdown of thylakoid membrane lipids and the accumulation of harmful byproducts like malondialdehyde (Djanaguiraman et al., 2018).

The impact of HT on wheat, particularly its effects on pollen development and lipid metabolism, is complex and varies across different genotypes. Understanding these mechanisms is crucial for developing wheat cultivars that are more resilient to HT and ensuring global food security in the face of climate change. Previous research has demonstrated the heat sensitivity of pollen during its developmental stages, particularly with respect to HTs, as well as the alterations in fatty acid composition under HT. However, there is a lack of comprehensive studies addressing the heat tolerance of lipids in different wheat cultivars.

This chapter examines two key hypotheses: first, that the reduction in wheat pollen viability under high-temperature conditions is associated with alterations in its lipid composition; and second, that the lipid profiles of pollen and leaves vary among wheat genotypes when subjected to HTs (35°C/22°C and 40°C/22°C). The primary objective of this study is to evaluate the effects of HT on the lipid composition of wheat pollen and leaves, investigate the relationship between pollen viability and elucidate the mechanisms underlying HT tolerance in pollen lipids across four distinct wheat cultivars.

7.2. Methods and materials

7.2.1. Experiment design

Wheat plants were grown in a controlled microclimate room at the Plant Breeding Institute (PBI) Cobbitty, maintained at 22°C/15°C day/night temperatures under natural lighting with 60–70% relative humidity. Seeds were sown in 4 L pots filled with a 1:1 peat/sand potting mix, with five seeds per pot and 20 replicate plants per treatment. At planting, a slow-release fertiliser (Osmocote®, NPK - 18:6:12) was applied, supplemented biweekly with liquid fertiliser (Aquasol®, NPK - 23:4:18) at a concentration of 8 g/L. Watering was conducted once daily using an automated sprinkler system (10 min duration, dependent on soil moisture levels). Irrigation was non-limiting and continued even during HT treatments. To mitigate pest infestations, aphids were controlled with a foliar spray of imidacloprid (Confidor®).

Four wheat cultivars were selected for this study: Cobra (heat-susceptible), Flanker (heat-tolerant), Sunchaser (moderately heat-susceptible) and Suntop, based on prior field and greenhouse experiments.

7.2.2. HT Treatment

HT was applied when the plants reached the meiosis stage (auricle distance of 4–8 cm). Plants were exposed to three treatments:

(a) 35/22°C (day/night) for three days,
(b) 40/22°C (day/night) for three days,
(c) ambient control at 22/15°C (day/night) with no heat treatment.

Following heat treatment, plants were returned to their original growing conditions (22°C/15°C day/night) in the microclimate room. Throughout all treatments, water and nutrients were provided to ensure optimal plant growth.

7.2.3. Leaves and Pollen sample collection

At the onset of wheat pollen maturity, both pollen and flag leaves are collected for analysis.

7.2.4. Pollen Viability

Pollen viability was assessed at anthesis (Bokshi et al, 2021). In brief, mature anthers were collected from wheat florets and placed in Eppendorf tubes containing 1 mL of Ampha buffer #6. The samples were mixed using a mechanical crusher for pollen dispersion, then filtered through a 70 µm filter into a 5 mL sample tube. An additional 1 mL of buffer was used to rinse the Eppendorf tube, and the filtrate was added to the sample tube. The prepared pollen-buffer mixture was immediately analysed using an Ampha Z32 cytometer, which distinguished between viable and non-viable pollen.

7.2.5. Hydrolyzed Fatty Acid Measurement in Leaves and Pollen

Fatty acids were extracted from wheat leaf and pollen tissues using acid-catalysed transesterification. This single-step reactive extraction method is faster and more efficient than protocols requiring separate extraction and esterification steps. For leaf samples, 50 mg of tissue, and for pollen samples, approximately 30 mg of anther/pollen (40 anthers), were weighed and placed in Teflon-lined screw-cap glass tubes. An internal standard (C17:0 fatty acid, 25 µg/mL, 10 µL) was added to each tube, followed by 1.0 mL of freshly prepared 2.5% H_2SO_4 in methanol. The tubes were incubated at 70°C for 1.5 h, with occasional shaking. After cooling to room temperature (~5 minutes), 500 µL of hexane and 1.5 mL of 0.9% NaCl solution were added to facilitate phase separation. The tubes were vortexed vigorously and centrifuged at 500 g for 5 minutes. A 50 µL aliquot of the organic phase was transferred to a glass vial with a low-volume insert for gas chromatography-mass spectrometry (GC-MS) analysis (Bokshi et al., 2022).

GC‑MS analyses were performed on a Shimadzu GCMS‑QP2010 system equipped with an Rxi‑5Sil MS capillary column (40 m × 0.25 mm i.d., 0.25 µm film thickness; Restek). Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min under linear velocity control (32.5 cm/s). The injection port was maintained at 220°C and operated in split mode (split ratio 10:1). The column oven temperature was initially set to 120°C and held for 1 min, then ramped at 4°C/min to 260°C, followed by an increase at 30°C/min to 330°C, where it was held for 10 min, resulting in a total run time of 48.33 min. The mass spectrometer was operated in electron ionisation (EI) mode (70 eV), with the ion source and interface temperatures set to 250°C and 280°C, respectively. Data acquisition began at 4.5 min, following a solvent cut time of 4.3 min, and continued until 48.0 min in scan mode (m/z 45‑650). The detector voltage was set to 0.05 kV above the tuning result, and the threshold was fixed at 100.

7.3. Data collection and analysis

7.3.1. Pollen Viability Data:

Pollen viability was determined using the Ampha Z32 cytometer, and the data were recorded using the accompanying software.

7.3.2. Lipid Data:

Fatty acid methyl esters (FAME) concentrations were quantified by correlating peak areas from GC-MS analysis with standard curves generated from the 37-component FAME mix. This relationship was used to convert peak areas into fatty acid concentrations (µg/g) (fresh tissue weight).

7.3.3. Data analysis

The experimental data were subjected to two-way of variance (ANOVA) using Statistix 8.1 and Genstat 22nd Edition to assess the main effects and interactions of factors. Where significant differences were observed ($p<0.05$), post hoc Tukey's Honestly Significant Difference (HSD) tests were applied to identify pairwise comparisons among treatment means. Correlations between components were analysed using Statistix 8.1 software to evaluate the strength and direction of relationships between variables, with correlation coefficients providing insights into potential dependencies.

7.4. Results

In a two-way ANOVA analysis of fatty acids in wheat leaves (Table 7.1), only palmitoleic acid (C16:1) exhibited a significant treatment-by-cultivar interaction (Figure 7.1). Overall, higher temperatures resulted in increased levels of palmitoleic acid, with the highest content observed in Cobra leaves under 40/20°C conditions.

Pollen fatty acids Lauric acid (C12:0), Myristic acid (C14:0), Palmitic acid (C16:0), Palmitoleic acid (C16:1), Stearic acid (C18:0), Arachidic acid (C20:0), total saturated fatty acids (SFA), total unsaturated fatty acids (UFA), saturated/unsaturated fatty acids ratio (SFA/UFA) and total fatty acids exhibited significant treatment-by-cultivar interactions, while fatty acids α-Linolenic acid (C18:3), Behenic acid (C22:0), and Lignoceric acid (C24:0) showed no significant interactions (Table 7.2).

Table 7.1. The main effects and interactions of treatment (22/15 °C, 35/22 °C and 40/22 °C) and cultivar (Cobra, Flanker, Sunchaser and Suntop) on wheat leaves were analysed, with corresponding p-values from the ANOVA.

	Treatment	**Cultivar**	**Treatment × Cultivars**
C12:0 (Lauric acid)	0.001	<.001	NS
C14:0 (Myristic acid)	NS	0.045	NS
C16:0 (Palmitic acid)	0.017	NS	NS
C16:1 (Palmitoleic acid)	NS	NS	0.029
C18:0 (Stearic acid)	0.005	<.001	NS
C18:3 (α-Linolenic acid)	NS	NS	NS
C20:0 (Arachidic acid)	0.03	NS	NS
C22:0 (Behenic acid)	0.014	0.048	NS
C24:0 (Lignoceric acid)	0.023	<.001	NS

12 Treatments =3 treatment × 4 cultivar; number of replications = 4; NS = p>0.05

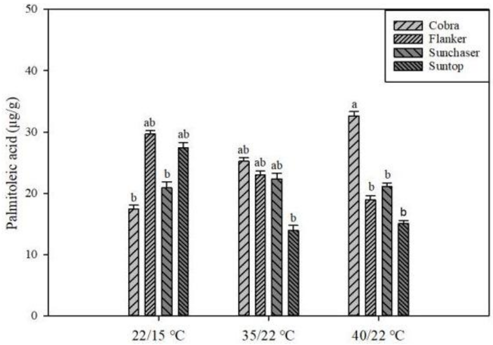


Figure 7.1. Effect of treatment and cultivar on palmitoleic acid (C16:1) for wheat leaves. Means followed by the same letters are not different at p<0.05 for treatment × cultivar interaction. Error bars represent standard error of the mean (n=4)

Table 7.2. The main effects and interactions of treatment (22/15 °C, 35/22 °C and 40/22 °C) and cultivar (Cobra, Flanker, Sunchaser and Suntop) on wheat pollen lipids were analysed, with corresponding p-values.

	Treatment	**Cultivar**	**Treatment × Cultivar**
C12:0 (Lauric acid)	<.001	<.001	0.002
C14:0 (Myristic acid)	<.001	<.001	<.001
C16:0 (Palmitic acid)	<.001	0.001	0.002
C16:1 (Palmitoleic acid)	<.001	<.001	0.011
C18:0 (Stearic acid)	<.001	<.001	0.002
C18:3 (α-Linolenic acid)	<.001	0.019	NS
C20:0 (Arachidic acid)	0.002	<.001	0.048
C22:0 (Behenic acid)	0.018	<.001	NS
C24:0 (Lignoceric acid)	0.012	<.001	NS
Viable pollen number	<.001	0.002	<.001
Total saturated fatty acids	<.001	<.001	<.001
Total unsaturated fatty acids	<.001	<.001	<.001
Saturated/unsaturated fatty acids ratio	<.001	0.001	<.001
Total fatty acids	<.001	<.001	<.001

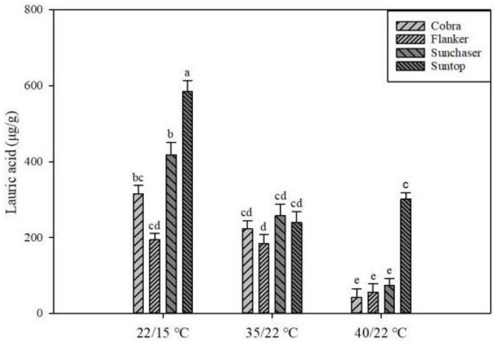
12 Treatments =3 treatment × 4 cultivar; number of replications = 4; NS = p>0.05

Overall, the contents of Lauric acid, Myristic acid, Palmitic acid, Palmitoleic acid, and Stearic acid decreased with increasing temperatures, with the highest levels observed in Suntop under 22/15°C conditions (584.8, 9836.3, 47445.7, 488.1, and 8494.9 µg/g, respectively). Among these fatty acids, Cobra showed the largest reduction ratios as temperatures increased. In contrast, the content of C20:0 was highest under 35/22°C conditions and lowest under 22/15°C conditions, with the maximum content observed in Cobra under 35/22°C (1.623 µg/g). The number of viable wheat pollen grains decreased with increasing temperature, except for Flanker, which reached its highest viability under 35/22°C conditions (Figure 7.2).

There are significant variations in the content of individual fatty acids among four wheat cultivars (Cobra, Flanker, Sunchaser, and Suntop) under three temperature regimes (22/15°C, 35/22°C, and 40/22°C). Lauric acid and myristic acid exhibited the highest levels under the 22/15°C condition, with Flanker and Sunchaser showing the greatest accumulation, respectively. However, their levels declined markedly as the temperature increased, with minimal differences between cultivars under 40/22°C (Figure 7.2 (a) (b)). Palmitic acid, the predominant saturated fatty acid, followed a similar trend, showing significantly higher levels in Cobra and Flanker under 22/15°C, which diminished sharply under higher temperatures (Figure 7.2 (c)). Palmitoleic acid, a monounsaturated fatty acid, showed less sensitivity to temperature changes compared to saturated fatty acids (Figure 7.2 (d)). Stearic acid displayed a similar decline under elevated temperatures, with Flanker maintaining the highest levels under 22/15°C (Figure 7.2 (e)). In contrast, arachidic acid showed a less pronounced response to temperature changes, with a peak observed in Flanker under 35/22°C conditions ((Figure 7.2 (f)).

For pollen viability, all genotypes exhibited high levels at 22/15°C; at 35/22°C, Cobra showed the highest viability; at 40/22°C, viability declined significantly across all genotypes, with Suntop and Sunchaser being most affected (Figure 2 (h). In terms of total fatty acid composition, SFA and UFA contents were significantly higher under the 22/15°C condition for all cultivars, with Flanker and Sunchaser demonstrating the highest levels. Elevated temperatures (35/22°C and 40/22°C) resulted in substantial reductions in both SFA and UFA levels across all cultivars (Figure 7.2 (i) (j)). The total fatty acid content followed a similar trend, with the highest accumulation observed under 22/15°C and a sharp decline under 40/22°C, where all cultivars showed minimal total fatty acid levels (Figure 7.2 (l)). Additionally, the saturated-to-unsaturated fatty acid ratio (SFA/UFA) increased significantly under higher temperatures, indicating a relative inhibition of UFA synthesis under HT. This trend was most pronounced in the Sunchaser cultivar, suggesting a differential cultivar response to high-temperature stress. These findings underscore the critical impact of HT on fatty acid metabolism, affecting both individual and total fatty acid profiles, which has potential implications for wheat cellular function and membrane integrity (Figure 7.2).

a


Lauric acid (µg/g)
0
200
400
600
800
22/15 °C
35/22 °C
40/22 °C
Cobra
Flanker
Sunchaser
Suntop
bc
cd
b
a
cd
d
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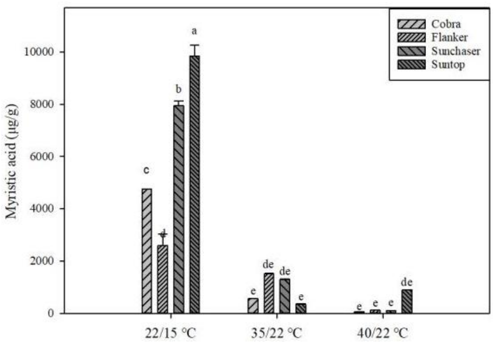
c

b


Myristic acid (µg/g)
0
2000
4000
6000
8000
10000
22/15 °C
35/22 °C
40/22 °C
Cobra
Flanker
Sunchaser
Suntop
c
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b
a
e
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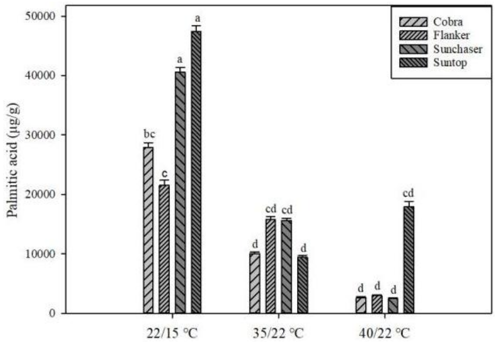
c


Palmitic acid (µg/g)
0
10000
20000
30000
40000
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22/15 °C
35/22 °C
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Cobra
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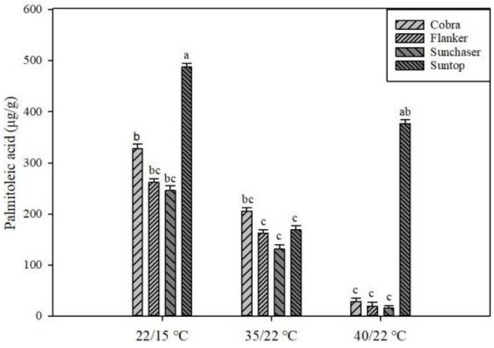
Palmitoleic acid (µg/g)
0
100
200
300
400
500
600
22/15 °C
35/22 °C
40/22 °C
Cobra
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Sunchaser
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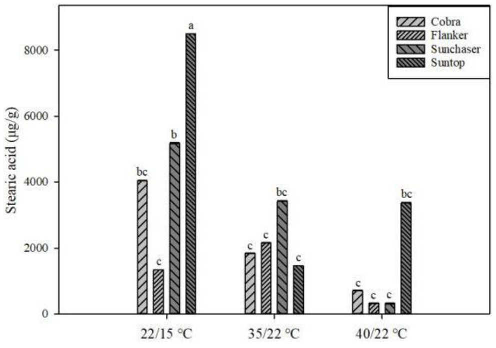
e


Stearic acid (µg/g)
0
2000
4000
6000
8000
22/15 °C
35/22 °C
40/22 °C
Cobra
Flanker
Sunchaser
Suntop
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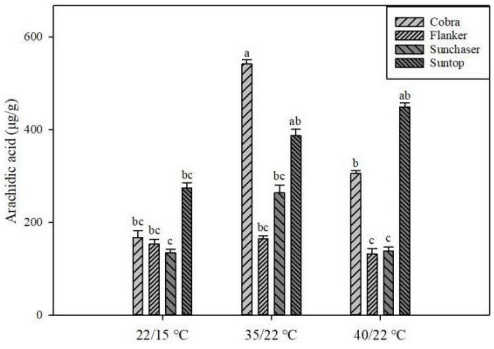
Arachidic acid (µg/g)
0
200
400
600
22/15 °C
35/22 °C
40/22 °C
Cobra
Flanker
Sunchaser
Suntop
bc
bc
c
bc
a
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ab
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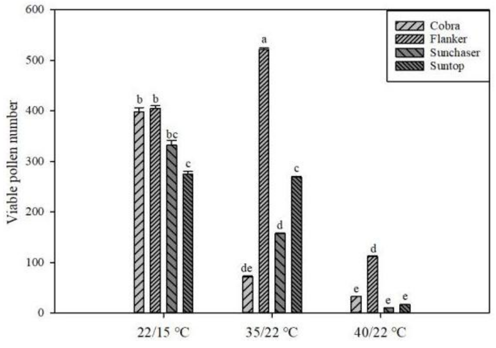
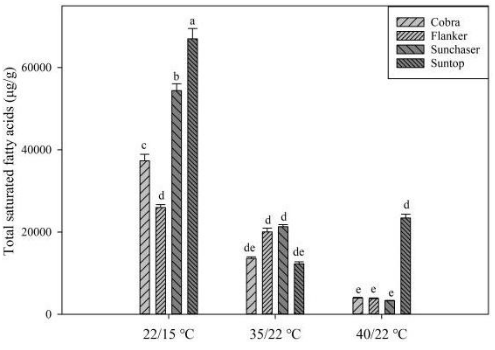
ab

h


Viable pollen number
0
100
200
300
400
500
600
22/15 °C
35/22 °C
40/22 °C
Cobra
Flanker
Sunchaser
Suntop
b
b
bc
c
de
a
d
c
e
d
e
e

i


Total saturated fatty acids (µg/g)
0
20000
40000
60000
22/15 °C
35/22 °C
40/22 °C
Cobra
Flanker
Sunchaser
Suntop
c
d
b
a
de
d
d
de
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e
d

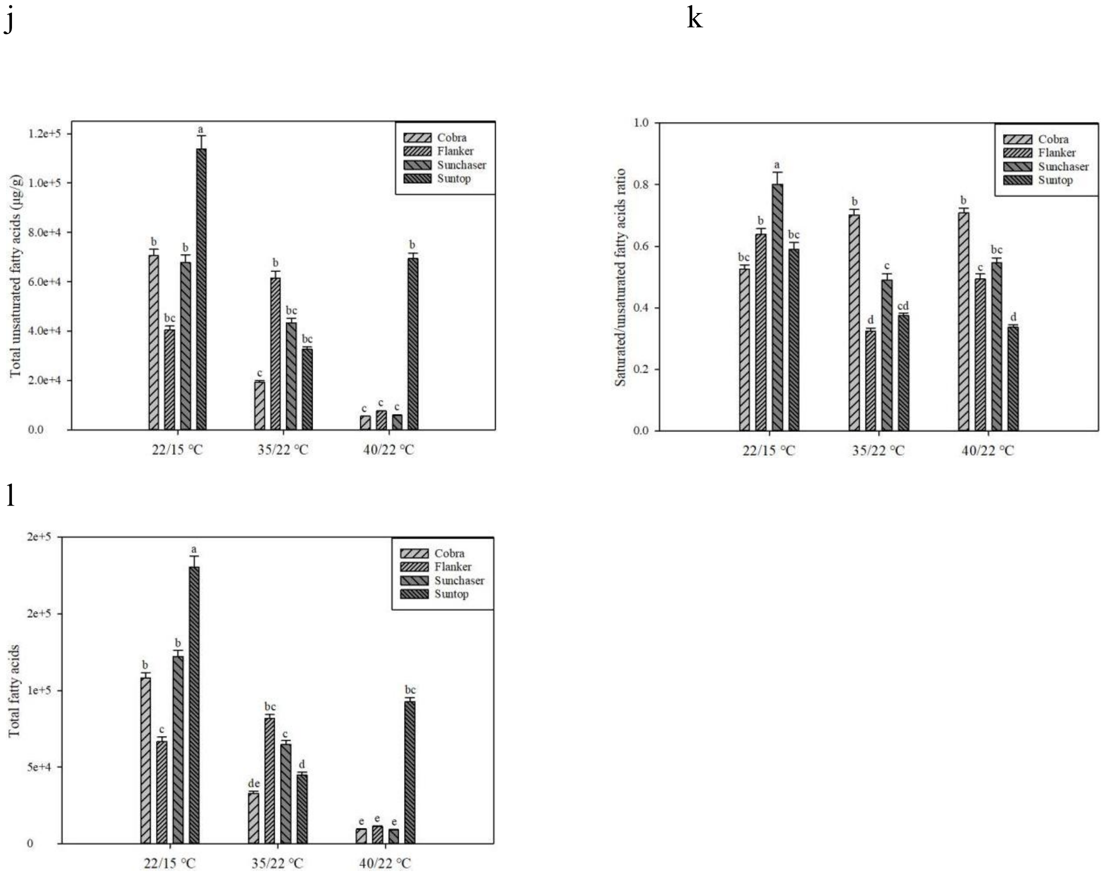

Figure 7.2. Effect of treatment and cultivar on (a) C12:0 (Lauric acid), (b) C14:0 (Myristic acid), (c) C16:0 (Palmitic acid), (d) C16:1 (Palmitoleic acid), (e) C18:0 (Stearic acid), (f) C20:0 (Arachidic acid) acid and (h) viable pollen number for wheat pollen. Means followed by the same letters are not different at p<0.05 for treatment × cultivar interaction. Error bars represent standard error of the mean (n=4).

Correlation analysis revealed strong, significant positive associations among fatty acids (Table 7.3). For instance, lauric acid correlated with total saturated fatty acids (SFA) (r=0.945, p<0.001), total unsaturated fatty acids (UFA) (r=0.917, p<0.001), and total fatty acids (r=0.948, p<0.001). Similar trends were observed for myristic, palmitic, palmitoleic, stearic, and α -linolenic acids. Notably, palmitic acid showed high correlations with total SFA (r=0.997, p<0.001) and high correlations with total UFA (r=0.908, p<0.001) and total fatty acids (r=0.963, p<0.001). Moreover, total SFA was strongly associated with total UFA (r=0.908, p < 0.001) and total fatty acids (r=0.964, p<0.001), while total UFA correlated with total fatty acids (r=0.986, p<0.001). Detailed data for individual fatty acids are provided in Table 7.3.

Table 7.3. The pairwise correlations between wheat pollen lipids and viable pollen number by Cobra, Flanker, Sunchaser and Suntop.

		C12:0	C14:0	C16:0	C16:1	C18:0	C18:3	C20:0	C22:0	C24:0	Viable pollen number	Total SFA	Total UFA	Total SFA/UFA	Total fatty acids
C12:0	*r*=	1													
	p=														
C14:0	*r*=	0.878	1												
	01	<0.001													
C16:0	*r*=	0.935	0.967	1											
	p=	<0.001	<0.001												
C16:1	*r*=	0.885	0.717	0.831	1										
	p=	<0.001	0.009	<0.001											
C18:0	*r*=	0.965	0.906	0.931	0.828	1									
	p=	<0.001	<0.001	<0.001	<0.001										
C18:3	*r*=	0.917	0.822	0.908	0.902	0.921	1								
	p=	<0.001	0.001	<0.001	<0.001	<0.001									
C20:0	*r*=	NS	NS	NS	NS	NS	NS	1							
	p=														
C22:0	*r*=	NS	NS	NS	NS	NS	NS	0.793	1						
	p=							0.002							

C24:0	*r*= *p*=	NS	NS	NS	NS	NS	NS	0.709 0.009	0.987 <0.001	1					
Viable pollen number	*r*= *p*=	NS	NS	NS	NS	NS	NS	NS	NS	NS	1				
Total SFA	*r*= *p*=	0.945 <0.001	0.974 <0.001	0.997 <0.001	0.826 <0.001	0.949 <0.001	0.908 <0.001	NS	NS	NS	NS	1			
Total UFA	*r*= *p*=	0.917 <0.001	0.822 0.001	0.908 <0.001	0.903 <0.001	0.921 <0.001	0.997 <0.001	NS	NS	NS	NS	0.908 <0.001	1		
Total SFA/UFA	*r*= *p*=	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	1	
Total fatty acids	*r*= *p*=	0.948 <0.001	0.899 <0.001	0.963 <0.001	0.893 <0.001	0.953 <0.001	0.986 <0.001	NS	NS	NS	NS	0.964 <0.001	0.986 <0.001	NS	1

Correlation and significance at *p* = 0.05, NS = p>0.05

7.5 Discussion

7.5.1. Impact of HT on Pollen Viability and Lipid Profiles

HT significantly reduced pollen viability in all wheat genotypes, with Cobra (thermosensitive) showing the greatest decrease, especially at 40/22°C. In contrast, Suntop (thermotolerant) showed the highest pollen viability under all temperature conditions. These findings are consistent with previous studies highlighting the thermosensitivity of pollen during meiosis and gamete formation, where elevated temperatures impair enzyme activities, disrupt starch metabolism, and induce oxidative stress, ultimately reducing viability (Prasad & Djanaguiraman, 2014; Saini & Aspinall, 1982; Zhang et al., 2010). Structural damage observed in pollen, such as collapsed grains and dented surfaces, further confirmed these deleterious effects (Farooq et al., 2011; Zoeller et al., 2012).

Genotype-specific differences in pollen viability may indicate that Suntop's thermotolerance stems from its ability to maintain lipid homeostasis under HT. In contrast, Cobra's high sensitivity highlights its limited adaptive capacity, especially at temperatures above 35°C. These observations are consistent with previous studies showing that 12-22°C is the optimal temperature range for wheat reproduction, with rapid decline in wheat reproduction beyond these thresholds (Shewry, 2009b; Narayanan et al., 2018).

Lipid profiles also showed significant disruptions under HT, with a substantial reduction in SFA and UFA in all genotypes. Cobra showed the most significant increase in SFA/UFA ratio, reflecting its impaired lipid homeostasis capacity. In contrast, the relatively stable SFA/UFA ratio in Suntop under HT conditions highlights its superior lipid homeostasis, which is critical for maintaining membrane integrity and cellular function (Narayanan et al., 2016a; Zhang et al., 2016).

7.5.2. Genotype-Specific Lipid Homeostasis and Its Role in Thermotolerance

This study found genotypic variation in lipid homeostasis under HT, with Suntop showing the strongest adaptation. At 40/22℃, Suntop only slightly decreased its lauric, myristic, and palmitic acid levels, while Cobra's levels dropped dramatically. These differences suggest that the enhanced heat tolerance in Suntop may be attributed to its ability to regulate lipid biosynthesis and reduce oxidative lipid peroxidation, consistent with previous findings (Narayanan et al., 2018; Djanaguiraman et al., 2018).

In contrast, Cobra showed a significant increase in the SFA/UFA ratio and a significant decrease in total fatty acid content. These changes suggest impaired lipid homeostasis and increased sensitivity to oxidative damage, as evidenced by elevated levels of malondialdehyde (MDA), a marker of lipid peroxidation (Farooq et al., 2011; Zoeller et al., 2012). The vulnerability of the Cobra lipid pathway under HT is consistent with earlier reports highlighting the key role of lipid homeostasis in thermotolerance (Narayanan et al., 2016b; Zhang et al., 2016).

Furthermore, this study is the first to systematically demonstrate that coordinated lipid homeostasis, as opposed to alterations in individual fatty acid levels, is pivotal for maintaining membrane integrity and cellular function under high-temperature stress. This novel insight establishes a theoretical basis for utilising lipid profiles as biomarkers in the screening and breeding of heat-tolerant wheat cultivars.

In addition, the stable lipid profile of Suntop under HT conditions may be related to its ability to maintain higher levels of unsaturated fatty acids such as linolenic acid. These findings are consistent with studies showing that UFAs can enhance membrane fluidity, reduce oxidative stress, and support pollen viability under HT (Welti et al., 2007; Narayanan et al., 2018).

7.5.3. Correlation Between Lipid Composition and Pollen Viability

Correlation analysis revealed strong correlations between fatty acids, especially between lauric and stearic acid (r=0.965, p<0.001), and between palmitic acid and total fatty acids (r=0.963, p<0.001). Although a direct metabolic pathway linking lauric and stearic acids has not been clearly established, their strong correlation might indicate coordinated regulatory mechanisms or shared upstream biosynthetic routes. These correlations suggest coordinated lipid metabolism under heat stress, consistent with previous reports (Narayanan et al., 2016b; Welti et al., 2002).

However, there were no significant correlations between individual fatty acids and pollen viability, highlighting the multifaceted nature of lipid homeostasis in thermotolerance. Notably, the increase in the SFA/UFA ratio, especially in Cobra, highlights the vulnerability of thermosensitive genotypes. This imbalance compromises membrane fluidity and exacerbates oxidative stress, thereby reducing pollen viability (Narayanan et al., 2016a; Zoeller et al., 2012). In contrast, Suntop's ability to maintain a stable SFA/UFA ratio under HT conditions reflects its robust lipid homeostasis, which mitigates oxidative damage and preserves cellular function.

7.5.4. Mechanistic Insights and Implications for Breeding

Lipid homeostasis under HT might be maintained through mechanisms such as reducing lipid saturation levels, adjusting membrane fluidity, or inhibiting oxidative damage; however, these remain speculative explanations, requiring further experimental validation. The stable lipid profile and enhanced UFA of Suntop suggest that its heat tolerance may be mediated by efficient lipid biosynthesis pathways and antioxidant defense (Narayanan et al., 2018; Zhang et al., 2016). These adaptations may minimise heat-induced membrane phase transitions and maintain cellular homeostasis.

In contrast, Cobra had impaired lipid homeostasis, reflected in its elevated SFA/UFA ratio and reduced pollen viability, highlighting its sensitivity to HT. This finding emphasise the need for breeding strategies targeting lipid metabolic pathways to enhance heat tolerance in heat-sensitive genotypes (Narayanan et al., 2016a,b; Djanaguiraman et al., 2018).

These findings have important practical implications. Lipid profiles in pollen and leaves can be used as biomarkers for selecting heat-tolerant genotypes. The robust lipid homeostasis ability of Suntop makes it a valuable resource for breeding programs aimed at improving wheat's resilience to global warming. Combining molecular studies of lipid metabolism with traditional breeding approaches could accelerate the development of wheat varieties that can maintain productivity under HT conditions (Welti et al., 2007; Narayanan et al., 2018).

7.5.5. Limitations and Future Directions

While this study provides valuable insights into the impact of HT stress on wheat pollen viability and lipid metabolism, it is limited by its controlled experimental conditions and the focus on four genotypes. Future research should explore a broader range of wheat varieties under field conditions to validate these findings and assess their applicability in diverse environmental contexts.

Additionally, molecular studies investigating the regulation of lipid biosynthesis pathways and their interaction with antioxidative defense systems could provide deeper insights into the mechanisms underlying thermotolerance. Identifying key genes involved in the coordinated changes in lipids could inform breeding strategies aimed at enhancing heat tolerance in wheat (Narayanan et al., 2016a; Mou et al., 2012).

7.6 Conclusion

This study highlights the detrimental effects of HT on wheat pollen viability and lipid metabolism, with significant genotype-specific variations. Elevated temperatures reduced pollen viability and disrupted lipid composition, particularly increasing the SFA/UFA ratio and reducing total fatty acid content. Suntop exhibited superior thermotolerance, maintaining stable lipid profiles and higher pollen viability under HT, while Cobra demonstrated pronounced susceptibility, marked by impaired lipid homeostasis and reduced pollen function. Correlations among fatty acids suggest that thermotolerance may involve maintaining a stable lipid composition through coordinated regulation of fatty acids, rather than relying on isolated changes in individual fatty acids. Suntop's robust lipid homeostasis capacity and resilience to HT offer promising traits for breeding heat-tolerant wheat cultivars. Lipid profiles may serve as biomarkers for selecting resilient genotypes. Future research should focus on the genetic regulation of lipid metabolism and the integration of molecular and traditional breeding approaches to develop cultivars capable of sustaining productivity under global warming. These insights are critical for ensuring wheat production and global food security in the face of climate change.

Chapter 8. General Discussion

8.1. Summary

This study aimed to investigate the impact of high temperature (HT) on wheat yield, quality, and nutritional components, particularly focusing on protein fractions, starch viscosity, crude fibre, mineral content, phytate levels, lipid metabolism, and pollen viability. Wheat, as a staple crop, plays a crucial role in global food security, yet its growth is threatened by high temperatures during critical developmental stages, such as grain filling. These conditions are expected to become more frequent due to climate change, posing a risk to both yield and nutritional quality. The research assessed wheat performance under HT conditions across three diverse Australian regions (Horsham, Narrabri, and Merredin) over two years (2019 and 2020), with a focus on environmental factors such as sowing time, soil properties, and genotype-specific responses.

Key findings of the study include several critical observations. Firstly, HT significantly reduced wheat yield and thousand kernel weight (TKW), particularly in Merredin, a location characterised by high temperatures and sandy soils. The data highlighted how HT, especially in combination with suboptimal soil types, leads to severe yield reductions. In contrast, wheat grown in cooler years (2020) showed more stable yields and improved quality, reinforcing the influence of temperature extremes on wheat performance. These findings also underscore the variability in wheat response to HT based on environmental factors, with some regions experiencing more pronounced adverse effects than others.

Additionally, the study revealed a marked decrease in protein quality due to HT, with changes in protein composition observed, particularly a reduction in glutenin-to-gliadin ratios. The starch and fiber properties of wheat were also affected, with HT leading to a reduction in starch gelatinisation and viscosity, alongside an increase in fibre content. These changes in starch behaviour can compromise the baking and processing qualities of wheat, ultimately impacting its suitability for food products.

A notable aspect of this research was the observed genotypic variation in wheat response to HT stress. Heat-tolerant cultivars, such as Berkut, Flanker, and Suntop, demonstrated superior yield stability, mineral content, and pollen viability under HT conditions compared with heat-sensitive cultivars like Sokoll. This suggests that specific genotypes have inherent traits that enable them to better cope with HT-induced stresses, offering a promising avenue for breeding heat-tolerant wheat cultivars in the face of rising global temperatures.

Soil properties, including pH and OM content significantly influenced the bioavailability of minerals, particularly zinc (Zn) and iron (Fe). Alkaline soils in Horsham and Narrabri were shown to limit the availability of these essential nutrients, leading to reduced mineral uptake by the wheat plants. On the other hand, sandy soils in Merredin exacerbated nutrient leaching, further reducing the availability of critical minerals to the plants. These findings reinforce the complex interaction between soil characteristics and HT stress in shaping the mineral nutrition of wheat, suggesting that soil management practices could play a key role in mitigating the negative impacts of HT.

Overall, this study provides valuable insights into the multifaceted effects of HT on wheat, revealing how environmental factors, genotype selection, and soil management can influence wheat yield, nutritional composition, and reproductive success under heat stress (Table 8.1).

Table 8.1 Summary of research on wheat responses to HT

Chapter	Objective	Key Findings	Research Results	Future Directions
Chapter 4	Investigate the impact of high temperature on wheat growth and development	High temperatures significantly reduce wheat yield and quality, particularly during flowering and grain filling; effects vary by genotype	High temperatures affect wheat phenology and physiological processes; genotype selection is essential for enhancing heat tolerance	Further research on genotype-environment interactions and improved agricultural practices to mitigate heat stress effects on wheat quality
Chapter 5	Assess the impact of high temperature on wheat starch and crude fibre	High temperatures reduce starch content and alter starch gelatinisation properties; crude fibre response is complex, varying by genotype and environmental factors	Heat-tolerant cultivars (e.g., Berkut and Flanker) show better starch and fibre stability compared with sensitive cultivars	To optimise sowing strategies and selecting heat-tolerant wheat cultivars with stable starch and fibre content

Chapter 6	Evaluate the effect of high temperature on wheat mineral content and phytate levels	High temperatures reduce wheat yield but enhance mineral concentrations (e.g., Zn and Fe) in some genotypes; phytate content decreases with high temperature	Heat stress alters mineral bioavailability and phytate levels, with genotype-specific variations; soil properties also influence mineral retention	To investigate soil management practices and breeding strategies to reduce phytate content and improve mineral bioavailability
Chapter 7	Explore the effects of high temperature on wheat lipid composition and pollen viability	High temperatures significantly reduce pollen viability and alter lipid profiles; heat-tolerant cultivars (e.g., Suntop), maintain higher pollen viability and stable lipid composition	Lipid metabolism is crucial for heat resilience, with Suntop showing better lipid homeostasis under heat stress	To investigate the genetic regulation of lipid biosynthesis and developing heat-tolerant wheat cultivars using molecular breeding techniques

8.2. High temperature effect on wheat

8.2.1. Effects of Environmental Factors on Yield and Quality

High temperature (HT) is one of the major environmental stressors affecting wheat growth, especially during critical developmental stages such as grain filling. The findings from Chapters 4, 5, and 6 of this study provide a comprehensive account of how HT, sowing time, and soil properties interact to affect wheat yield, quality, and nutrient content. Specifically, HT significantly reduced wheat yield and thousand kernel weight (TKW), with evidence suggesting that these reductions were primarily due to a shortened grain filling period, impaired pollen viability, and altered nutrient distribution under stress.

Sowing time, especially late sowing under HT conditions (TOS2), had a significant impact on wheat yield, further exacerbating the response to HT. Late sowing reduced the ability of wheat plants to properly take up nutrients and support grain filling, resulting in a significant reduction in yield. This is consistent with earlier studies showing that poor pollen viability under HT compromises successful fertilisation of flowers, further reducing yield potential (Saini & Aspinall, 1982). The effects of sowing time on wheat yield and quality highlight the importance of optimising sowing dates to mitigate the adverse effects of HT and improve crop stress tolerance.

In addition to reduced yield, high temperatures altered several key quality parameters of wheat. For example, HT negatively affected protein composition, especially the ratio of glutenin to gliadin, with an increase in glutenin (the protein fraction responsible for dough strength) and a greater increase in gliadin. Such changes in protein composition at HT can significantly affect the baking quality of wheat, a key aspect of wheat products (Spiertz et al., 2006). In addition, HT also affected starch properties, including an increase in gelatinisation temperature and a decrease in viscosity, indicating that starch functionality in baking is impaired.

While HT negatively affected protein and starch properties, it led to increased mineral concentrations, especially zinc (Zn) and iron (Fe) in some areas, especially when sown late. However, these increases in mineral concentrations were offset by decreased yield and nutrient retention, especially in areas with sandy soils such as Merredin. Sandy soils are characterised by low cation exchange capacity, which can aggravate nutrient loss and lead to poor retention of important minerals such as Zn and Fe. This highlights the importance of soil management practices to maintain mineral bioavailability under HT conditions.

The interaction between genotype and environment was another key factor determining how wheat performed under high temperatures. Heat-tolerant genotypes, such as Berkut and Suntop, performed well under heat stress, maintaining higher yield levels, stable mineral concentrations, and better pollen viability. Berkut, in particular, consistently outperformed the heat-sensitive cultivar Sokoll in terms of yield and mineral stability. This genotypic variation highlights the potential for breeding heat-tolerant cultivars to ensure stable wheat yields in the face of climate change. In addition, soil texture plays a crucial role in influencing nutrient retention. Clay soils, such as those at Narrabri, showed better retention of essential minerals, while sandy soils at Merredin exacerbated nutrient loss, further reducing mineral availability. These findings highlight the complex interactions between soil properties, genotype, and heat stress, and the need for site-specific agronomic practices to optimise wheat yield.

8.2.2. Interrelationships Between Traits

The study also revealed several significant trade-offs and interrelationships between wheat traits, which are crucial for understanding the broader implications of HT stress on wheat quality and function. These interrelationships demonstrate the complexity of wheat's response to HT and emphasise the need for a multi-trait approach in breeding programs.

One key trade-off observed in this study was between protein content and yield. There was a negative correlation between protein content and TKW, suggesting that higher protein levels, particularly in heat-stressed environments, may come at the expense of grain filling and yield potential. This trade-off highlights the challenge of optimising both yield and nutritional quality under HT conditions. Elevated protein content, particularly in heat-prone regions, may lead to reduced starch accumulation and overall yield potential, which can compromise both crop productivity and the quality of wheat for food production (Spiertz et al., 2006).

Another significant relationship explored in this study was between starch and fibre content. Under HT conditions, starch and fibre exhibited a competitive relationship, with high fibre content often associated with reduced starch accumulation. This finding is consistent with the hypothesis that under stress, wheat plants prioritise survival mechanisms, such as fibre production, over optimal growth and starch accumulation (Farooq et al., 2011). The increased fibre content under HT conditions may serve as a protective mechanism to help the plant maintain structural integrity under heat stress, although it may negatively affect the overall quality of the wheat for milling and baking purposes.

A crucial aspect of this study was the relationship between phytate content and mineral bioavailability. While lower phytate levels generally enhance the bioavailability of essential minerals such as zinc and iron, they also reduce the phosphorus stored in the grain. This reduction is significant in an agronomic context, as it determines the amount of phosphorus removed at harvest and the extent of fertiliser replenishment needed to maintain soil fertility (Sinclair & Krämer, 2012). The balance between phytate content and mineral uptake is therefore critical for ensuring both nutritional quality and long-term plant development. The study found that while HT conditions, particularly in Merredin, led to lower phytate levels, which could improve mineral bioavailability, this came at the cost of phosphorus storage, which is crucial for subsequent growth cycles (Raboy, 2009).

Finally, this study examined the role of lipid metabolism in maintaining pollen viability under HT stress. Effective lipid homeostasis is crucial for preserving cell membrane stability and consequently, pollen viability. Although HT conditions tend to increase the saturated/unsaturated fatty acid (SFA/UFA) ratio, a shift that can compromise membrane integrity, my data indicate that wheat genotypes with higher lipid homeostasis counteract these deleterious changes, thereby sustaining pollen function (Zhang et al., 2016). Heat-tolerant cultivars, such as Suntop, demonstrated higher lipid homeostasis, which contributed to higher pollen viability under HT conditions. This finding supports the idea that lipid homeostasis plays a key role in thermotolerance, particularly in maintaining the integrity of cellular membranes and ensuring reproductive success under heat stress (Djanaguiraman et al., 2018).

These interrelationships between traits highlight the complexity of wheat's response to HT and underscore the importance of considering multiple traits simultaneously when developing breeding strategies to mitigate the negative effects of climate change. Breeding approaches that prioritise traits such as heat tolerance, lipid homeostasis, and balanced mineral uptake, while minimising negative trade-offs, will be critical for ensuring sustainable wheat production under future climate conditions.

8.3. Comparison with Previous Studies

This study provides new insights into the effects of HT on wheat yield, quality, and nutritional content, especially protein composition, starch behaviour, mineral content, and lipid metabolism. My data are consistent with previous studies, while some provide deeper or more context-specific insights, especially regarding the interactions between HT, soil properties, genotypes, and environmental factors.

8.3.1. Effects of HT on Wheat Yield and Protein Quality

Multiple studies have confirmed that HT negatively affects wheat yield, especially during critical growth stages such as flowering and grain filling. The data of my study are consistent with these previous reports, which highlight that HT reduces wheat yield by shortening the grain filling period, impairing pollen viability, and disrupting nutrient uptake (Zahra et al., 2021; Velu et al., 2016). In line with Dias and Lidon

(2009a), my study found that late sowing (TOS2) under HT conditions resulted in yield reduction, which may be due to poor pollen viability and impaired nutrient mobilisation, especially in heat-sensitive genotypes. This finding supports earlier studies that highlighted the role of temperature-induced stress in reducing wheat reproductive success, leading to yield losses.

In addition to its effects on yield, high temperatures have long been known to alter the protein composition of wheat. Specifically, high temperatures disrupt the glutenin to gliadin ratio, reducing gluten strength and baking quality. The present study confirms these findings, showing that high temperature conditions in some regions (e.g., Merredin) leading to reduced glutenin content, which has implications for industrial applications of wheat (Velu et al., 2016). Reduced gluten strength, as well as altered starch functionality (gelatinisation temperature, viscosity), may affect the end-use quality of wheat, confirming that high temperatures can affect wheat yield and quality (Spiertz et al., 2006).

8.3.2. Starch and Fibre Under Heat Stress

The effects of high temperatures on starch and fibre content have been extensively studied, especially the competition between these two components under stress conditions. My data are consistent with the observations of Dias and Lidon (2009a), who reported that high temperatures promoted fibre synthesis at the expense of starch accumulation. The increase in fibre content under high temperatures in this study is consistent with the hypothesis that stress conditions prioritise survival mechanisms such as structural strengthening through fibre production over optimal growth, which generally favors starch accumulation (Farooq et al., 2011). The trade-off between fibre and starch under high temperatures in this study further emphasises the complexity of wheat's metabolic response to environmental stress and the need to develop breeding strategies that balance these traits to optimise yield and nutritional quality.

This study also extends the literature by quantifying these dynamics across multiple genotypes and environmental conditions. The differences in starch and fibre content between genotypes such as Berkut (heat tolerant) and Sokoll (heat sensitive) provide new evidence that heat tolerant cultivars are better able to maintain starch accumulation under high temperatures, suggesting a potential breeding target to improve starch resilience under climate change conditions.

8.3.3. Mineral and Phytate Under Heat Stress

One of the most important contributions of this study is the exploration of the relationship between HT, soil characteristics, genotype, and mineral bioavailability, particularly with respect to phytate content. While previous studies have shown that HT can increase the bioavailability of minerals such as zinc (Zn) and iron (Fe) due to stress-induced nutritional changes (Samineni et al., 2022), this study adds nuance by demonstrating that these effects are context-specific. For example, under late sowing conditions (TOS2), HT resulted in higher concentrations of Zn and Fe, particularly where soil conditions were more favourable (Narrabri). However, in sandy soil areas such as at Merredin, the benefits of increased mineral concentrations were offset by yield reductions and poor mineral retention, which exacerbated nutrient leaching (Peck & McDonald, 2010).

Soil characteristics, such as pH and OM content, play a key role in the availability of minerals in wheat. These findings build on research by Kabata-Pendias (2004), who found that alkaline soils limit the bioavailability of key micronutrients such as zinc and iron. In contrast, slightly acidic soils, such as those at Merredin, promote the dissolution of these minerals but also result in reduced phosphorus (P) storage capacity due to reduced phytic acid content. This study highlights the need for a deeper understanding of how soil properties interact with environmental stresses to influence mineral dynamics and bioavailability in wheat.

While previous studies have typically focused on the effects of HT on either mineral content or phytic acid levels separately, this study focused on both factors simultaneously across multiple genotypes at different locations, providing a more complete understanding of how HT affects the nutritional quality of wheat. The data highlight the importance of balancing phytic acid content and mineral bioavailability to ensure both short-term and long-term plant development (Raboy, 2009).

8.3.4. Pollen Viability and Lipid Under Heat Stress

The effects of heat on lipid metabolism and pollen viability are another area to which this study makes a significant contribution. Previous studies reported that high temperatures disrupt lipid metabolism, especially in pollen, by increasing the ratio of saturated to unsaturated fatty acids, thereby impairing membrane stability and pollen viability (Zhang et al., 2016; Djanaguiraman et al., 2018). This study confirmed these findings, showing that heat-tolerant cultivars such as Suntop maintained lipid composition and this helped maintain pollen viability under high temperature conditions. In contrast, more heat-sensitive cultivars such as Cobra showed a significant increase in the saturated/unsaturated fatty acid ratio, which may have contributed to their reduced pollen viability and reproductive success.

The novel contribution of this study is that it focuses on lipid homeostasis in pollen under high temperatures, further deepening the understanding of how lipid composition affects heat tolerance. By identifying the specific role of lipid homeostasis in heat tolerance, this study provides a new perspective on how to target lipid metabolism in breeding programs to enhance wheat resilience to climate change.

8.3.5. Multi-environment Approach and Genotype-environment Interactions

A major strength of this study is its multi-environment approach, which examined wheat performance under high temperatures across multiple locations and years. This approach provides a deeper understanding of genotype-environment interactions and how environmental factors such as soil properties, temperature, and sowing time affect wheat performance. Previous studies have often focused on one location or one stressor, but this study combined data from different locations and under different environmental conditions, adding depth to the findings. For example, the contrasting results observed in locations such as Merredin (hot temperatures and sandy soils) and Narrabri (milder temperatures and clay soils) highlight the importance of local environment in breeding and agronomic practices.

By evaluating wheat performance under high temperatures across multiple locations, this study highlights the importance of genotype-environment interactions, emphasising that breeding programs aimed at improving wheat resilience to climate change must consider regional differences in soils and climate. These findings are consistent with previous research on genotype-environment interactions in wheat, which suggests that the effectiveness of breeding strategies can be significantly influenced by environmental factors. The data of this study contribute to a growing literature that highlights the need for site-specific agronomic practices and breeding strategies to optimise wheat production under climate change conditions.

8.4. Limitations and Future Directions

8.4.1 Limitations of The Study

Despite the comprehensive nature of this study, several limitations must be acknowledged. Firstly, the study was conducted over two years (2019 and 2020), limiting its ability to capture the full variability in climate conditions over a longer period. A multi-year study could better account for climate variation and its effects on wheat performance. Secondly, while the study encompassed multiple locations, the temperature ranges experienced during these experiments (e.g., maximum daily temperatures between approximately 19 ℃ and 35 ℃) may not fully represent the broader spectrum of conditions under which wheat is grown in Australia or world. Consequently, caution is required when generalising these findings to environments with more extreme heat conditions. Field-based studies that account for additional stressors like pest pressures and water availability would complement the findings. Additionally, while the study focused on key nutritional components such as minerals and phytate, other factors like antioxidants or micronutrient interactions were not assessed. Expanding the analysis to include these factors would provide a more comprehensive understanding of wheat nutritional quality under HT stress.

8.4.2. Future Research Directions

Future research should focus on extending the study over multiple years to capture the variability in HT stress across different climatic conditions. Incorporating field-based experiments would allow for a more realistic assessment of HT stress under actual agricultural conditions. Furthermore, understanding the genetic basis of heat tolerance remains a key area for future investigation. Identifying genes associated with heat resilience in wheat, particularly those related to protein composition, lipid metabolism, and pollen viability, will be essential for breeding heat-tolerant cultivars. Research into soil management practices, such as the use of organic amendments, should also be explored to mitigate the effects of HT on wheat's mineral bioavailability. Lastly, expanding the scope to include a broader range of nutritional components, including micronutrients and antioxidants, would help identify strategies to improve the nutritional quality of wheat under HT stress, addressing global food security and micronutrient deficiencies.

8.5. Conclusions

This study systematically evaluated the effects of high temperature (HT) stress on wheat yield, quality, and nutritional components over two years across multiple locations of Australia. HT significantly reduced total yield and thousand kernel weight, primarily by shortening the grain filling period and impairing reproductive processes such as pollen viability. These thermal stresses induced notable changes in wheat biochemical properties, including a shift in the glutenin-to-gliadin ratio that compromised dough strength and altered starch functionality evidenced by increased gelatinisation temperatures, reduced peak viscosity, and modified crude fibre content. Furthermore, HT affected lipid metabolism by increasing the ratio of saturated to unsaturated fatty acids, while variations in phytate levels influenced the bioavailability of essential minerals like zinc and iron.

Environmental factors and genotype emerged as key modulators of wheat's response to HT. Heat-tolerant cultivars, such as Berkut, Flanker, and Suntop, maintained more stable yields, nutrient profiles, and pollen viability compared to lower heat tolerant cultivars like Sokoll and heat sensitive cultivars like Cobra. Soil properties also played a critical role; for instance, clay soils supported improved mineral retention relative to sandy soils, which tended to exacerbate nutrient leaching. Despite these valuable insights, limitations such as the study's two-year duration and controlled experimental conditions suggest that future research should extend over multiple years and incorporate additional stressors, including pest pressures and water availability. Expanding investigations to encompass other nutritional factors and elucidating the genetic basis of heat tolerance will be essential for guiding breeding programs and agronomic practices aimed at sustaining wheat production under a warming climate.

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