Improving the Adaptation of Wheat (*Triticum aestivum*) to Heat Stress Conditions

By

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Publications

This thesis contains three original research articles published in peer-reviewed journals. A Statement of authorship is included at the beginning of each chapter containing an original research article.

Telfer, P., J. Edwards, D. Bennett, D. Ganesalingam, J. Able and H. Kuchel (2018). "A field and controlled environment evaluation of wheat (Triticum aestivum) adaptation to heat stress." Field Crops Research **229**: 55-65. doi:10.1016/j.fcr.2018.09.013

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Abstract

Heat stress is a significant abiotic stress limiting crop production in many regions globally, including in the Mediterranean-type environments of southern Australia. Various approaches have been used to understand the negative impacts of heat stress conditions on plant function and crop productivity, with several loci identified with proposed benefits for adaptation to heat stress conditions. However, there has been little uptake of targeted selection for heat stress traits or loci by breeding programs.

This study used a combination of controlled environment evaluation targeting heat stress conditions during grain filling (three consecutive days of 36°C with a wind speed of 40 km h^{-1} starting 10 days post anthesis), and evaluation over multiple representative field environments. Field environments were targeted to achieve a range of heat stress conditions during the sensitive anthesis and grain filling developmental stages and were conducted within the South Australian cereal producing region, with temperature co-variates used to quantify the level of stress in each environment. A series of experiments evaluated relevant Australian varieties and advanced breeding lines to evaluate the level of adaptation to heat stress conditions currently available in adapted germplasm. This allowed the impacts of heat stress on grain yield, and the role of heat stress during anthesis and grain filling on variety performance to be evaluated. In a second component of the study, seven doubled haploid mapping populations were evaluated to identify QTL for adaptation to heat stress conditions. The QTL analysis was conducted to identify performance (QTL with stable performance regardless of heat stress treatment, or across a range of stress conditions in the field), and to identify responsiveness (QTL with a favourable response to heat stress treatments in a controlled environment, or a favourable response to increasingly stressful conditions

experienced in the field), with numerous QTL identified in both controlled environment and field conditions.

The QTL identified provides opportunities for breeders to target improved adaptation to heat stress conditions through two mechanisms: performance QTL for stable and elite adaptation across all environments, and responsiveness for specific adaptation allowing selection of a favourable response to stressed conditions. This study proposes that assessing adaptation to heat stress conditions as the combination of performance and responsiveness is an improved definition and framework to assess tolerance to heat stress conditions, and is of greater relevance to breeders' selection objectives.

Statement of Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

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I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Scholarship.

Paul Robert Telfer 21/04/2022

Statement of Acknowledgment

Many people have supported me through the process of completing my Ph.D., all of them requiring more thanks than can be done justice here. I was warned that conducting a Ph.D. part-time while working full-time would be challenging, something that I can confirm to be acutely true. However, the support from my wife, family, supervisors, friends, and colleagues has made the process much more manageable and enjoyable. Additionally, the opportunities offered along the way have made it all the more rewarding, as I have had both academic and practical on the job experience in my training as a plant breeder that has been invaluable.

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Everyone at AGT, particularly the personnel based at Roseworthy (including Simeon, Rowan, Jake, Stacey, Robert, and Kath), your support and assistance has been incredible, without the assistance of managing trials, none of this would have been possible. A special thanks to Cassandra whose efforts in helping to manage the controlled environment experiments were huge. Also thank you to Dion Bennett for the contribution to the AGT heat stress projects before I joined that formed the basis for much of this project, and for being a sounding board for ideas for many years.

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has been amazing! Thanks also to my family, particularly my parents Greg and Pam, who have always supported me in all my endeavours and instilled a strong work ethic and to always push to succeed to the best of one's abilities.

Chapter 1. Introduction and Literature Review

1.1 Australian Wheat Production

Bread wheat (*Triticum aestivum* L.) is the largest cereal commodity in Australia, with production averaging 24.6 million tonnes per annum over the last 10 years, produced from an average of 12.0 million ha (2010-2011 to 2020-2021), with an average yield of 2.0 tonnes per hectare (ABARES 2021) across the Australian wheat belt (Figure 1). From 2010-2011 through to 2020-2021 Australian wheat exports were valued at approximately $5.36 billion per annum for the Australian economy with an average of 17 million tonnes exported annually (ABARES 2021). Relative to the rest of the world Australian wheat production is quite small, with global production averaging 733 million tonnes annually. But with global wheat exports totalling an average of approximately 170 million tonnes annually, Australia contributes a relatively large proportion of the global wheat trade (ABARES 2021). However, with increasing input costs, improved productivity is required to ensure the long-term sustainability and profitability of Australian farms.

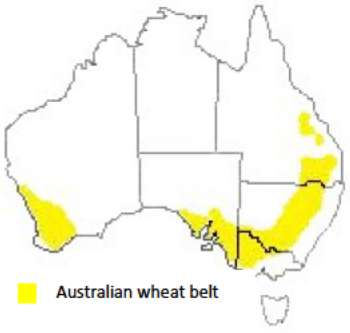

Figure 1. The Australian wheat belt (AWB 2012).

Achieving this is crucial not only to ensure profitable agricultural production in Australia but to increase the food supply for a growing global population and sectors of the world's population which are currently undernourished (FAO 2013).

1.2 Production Constraints in Southern Australia

Several factors limit agricultural production in southern Australia. These include drought stress, heat stress, frost, hostile soil conditions, and plant diseases. The southern Australian environment is classed as a Mediterranean-type environment, characterized by hot dry summers and cool wet winters. Winter crops are sown into moist soil from late autumn to early winter to utilise winter rainfall, with crops harvested in early summer (Castri, Mooney et al. 1973, French and Schultz 1984). Variable rainfall and temperature throughout the growing season can restrict crop productivity, stressing plants, particularly leading up to, during, and post-anthesis. Such variability in Australia's climate is related to the El Niño, La Niña, Southern Annular Mode, and the Indian Ocean Dipole climate phenomena (Montazerolghaem, Vervoort et al. 2016). There is also evidence to suggest that climate variability could be increasing, associated with atmospheric carbon dioxide levels and other greenhouse gases (Easterling, Evans et al. 2000, Murphy and Timbal 2008, Fierro and Leslie 2013), which may further exacerbate the frequency and severity of abiotic stress conditions experienced by crops.

1.2.1 Heat stress

Heat stress occurs when plants experience temperatures above optimum for plant function resulting in reduced growth or physical damage. During anthesis the optimal temperature for plant function is 21°C, with a maximum temperature of 31°C, beyond which plant function virtually stops with limited ability for plant function to recover. During grain filling the optimal temperature is 20.7°C with a maximum of 35.4°C (Porter, Nguyen et al. 1995). Heat stress and its effects on plants is a complex interaction between the intensity, duration, and rate of temperature increase (Wahid, Gelani et al. 2007). Wheat is affected differently at different

stages of growth. The reproductive tissues are the most sensitive to heat stress, including at the early stages of pollen development during the young microspore stage (Saini, Westgate et al. 2000, Dolferus, Ji et al. 2011). During anthesis heat stress can lead to reduced fertility (pollen viability), and induced sterility (floret abortion), resulting in reduced grain number (Barnabas, Jager et al. 2008). Heat stress during grain filling can lead to reduced grain size as a result of faster plant maturation (Wardlaw 1994, Wollenweber, Porter et al. 2003), accelerated senescence, and reduced plant photosynthetic capacity (Tewolde, Fernandez et al. 2006). In Australian farming systems crops are more frequently exposed to temperature stress during the anthesis and grain filling stages of crop development (Alexander, Hayman et al. 2010). These plant responses are largely a function of more localised and specific responses to heat stress within the plant tissue, such as enzyme denaturation, altered membrane fluidity, and creation of reactive oxygen intermediates (Wollenweber, Porter et al. 2003, Wahid, Gelani et al. 2007).

Plants have several mechanisms which limit the damage caused by heat stress and induce thermotolerance to reduce the potential risk of additional damage under further heat stress conditions. An example is the accumulation of heat shock proteins as discussed by Kotak, Larkindale et al. (2007). These heat shock proteins aid in protecting proteins, particularly those important for respiration and photosynthesis, and other cell structures against heat-related damage, and repair minor damage after the stress has occurred. Various plant hormones, particularly ethylene and abscisic acid, have also been reported as having a role in plant responses to heat stress. Plants with increased levels of abscisic acid display improved heat stress tolerance through regulation of sink size during stress, in a similar fashion to drought and cold stress (Wahid, Gelani et al. 2007, Dolferus, Ji et al. 2011).

Various studies have observed the negative impact of heat stress events on grain yield (Gibson and Paulsen 1999, Tewolde, Fernandez et al. 2006, Kuchel, Williams et al. 2007, Bennett,

Izanloo et al. 2012, Gourdji, Mathews et al. 2012, Guan, Lu et al. 2018, Li, Mao et al. 2019, Liu, Sukumaran et al. 2019, Tadesse, Suleiman et al. 2019, Thistlethwaite, Tan et al. 2020). Gibson and Paulsen (1999), identified a three to five percent reduction in yield for every one degree increase in temperature over 15°C under controlled environment conditions. Bennett, Izanloo et al. (2012) identified a reduction of 187 kg ha^{-1} for every one degree increase in average temperature during anthesis and grain filling. This shows that heat stress does have considerable negative effects on agricultural production and is an area of crop adaptation that needs to be investigated. However, given the complexity of this trait, it has been difficult to investigate. Nonetheless, due to the potential losses that can be incurred as a result of this stress, it is one of the most important abiotic stress limitations to overcome in order to improve yield potential in southern Australia.

1.2.2 Drought stress

Like heat stress, drought stress (moisture depravation) during the growing season is an issue for large areas of land used for agricultural production throughout the world. There are similarities in how drought and heat affect plants, and often the two stresses coincide in the growing environment (Machado and Paulsen 2001, Shah and Paulsen 2003, Toreti, Cronie et al. 2019). It has also been suggested that heat and drought stress interact, by altering plant water status and reducing water use efficiency (Machado and Paulsen 2001, Shah and Paulsen 2003), and in combination contribute to larger crop losses (Liu, Able et al. 2019). In Australia, drought is a common problem, particularly terminal drought, when regular rainfall stops late in the season exposing plants to water stress during grain filling (Richards, Rebetzke et al. 2010). This stress is often severe, causing reductions in plant growth, and potentially large reductions in grain yield (Hsiao and Acevedo 1974, Debaeke and Aboudrare 2004, Rajala, Hakala et al. 2009).

There is considerable variation in plant performance under drought conditions present in several plant species, indicating the potential to improve crop performance under drought conditions (Richards 2006). Large improvements in adaptation to drought-prone environments in Australia have been achieved through the development of crops with appropriate phenology. It was noted very early in Australia by Farrer (1898) that long-season varieties from elsewhere in the world were not suited to many wheat-growing regions in Australia. Farrer sourced lines from India and South Africa to use in crossing, producing the variety Federation in 1901 (Macindoe and Walken Brown 1968, Eagles, Cane et al. 2009). Since then, correctly matched phenology has been identified as a key adaptation trait (Reynolds and Tuberosa 2008, Eagles, Cane et al. 2009, Richards, Rebetzke et al. 2010, Flohr, Hunt et al. 2017).

During drought stress plants can become desiccated to the point of being irreversibly damaged, whereby normal function is not resumed upon rewatering (Hsiao and Acevedo 1974). There are numerous functions affected, which create a complex response within plants. These include adverse effects on cell growth, cell wall synthesis, protein synthesis, abscisic acid accumulation, stomata control, and respiration (Hsiao and Acevedo 1974). Several physiological traits have been investigated to potentially improve drought performance relating to plant water use, water use efficiency, and harvest index (Reynolds and Tuberosa 2008). These traits have the potential to contribute to improved drought performance. However, an in-depth understanding of how traits affect performance, their interactions with the environment, and how trait value can change with different environments; needs to be better understood (Richards, Rebetzke et al. 2010). Some physiological traits that may provide drought stress tolerance include transpiration efficiency (measured by carbon isotope discrimination), canopy temperature, reduced tillering, glaucousness, high early vigour, carbohydrate storage and immobilisation, green leaf area maintenance, root vigour and structure, and the presence or absence of awns (Blum, Shpiler et al. 1989, Richards 2000, Richards, Rebetzke et al. 2002, Richards, Rebetzke et al. 2010). All of these traits remain areas

of current research. However, carbon isotope discrimination, conferring improved efficiency of CO_2 fixed for every unit of water transpired during transpiration (Rebetzke, Condon et al. 2002), has resulted in a commercial variety, Drysdale, with improved drought adaptation.

1.2.3 Hostile soils

Numerous soil constraints act to reduce production across the Australian wheat belt. These include salinity, sodicity, soil acidity, and nutrient toxicities such as boron and aluminium toxicity as well as nutrient deficiencies including zinc and manganese. Some of these stresses, such as aluminium toxicity in acid soils has been shown to increase the severity of drought (Yang, Rao et al. 2013), and the presence of the aluminium tolerance gene *TaALTMT1-V*, may confer improved performance under spring heat stress conditions (Eagles, Cane et al. 2014).

Saline soils cover up to 60% of the cropping area of Australia, with sodium chloride being the most common salt contributing to reduced yield potential. Salinity reduces plant growth through osmotic changes within cells and tissues, and direct toxicity (Rengasamy 2002). If sodium levels are high enough, soil structure and porosity are adversely affected, which in turn reduces plant available water, decreasing transpiration, decreasing root turgor, and reducing cell membrane pressure. This leads to stressed leaves and ultimately reduced grain yield (Rengasamy 2002, Munns, James et al. 2006).

Boron is an essential micronutrient for plant function and is often deficient in agricultural soils internationally (Gupta, Jame et al. 1985). However, widespread areas of southern Australia have soil boron levels that are toxic (Cartwright, Zarcinas et al. 1986). Toxicity symptoms include reduced shoot vigour, root growth inhibition, leaf yellowing, and mottling, resulting in reduced plant biomass and grain yield (Cartwright, Zarcinas et al. 1984, Gupta, Jame et al. 1985, Cartwright, Zarcinas et al. 1986).

1.2.4 Other stresses affecting production

In addition to heat and drought stress and the numerous stresses involved with hostile soils, there is a myriad of other stresses adversely affecting crop production. These stresses may interact with one another as well as with abiotic stresses such as heat stress, increasing plant susceptibility, and the extent to which plant function and production is affected (Reynolds and Tuberosa 2008, Richards, Rebetzke et al. 2010). Understanding their role in plant health needs to be considered as a confounding effect when investigating abiotic stresses.

Frost stress can adversely affect all stages of crop growth. In Australia frost is common during spring when frost forms under clear night skies where radiation from the crop exceeds incoming radiation, causing temperatures to decline, and damage to plants below -2°C (Marcellos and Single 1975, Reinheimer, Barr et al. 2004). These conditions hinder male and female gametophyte development, adversely affect anthesis, fertilisation, and grain formation, and can lead to floret abortion and damaged grains (Thakur, Kumar et al. 2010). Various options to improve tolerance have been proposed including tolerance genes (Sutka 2001, Reinheimer, Barr et al. 2004) and manipulating metabolite accumulation (Galiba 1994, Sutka 2001, Thakur, Kumar et al. 2010). However, in farming systems in Australia, producers endeavour to use the correct combination of crop planting time and phenology such that anthesis occurs in the optimal risk window to minimise exposure to frost conditions in spring and temperature stress conditions as the season progresses (Bassu, Asseng et al. 2009, Zheng, Chenu et al. 2012, Flohr, Hunt et al. 2017).

Cereal cyst nematode (*Heteroda avenae*) lives in, and produces cysts on, the roots of cereals, infecting up to two million hectares of Victoria and South Australia (Brown 1984). Lighter soils are more commonly affected, with symptoms including leaf yellowing and crop patchiness resulting in stunted growth and reduced grain yield (Brown 1984, Vanstone, Hollaway et al. 2008). Cereal cyst nematode is normally managed through crop rotation with break crops and

through resistance genes including the *Cre1*, *Cre3*, *Cre5,* and *Cre8* genes (Ogbonnaya, Subrahmanyam et al. 2001, Safari, Gororo et al. 2005, Vanstone, Hollaway et al. 2008).

Three species of rust, a fungal pathogen, are prevalent in Australia, stem rust (*Puccinia graminis*), leaf rust (*Puccinia recondita*), and stripe rust (*Puccinia striiformis*). Stipe rust is the most significant of the three rusts to the Australian wheat crop, with estimated losses in the order of $127 million per annum. However, is estimated to be much higher at $994 million per annum if management options were not utilised (Murray and Brennan 2009). Yellow leaf spot (*Pyrenophora tritici-repentis*) is another fungal pathogen affecting all regions of the Australian wheat belt (Murray and Brennan 2009), producing necrotic lesions on leaves adversely affecting photosynthesis and grain yield.

Soil-borne fungal pathogens contribute considerable losses to crop production. Three notable examples include rhizoctonia root rot or bare patch (*Rhizoctonia solani*), crown rot (*Fusarium pseudograminearum),* and Take-all (*Gaeumannomyces graminis* var. *tritici*). Rhizoctonia is estimated to cause an average two percent reduction in grain yield across southern Australia annually. Symptoms include bare patches or patches of severely stunted plants with reduced tillering. Crown rot is estimated to cause an average yield reduction of 1.8% across southern Australia annually (Murray and Brennan 2009), with typical symptoms including brown colouring of stems near the crown and white coloured heads after heading. Both of these diseases need to be considered when investigating abiotic stresses, as their root-trimming effects reduce plant water availability and produce plant phenotypes much like drought and heat stress.

1.3 Improving Adaptation and Production Through Genetic Variation and Breeding Methodologies

Breeding programs aim to improve the yield (productivity) and quality (value) of crops through repeated cycles of cross hybridisation and selection to exploit variation within available

germplasm. Various breeding methodologies can be employed to achieve this. Some are more effective for qualitative traits and others have advantages when targeting quantitative traits such as abiotic stresses, or a target population of environments with high levels of genotype by environment interactions.

1.3.1 Variation for adaptation within Australian and International germplasm

There is extensive genetic variation for tolerance to many production-limiting factors within Australian germplasm. Fleury, Jefferies et al. (2010) described elite drought tolerant lines including RAC875, Excalibur, and Gladius, while Esten Mason, Mondal et al. (2011) described the heat stress tolerant line Halberd. In addition to the variation that we are currently aware of, the potential exists to identify sources of tolerance from sources that have had reduced influences from domestication, with more than six million agricultural crop and close relative accessions being stored worldwide (Hammer, Arrowsmith et al. 2003, Glaszmann, Kilian et al. 2010). Through the focused identification of germplasm strategy (FIGS), germplasm originating from areas that often experience the conditions of interest can be targeted to identify novel sources of tolerance (El Bouhssini, Street et al. 2011). Additionally, pre-domestication sources of genetic diversity have been investigated as options for additional sources of novel genetics for adaptation, including heat stress, through the use of synthetic hexaploid wheat. Synthetic hexaploid wheat is created by recreating the natural hybridisation events of approximately 8000 years ago that combined tetraploid *Triticum turgidum ssp. durum* L. and diploid *Aegilops tauschii* (Cossani and Reynolds 2015, Bhatta, Morgounov et al. 2018). Similarly, close relatives of domesticated hexaploid wheat have been investigated as potential sources of exotic adaptation to stress conditions including heat stress. For example, Emmer wheat (*Triticum dicoccon* Schrank) has been identified by Ullah, Bramley et al. (2018) as a potential source of novel adaptation to heat stress conditions. These sources of variation can then be exploited through breeding programs to improve crop adaptation.

1.3.2 Genotype by Environment interactions (GxE)

The selection of superior varieties best occurs in target environments where the varieties will be grown (Richards, Rebetzke et al. 2010). However, the target population of environments may consist of sub-sets of highly heterogeneous environments. Variation may be both spatial and temporal (Finlay and Wilkinson 1963, Basford and Cooper 1998). Given environmental variation, it can be difficult to gain a true representation of how a variety will perform without testing the variety over a range of environments and seasons. This interaction between genotype performance, the location, and the season is referred to as a genotype by environment interaction (GxE). The environment component is a complex interaction of soil properties, climate, disease profiles (Reynolds and Tuberosa 2008), and crop management practices (Reynolds and Tuberosa 2008, Passioura, Angus et al. 2010).

An understanding of the stability of a variety's performance across a range of environments is important in understanding how adapted a variety is. Finlay and Wilkinson (1963) discussed how the performance ranking of varieties can change based on their adaptation profile and yield stability under favourable and unfavourable environments. There are four basic adaptation profiles, with a continuum between each. These are; well adapted to all environments, specifically adapted to favourable environments, specifically adapted to unfavourable, and poorly adapted to all environments. In Australia, stressful conditions are common and therefore adaptation to unfavourable environments is desirable in a variety. In the same way a variety can have a GxE interaction for grain yield, so too can genes or QTL (Quantitative Trait Locus). Tura, Edwards et al. (2020), and Bonneau, Taylor et al. (2013) identified the expression of QTL for grain yield and other performance traits, changing across their sample environments. Further, instances have been identified where QTL expression was found to interact with various environmental co-variates sampled (including temperature), more deeply explaining components of the environment influencing gene or QTL expression

(Baenziger, Budak et al. 2004, Malosetti, Voltas et al. 2004, Kuchel, Williams et al. 2007). Understanding the interaction of heat stress tolerance traits with the environment will be crucial to understanding plant adaptation to heat stress conditions and being able to effectively select for improved adaptation within a breeding program.

1.3.3 Breeding methodologies

Various breeding methodologies have been used by breeding programs, with each offering certain advantages and disadvantages when selecting for adaptation to heat stress conditions and other abiotic stress conditions. The pedigree method has been the basis of many programs, including that of CIMMYT (International Maize and Wheat Improvement Center) when it was in its infancy (Ginkel, Trethowan et al. 1998). This method (Figure 2) involves having F_1 seed grown as a bulk, with single head or plant selections occurring within the F_2 generation and subsequent generations until families become homozygous, at which point larger scale yield plot evaluation can occur to select preferred progeny (Allard 1960, Ginkel, Trethowan et al. 1998, Gupta 2009). During the generations of single plant parentage, culled siblings can be bulked to allow yield testing of families to improve selections of the next generation. Advantages of the pedigree system include the ability to take advantage of maximum heterozygosity in the F_2 generation, information from previous generations can be used to aid in selections, and is effective for simply inherited traits (Gupta 2009). However, drawbacks include that selection for complex traits such as yield and broad adaptation cannot occur within early generations (Allard 1960, Ginkel, Trethowan et al. 1998).

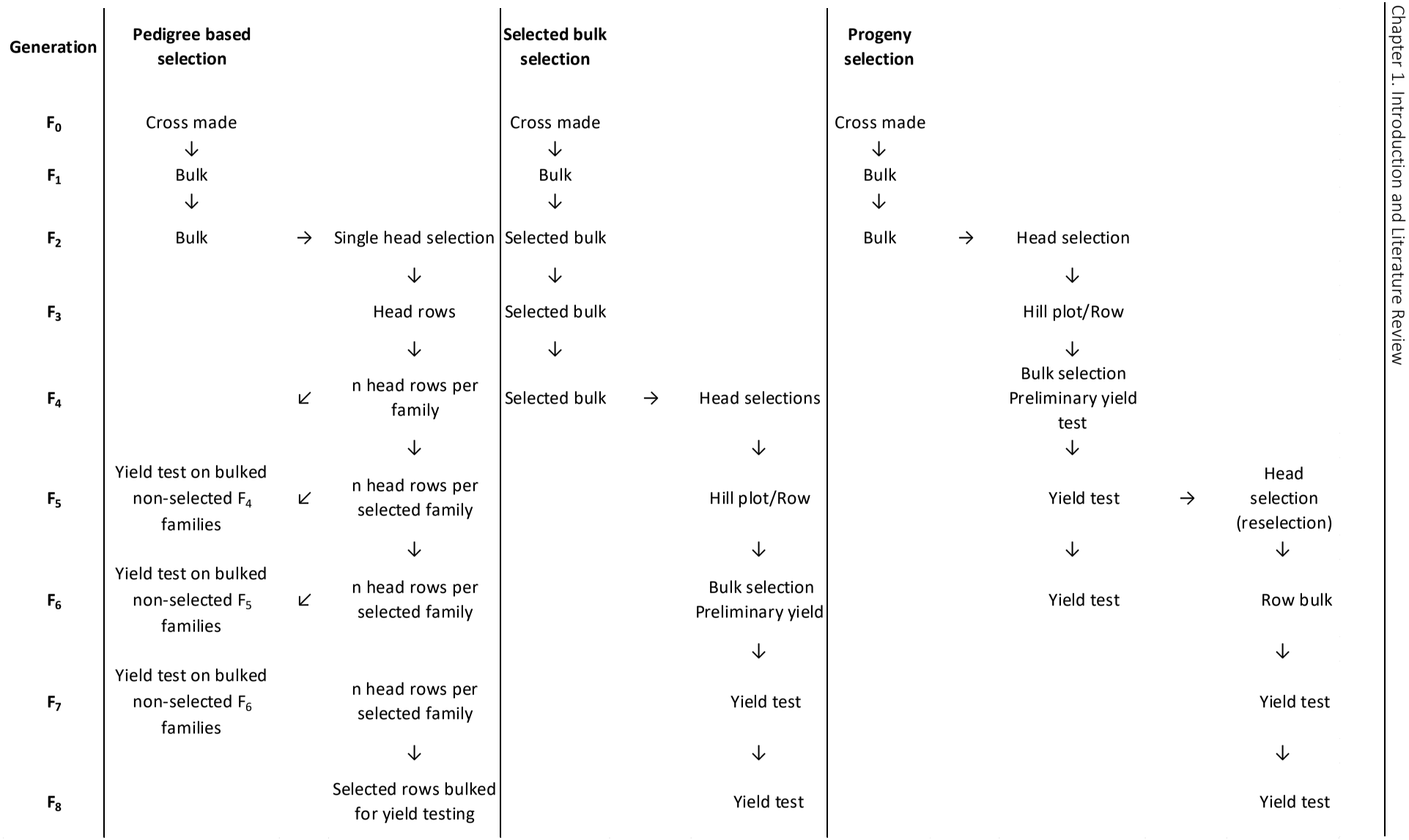

Figure 2. A stepwise diagram of the selection processes involved with pedigree selection, selected bulk selection, and progeny selection.

An alternative to the pedigree method is the selected bulk method. This method (Figure 2) involves each generation being harvested as a bulk from the F_2 through to the F_4. Following this, single plant selections are made and planted in rows or hill plots, the progeny of which are first yield tested in the F_6 (Allard 1960, Kuckuck, Kobabe et al. 1985, Gupta 2009). Advantages of this method include simplicity and reduced cost, record keeping, and breeder input in early generations. However, disadvantages include limited selection opportunities (except for traits able to be assessed on single plants), particularly that of yield and quality, up until the F_6 generation (Gupta 2009). This subsequently means that the method takes longer than the pedigree method as extensive yield, quality screening and assessment of broad adaptation occurs at a later generation.

In a bid to overcome the drawbacks of both the pedigree and bulk breeding methods various combinations of the two have been implemented. One such example is F_2 Progeny selection, as has been used by the University of Adelaide breeding programs (Hollamby and Bayraktar 1996). Progenies of F_2 lines are selected based on characteristics of the parent lines and bulked in the F_3 generation before yield testing in the F_4 generation (Figure 2). Yield testing in earlier generations relative to pedigree and bulk breeding methods is an advantage when breeding for diverse environments, prone to various and variable stresses such as heat and drought stress. A reselection in the F_5 generation, such as what occurs in the Roseworthy wheat breeding program (Haydn Kuchel, *pers. comm.*), allows line uniformity to be ensured, overcoming any lack of homozygosity resulting from an F_2 selection.

1.3.4 Trait-based breeding

Specific physiological traits are often suggested as being important for improving varietal performance for grain yield and performance under stressful conditions, such as heat stress. These generally aim to improve the yield potential, through improved water use, increased plant productivity through increased biomass production with a maintained harvest index, and

increased radiation capture and use (Reynolds, Bonnett et al. 2011). This has been discussed in relation to improving adaptation to drought conditions through traits such as carbon isotope discrimination; resulting in the wheat variety Drysdale (Rebetzke, Condon et al. 2002, Richards 2002), stem water-soluble carbohydrate accumulation (Richards, Rebetzke et al. 2010), early vigour (Rebetzke and Richards 1999), extended crop duration (Richards 2000, Richards 2006, Hunt, Lilley et al. 2019), and increased coleoptile length (Condon, Richards et al. 2004, Richards, Rebetzke et al. 2010).

Traits identified as important and given priority for selection within a breeding program, are often incorporated into an adapted background using backcrossing and selection tools such as physical assays or marker-assisted selection (Richards 1996). An example of this is the incorporation of boron tolerance into wheat in southern Australia. Superior, tolerant lines (Halberd) were identified using a physical assay, crossed to elite broadly adapted germplasm (Schomburgk), progeny tested for tolerance, and backcrossed to the recurrent parent, resulting in the commercial release of the BT-Schomburgk variety and conferred an 8.5% increase in yield over the recurrent parent (Rathjen, Moody et al. 1995).

1.3.5 Adaptation-based breeding

A different philosophy from targeting specific traits is to prioritise the selection of grain yield while ensuring high levels of genetic variability in the parent germplasm pool, including potential novel traits of interest (Hollamby 1973, Hollamby and Bayraktar 1996). When this occurs over a wide range of environments it should ensure wide adaptation of varieties. Along with this, adequate levels of quality and agronomic traits such as disease resistance need to be maintained to meet producer and end-user requirements (Hollamby and Bayraktar 1996). This is of relevance to abiotic stresses, as selecting for yield under such conditions, will confer improved adaptation to these conditions, if there is sufficient genetic variability available in the parent germplasm pool.

1.4 Tools to Aid in Breeding and Selection for Improved Abiotic Stress Adaptation

In addition to the breeding methodologies previously discussed, there are numerous tools that breeders can implement at various stages of the breeding program to aid in selection.

1.4.1 Recurrent and early generation selection

Recurrent selection is a cyclical breeding strategy that enables the concentration of favourable alleles scattered among multiple individuals to be combined over multiple generations of crossing and favourable trait selection while maintaining genetic variability within a breeding population. In essence, this results in favourable selections being crossed in all possible combinations before selection occurs again in the subsequent self-pollinated generation (Allard 1960). This method was first successfully employed by Sprague and Brimhall (1950) who used the recurrent selection technique in maize to increase the oil content of the grain thereby improving an important grain quality component. A two to six-fold increase in breeding efficiency for grain oil content was shown when compared to traditional breeding techniques.

Recurrent selection has also been successfully used in wheat; examples include Loffler, Busch et al. (1983), who used recurrent selection techniques to increase grain protein content by 2.5% over two cycles of selection. Avey, Ohm et al. (1982) used recurrent selection techniques to select for early maturing plant types, successfully reducing the duration to heading, with the greatest gains in the first cycle of selection. Similarly, Wiersma, Busch et al. (2001) showed that the grain weight of wheat could be increased on average by 4.5% per cycle.

Early generation selection, with selection in successive generations of a segregating population, is an alternative to the traditional recurrent selection, where selection is performed over multiple generations within a segregating population. This method is distinct from recurrent selection techniques, as selected lines are not inter-crossed between generations where selection takes place. This method was used to select for higher levels of

grain dormancy as a way of reducing pre-harvest sprouting in wheat (Hickey, Dieters et al. 2010). Levels of grain dormancy were successfully increased with selection occurring over the F_2 and F_3 generations and population shift assessed in the F_4. However, the maximum level of dormancy achieved was only equal to that of the dormant parent, with the accumulation of all dormancy alleles from the dormant parent being the maximum level of dormancy possible. If the parents used to create the segregating populations combine different genes controlling a trait, they can undergo transgressive segregation, and it may be possible to achieve trait levels in excess of either of the two parents.

Drawbacks of recurrent selection include the time and resources required to facilitate the inter-crossing of all lines, particularly in wheat which is a self-pollinated crop. Also, selection is usually targeted at one key trait of interest, potentially leaving other traits to decline. This was discussed by Wiersma, Busch et al. (2001), where traits including grain number per spike and tiller number declined in response to selection for grain weight. As a result, this method of recurrent selection is of limited use except for producing parent material to incorporate into a breeding population as a donor parent for traits of interest (Loffler, Busch et al. 1983).

Recurrent selection or early generation selection has the potential to be used in the selection of material adapted to abiotic stresses such as heat stress. Heat stress is a complex trait, with many sub-traits influencing tolerance. However, if a controlled environment assay that exposes plants to a consistent stress representative of field conditions, is employed as a selection tool to select for improved heat stress performance; it may be possible to generate elite parental material for a breeding program.

1.4.2 Genetic tools

Genetic markers are identifiable and variable (polymorphism) sequences of DNA that may be linked to genes responsible for traits of interest. Numerous markers have been identified for

traits across many crop species. However, progress in wheat has been slower due to the size and complexity of the wheat genome and the smaller number of polymorphisms identifiable (Langridge, Lagudah et al. 2001, Xu 2010).

Traditional breeding techniques can be assisted and accelerated through a greater understanding of the underlying genetics and the use of genetic markers in selection for superior plant types and traits (Reynolds, Bonnett et al. 2011). The use of genetic markers as an aid in selection is known as marker-assisted selection (MAS) and has been applied in numerous instances, but primarily for simple qualitative traits controlled by a single gene (Eagles, Bariana et al. 2001, Francia, Tacconi et al. 2005, Xu 2010). However, many agronomically important traits are quantitative, controlled by a combination of numerous minor genes. Regions containing genes contributing to these traits are mapped using quantitative trait locus (QTL) analysis (Collard, Jahufer et al. 2005, Francia, Tacconi et al. 2005). Yield and traits related to abiotic stresses (such as heat stress) are often quantitative traits, with numerous sub-traits and genes contributing to overall performance and adaptation. QTL are identified using populations derived from parents contrasting for the trait of interest. This allows for an association between genotype and phenotype to be identified for that particular trait (Collard, Jahufer et al. 2005). Using marker information, a linkage map is developed to indicate the relative distance between markers. This in combination with phenotype data for various traits, is used for QTL analysis (Eckermann, Verbyla et al. 2001).

Before marker selection of QTL can be implemented within a breeding program validation needs to occur (Yun, Gyenis et al. 2006) to ensure markers are sufficiently linked to traits, representative of germplasm pools in which it would be used, and all GxE interactions are understood (Eckermann, Verbyla et al. 2001, Narasimhamoorthy, Gill et al. 2006, Kuchel, Williams et al. 2007). Numerous QTL identified have failed to be implemented as a result of not meeting these criteria (Eagles, Bariana et al. 2001, Sharp, Johnston et al. 2001). However,

examples of the successful application of single-gene markers exist including Kuchel et al. (2007) who successfully used markers in wheat to select for two rust genes *Lr34/Yr18* and *Lr46/Yr29*, height regulating genes, and grain protein genes *Glu-D1* and *Glu-A3.* Potential benefits include time savings, reduced reliance on field trials, reduced reliance on variable phenotypic evaluation, gene pyramiding, and increased selection accuracy for traits with low heritability (Collard, Jahufer et al. 2005, Kuchel, Fox et al. 2007).

Genetic modification (GM) is another genetic tool available to breeders, allowing opportunities to source genetic diversity beyond the scope of the species of interest (Nelson 2001, Tester and Langridge 2010). The most notable examples of GM application to date include insect resistance, chemical resistance, improved crop quality, and bio-fortification. Commercial crops containing novel genes introduced through GM are used extensively, particularly in the Americas, where *Bt* corn and cotton, as well as Roundup-Ready soybean, corn, and canola, are common (Nelson 2001, James 2010). Alternatively, GM has been used for the bio-fortification of crops, including β-carotene bio-fortified rice (or Golden rice as it is otherwise known), to help alleviate vitamin A deficiency in rice-dependent diets (Paine, Shipton et al. 2005). More recently, the *HaHB4* gene identified in sunflower has been transformed through GM technology into wheat and soybean, with the potential to improve crop performance in drought conditions. Evaluation of this trait continues to further understand what role it may have during drought and what environmental conditions it may confer an advantage (González, Rigalli et al. 2020). In the future, there may be additional GM traits identified which may allow for new novel sources of abiotic stress tolerance to be sourced from other species to improve adaptation.

1.4.3 Genomic selection

Genomic selection (GS) or whole genome selection is a relatively new genetic tool being explored by breeders to aid in selection and breeding, particularly for quantitative traits; grain

yield, and potentially abiotic stresses such as heat and drought tolerance. Two phases are involved with GS, firstly generating breeding values for markers within a training population, then using those estimated values to select between related individuals with no, or limited phenotype data, based on their marker profiles (Reynolds, Bonnett et al. 2011, Zhao, Gowda et al. 2012). Genomic selection allows all genetic effects, regardless of size to be included in the genetic estimates, resulting in greater efficiency of selection (Meuwissen, Hayes et al. 2001) compared to marker selection for single genes or QTL. Early research into the application of GS in plant breeding occurred in bi-parental populations or similar, to simulate the application of GS within a breeding population (Reynolds, Bonnett et al. 2011). However, breeding populations are much more complex, consisting of individuals with complex interactions of relatedness that have been built up over many cycles of crossing (Zhao, Gowda et al. 2012). More recently, studies like that of Norman, Taylor et al. (2017) have used subsets of breeding programs to validate on a larger and more representative scale, showing that GS techniques can be effective. The effective inclusion of GxE in selection models remains a current area of research.

There are similarities between QTL analysis and GS. The same genotype and phenotype information is required. The difference for GS is that estimates of genetic effect are calculated for the whole genome, rather than just QTL, and these are referred to as genomic estimated breeding values (GEBVs) (Tester and Langridge 2010, Reynolds, Bonnett et al. 2011, Nakaya and Isobe 2012). Various models have been proposed to generate the GEBVs including least square analysis, random regression best linear unbiased prediction (RR-BLUP) analysis, and Bayesian shrinkage (Bayes) methods (Meuwissen, Hayes et al. 2001, Heslot, Yang et al. 2012, Zhao, Gowda et al. 2012, Habier, Fernando et al. 2013). Accuracy of selections using GEBVs has been shown to increase with higher marker density and increased training population size, particularly with low heritability traits (Nakaya and Isobe 2012, Zhao, Gowda et al. 2012, Norman, Taylor et al. 2018). Genomic selection methodology has been tested in wheat for

grain yield and related traits (Crossa, de Los Campos et al. 2010) with improved selection accuracy over MAS (28%) and phenotypic selection (14%) (Heffner, Jannink et al. 2011).

1.5 Previous Research Targeting Adaptation to Heat Stress Conditions

1.5.1 Previous methodologies used to evaluate heat stress adaptation

Various methods have been developed to study plant response to high-temperature growing environments and terminal temperature stress conditions. This includes using controlled environment assays, where all growing conditions are managed to allow for consistency and repeatability across experiments. In contrast to field conditions where variability in conditions within a season may mean that plants with a different time to anthesis may experience different stress levels, with further variation between seasons additionally impacting on repeatability. Controlled environment methods were used by Stone and Nicolas (1995), who screened heat stress responses in wheat using controlled environments with maximum temperatures of 40°C, 10 and 30 days after anthesis for three consecutive days, with irrigation to ensure no drought stress. This study showed susceptible lines reduced grain mass under heat stress conditions. Similarly, Esten Mason, Mondal et al. (2011) used 38°C, 10 days after anthesis for three consecutive days (nine hours a day), detecting differences in grain number and grain size under the stress conditions. However, despite the advantages of improved repeatability of controlled environment experiments, care needs to be taken in managing and interpreting the results of such studies as other confounding factors may be introduced. These include damage caused by the manual handling of plants when imposing stress treatments, CO_2 depletion if using recirculated air when, and keeping all growth and management the same for control and stressed plants throughout experiment cycles).

In a similar fashion to the controlled environment assays discussed, Australian Grain Technologies developed a controlled environment assay to target post-anthesis heat stress (D Bennett *pers. comm.*). Compared to previous studies similar developmental timing (10 days

post anthesis), temperature duration (three consecutive days for eight hours), and temperature (36 °C Day temperature) are used, with plant water status managed to remove drought effects. The point of difference with this assay is the optional addition of 40 km hr^{-1} winds and a lower humidity level than can be achieved in conventional growth rooms. The conditions created intend to target conditions representative of the heat stress conditions experienced during anthesis to early grain filling in southern Australia (Alexander, Hayman et al. 2010, Talukder, McDonald et al. 2013).

Although controlled environment conditions do offer advantages to consistency and repeatability of experimental conditions, evaluation under field conditions ensures relevance to 'real-world' growing conditions experienced by plants. A common method used to evaluate performance under terminal heat stress conditions is to use delayed-sowing (Reynolds, Pierre et al. 2007, Pinto, Reynolds et al. 2010, Bennett, Reynolds et al. 2012, Esten Mason, Hays et al. 2013, Sadras, Vadez et al. 2015, Pinto, Lopes et al. 2016). Delaying sowing time, delays plant development shifting the more sensitive reproductive and grain-filling stages later in the growing season to coincide with a higher incidence of heat stress. However, this also alters plant exposure to several other environmental conditions including longer photoperiod and altered plant available water (Sadras, Vadez et al. 2015), creating unrepresentative growing conditions. Care, therefore, needs to be taken when considering results from studies using delayed sowing and relevance to agronomically recommended sowing dates.

Increased consistency, repeatability, and relevance of field-based studies can be achieved by using agronomically recommended sowing dates and then imposing heat stress conditions at relevant developmental stages using plot-based heat chambers within field experiments (Alexander, Hayman et al. 2010, Talukder, McDonald et al. 2013, Thistlethwaite, Tan et al. 2020). However, the advantages are somewhat offset by the physical encumbrance of manhandling heat chambers in the field. This restricts the methodology to smaller more

detailed studies with a reduced number of lines evaluated, rather than large breeding populations.

It is well understood that there is a relatively high amount of GxE in environments such as Australia, making field studies conducted over a range of environments and seasons more important, and this is no different when examining adaptation to abiotic stresses such as heat stress. However, it can be difficult to attribute differential adaptation to stress conditions when there are numerous factors present across the target population of environments. Kuchel, Williams et al. (2007) were able to attribute variable QTL effects in different environments to changing environmental conditions across each environment, including to changing temperature conditions. Similarly, Malosetti, Voltas et al. (2004), and Baenziger, Budak et al. (2004) were able to use environmental co-variates to explain GxE and QTL by environment interactions.

1.5.2 Defining heat stress adaptation

Identifying the superior genotype that has improved performance under stress conditions is an important part of abiotic stress studies, to ensure the desired outcomes are achieved for improved adaptation. This is often defined as tolerance, resistance, or susceptibility. To achieve this several methods have been developed to define and measure differences in performance under stress conditions. Fischer and Maurer (1978) defined a susceptibility index comparing the performance of varieties under both water-limited and non-stressed growing conditions when evaluating adaptation to drought conditions. This method has been applied in heat stress studies by Esten Mason, Mondal et al. (2010), and Esten Mason, Hays et al. (2013). Later, Rosielle and Hamblin (1981) compared the mean performance of varieties under stressed and non-stressed environments, and this was applied in a heat study evaluating the impact of heat stress resulting from delayed sowing by Bennett, Reynolds et al. (2012).

However, these different methods or selection indices offer varying levels of effectiveness and potential unintended implications as studies by Sio-Se Mardeh, Ahmadi et al. (2006), and Mohammadi, Armion et al. (2010) have shown. Mohammadi, Armion et al. (2010) demonstrated that a susceptibility index, as defined by Fischer and Maurer (1978), favoured lines that have a low penalty in performance under stress conditions relative to unstressed conditions, but inadvertently also favoured lines that have an inherently low performance ability. This is an unfavourable combination not suitable for breeders to use in a selection program aiming to improve overall performance and adaptation to stressful growing conditions.

Lemerle, Smith et al. (2006) further defined tolerance as a positive deviation from the response expected under stress conditions relative to non-stressed conditions, after noting the frequent high correlation of performance under both stress and non-stressed conditions. This method, applied by Dolferus, Thavamanikumar et al. (2019), identifies tolerance in terms of responsiveness of a variety or gene to changing stress conditions separate from the overall performance. This is comparable to Blum (2005), who recognised that there needs to be concurrent and independent selection for yield potential and abiotic stress resistance to maximise the value of breeding efforts.

1.5.3 Understating the genetics of heat stress adaptation

Numerous studies have focused on the underlying genetic drivers of adaptation to heat stress conditions, identifying numerous QTL for a range of performance traits in wheat grown under heat stress conditions, as summarised in Tables 1 and 2. These studies have used the various controlled environment and field-based methods discussed previously. QTL for grain yield achieved in high-temperature conditions in the field has been identified by (Pinto, Reynolds et al. 2010, Bennett, Reynolds et al. 2012, Bonneau, Taylor et al. 2013, Esten Mason, Hays et al. 2013, Pinto, Lopes et al. 2016, Bhusal, Sarial et al. 2017, Tahmasebi, Heidari et al. 2017, Guan,

Lu et al. 2018, Hassan, Solouki et al. 2018, El Hassouni, Belkadi et al. 2019, Liu, Sukumaran et al. 2019, Tadesse, Suleiman et al. 2019). Additionally, in field conditions QTL have been identified for grain size (Pinto, Reynolds et al. 2010, Bennett, Reynolds et al. 2012, Paliwal, Röder et al. 2012, Pinto, Lopes et al. 2016, Bhusal, Sarial et al. 2017, Tahmasebi, Heidari et al. 2017, Guan, Lu et al. 2018, El Hassouni, Belkadi et al. 2019, Li, Mao et al. 2019, Liu, Sukumaran et al. 2019, Tadesse, Suleiman et al. 2019), grain number (Pinto, Reynolds et al. 2010, Pinto, Lopes et al. 2016, Bhusal, Sarial et al. 2017, Tahmasebi, Heidari et al. 2017, Guan, Lu et al. 2018, El Hassouni, Belkadi et al. 2019, Liu, Sukumaran et al. 2019, Tadesse, Suleiman et al. 2019), grain fill duration (Paliwal, Röder et al. 2012, Pinto, Lopes et al. 2016, Bhusal, Sarial et al. 2017), as well as for leaf senescence and stay-green phenotype (Pinto, Reynolds et al. 2010, Pinto, Lopes et al. 2016) (Table 1 and Table 2). Under controlled environment conditions QTL for grain number and grain size have been identified by Esten Mason, Mondal et al. (2010), Esten Mason, Mondal et al. (2011), Mohammadi, Zali et al. (2008), and Shirdelmoghanloo (2014), as well as for leaf senescence and stay green (Vijayalakshmi, Fritz et al. 2010, Shirdelmoghanloo 2014, Talukder, Babar et al. 2014) and grain fill duration (Shirdelmoghanloo 2014) (Table 1 and Table 2).

While several studies have identified QTL associated with performance under heat stress conditions, there are no known reports of routine use of these loci as a selection tool within a breeding program. This may be due to the complex nature of these traits and likely GxE interactions. Such GxE interactions were noted by Bonneau, Taylor et al. (2013) who looked to understand the interactions of heat and drought on QTL performance across multiple field environments. A better understanding of how these QTL interact with other environmental drivers of performance, or other adverse impacts on variety performance may improve adoption within breeding programs.

Table 1. Previous studies that have identified QTL for grain yield, grain size, grain number, grain fill rate, harvest index, senescence rate, and maturation rate where an interaction with heat stress has been identified or has been identified under heat stress conditions. Studies that have previously identified QTL related to heat stress adaptation are organised by chromosome (CH) and trait.

CH	Grain yield	Grain size	Grain number	Grain fill rate	Harvest index	Senescence rate	Maturation rate
1A	El Hassouni, Belkadi et al. (2019) Hassan, Solouki et al. (2018)	Esten Mason, Mondal et al. (2010) Pinto, Lopes et al. (2016) Liu, Sukumaran et al. (2019) Tadesse, Suleiman et al. (2019)	Liu, Sukumaran et al. (2019) El Hassouni, Belkadi et al. (2019)		El Hassouni, Belkadi et al. (2019)		
1B	Pinto, Reynolds et al. (2010) Pinto, Lopes et al. (2016) Hassan, Solouki et al. (2018) Tahmasebi, Heidari et al. (2017)	Mohammadi, Zali et al. (2008) Esten Mason, Mondal et al. (2011) Tahmasebi, Heidari et al. (2017) Tadesse, Suleiman et al. (2019) Li, Mao et al. (2019)	Pinto, Reynolds et al. (2010) Pinto, Lopes et al. (2016) Tahmasebi, Heidari et al. (2017) El Hassouni, Belkadi et al. (2019)	Pinto, Lopes et al. (2016)	El Hassouni, Belkadi et al. (2019)	Pinto, Lopes et al. (2016)	
1D	Tahmasebi, Heidari et al. (2017)	Li, Mao et al. (2019)					
2A	Bhusal, Sarial et al. (2017) El Hassouni, Belkadi et al. (2019)	Esten Mason, Mondal et al. (2010) Bhusal, Sarial et al. (2017) Tahmasebi, Heidari et al. (2017) Guan, Lu et al. (2018) Liu, Sukumaran et al. (2019)	Bhusal, Sarial et al. (2017) Liu, Sukumaran et al. (2019) El Hassouni, Belkadi et al. (2019)		El Hassouni, Belkadi et al. (2019)	Vijayalakshmi, Fritz et al. (2010) Pinto, Lopes et al. (2016)	Bhusal, Sarial et al. (2017)
2B	Hassan, Solouki et al. (2018)	Paliwal, Röder et al. (2012) Pinto, Lopes et al. (2016)	Esten Mason, Mondal et al. (2010) Liu, Sukumaran et al. (2019) Tadesse, Suleiman et al. (2019) Tahmasebi, Heidari et al. (2017) Guan, Lu et al. (2018)	Paliwal, Röder et al. (2012) Pinto, Lopes et al. (2016)		Pinto, Lopes et al. (2016)	
2D	Esten Mason, Hays et al. (2013) Hassan, Solouki et al. (2018)	Esten Mason, Mondal et al. (2011) Pinto, Lopes et al. (2016) Guan, Lu et al. (2018)		Pinto, Lopes et al. (2016)		Pinto, Lopes et al. (2016)	
3A	Bennett, Reynolds et al. (2012) Hassan, Solouki et al. (2018)	Bennett, Reynolds et al. (2012) Tadesse, Suleiman et al. (2019)	Liu, Sukumaran et al. (2019) El Hassouni, Belkadi et al. (2019)		El Hassouni, Belkadi et al. (2019)	Vijayalakshmi, Fritz et al. (2010)	

CH	Grain yield	Grain size	Grain number	Grain fill rate	Harvest index	Senescence rate	Maturation rate
3B	Pinto, Reynolds et al. (2010) Esten Mason, Hays et al. (2013) Bennett, Reynolds et al. (2012) Bonneau, Taylor et al. (2013) Pinto, Lopes et al. (2016) Tahmasebi, Heidari et al. (2017) Tadesse, Suleiman et al. (2019)	Pinto, Reynolds et al. (2010) Esten Mason, Mondal et al. (2011) Shirdelmoghanloo (2014) Shirdelmoghanloo, Taylor et al. (2016) Pinto, Lopes et al. (2016) Tahmasebi, Heidari et al. (2017) Tadesse, Suleiman et al. (2019)	Esten Mason, Mondal et al. (2010) Pinto, Reynolds et al. (2010) Pinto, Lopes et al. (2016) El Hassouni, Belkadi et al. (2019)	Shirdelmoghanloo (2014) Pinto, Lopes et al. (2016)	Shirdelmoghanloo (2014) El Hassouni, Belkadi et al. (2019)	Shirdelmoghanloo (2014)	
3D	Esten Mason, Hays et al. (2013) Bennett, Reynolds et al. (2012) Liu, Sukumaran et al. (2019)	Liu, Sukumaran et al. (2019)					
4A	Pinto, Reynolds et al. (2010) Pinto, Lopes et al. (2016) Tadesse, Suleiman et al. (2019) Tahmasebi, Heidari et al. (2017)	Esten Mason, Mondal et al. (2011) Pinto, Reynolds et al. (2010) Tadesse, Suleiman et al. (2019) Guan, Lu et al. (2018) Tahmasebi, Heidari et al. (2017)	Pinto, Reynolds et al. (2010) Pinto, Lopes et al. (2016) Guan, Lu et al. (2018)	Shirdelmoghanloo (2014) Pinto, Lopes et al. (2016)			
4B	Pinto, Lopes et al. (2016)	Pinto, Reynolds et al. (2010) Pinto, Lopes et al. (2016) Guan, Lu et al. (2018)	Pinto, Lopes et al. (2016) Tadesse, Suleiman et al. (2019)	Pinto, Lopes et al. (2016)		Shirdelmoghanloo (2014) Vijayalakshmi, Fritz et al. (2010)	
4D	Bennett, Reynolds et al. (2012)	Li, Mao et al. (2019) Tahmasebi, Heidari et al. (2017)					
5A	Esten Mason, Hays et al. (2013) Tadesse, Suleiman et al. (2019) El Hassouni, Belkadi et al. (2019)	Esten Mason, Mondal et al. (2011) Ali, Ibrahim et al. (2013) Tadesse, Suleiman et al. (2019) Guan, Lu et al. (2018) Li, Mao et al. (2019)	Guan, Lu et al. (2018) Tahmasebi, Heidari et al. (2017) El Hassouni, Belkadi et al. (2019)	Shirdelmoghanloo (2014)			Shirdelmoghanloo (2014)
5B	Bennett, Reynolds et al. (2012) El Hassouni, Belkadi et al. (2019)	Mohammadi, Zali et al. (2008) Esten Mason, Mondal et al. (2011) Pinto, Lopes et al. (2016) Tadesse, Suleiman et al. (2019)	Esten Mason, Mondal et al. (2011) Pinto, Reynolds et al. (2010) El Hassouni, Belkadi et al. (2019) Tahmasebi, Heidari et al. (2017)				
5D	Esten Mason, Hays et al. (2013)	Li, Mao et al. (2019)					

CH	Grain yield	Grain size	Grain number	Grain fill rate	Harvest index	Senescence rate	Maturation rate
6A		Tadesse, Suleiman et al. (2019) Guan, Lu et al. (2018) Tahmasebi, Heidari et al. (2017)				Vijayalakshmi, Fritz et al. (2010)	
6B	Pinto, Lopes et al. (2016) El Hassouni, Belkadi et al. (2019)	Shirdelmoghanloo, Taylor et al. (2016) Shirdelmoghanloo (2014) Pinto, Lopes et al. (2016) Li, Mao et al. (2019) El Hassouni, Belkadi et al. (2019) Tadesse, Suleiman et al. (2019)	Pinto, Reynolds et al. (2010) Pinto, Lopes et al. (2016) Li, Mao et al. (2019) Tahmasebi, Heidari et al. (2017)	Pinto, Lopes et al. (2016)	El Hassouni, Belkadi et al. (2019)	Vijayalakshmi, Fritz et al. (2010)	
6D	(Liu, Sukumaran et al. 2019) Tahmasebi, Heidari et al. (2017)	Esten Mason, Mondal et al. (2011) Pinto, Lopes et al. (2016) Li, Mao et al. (2019) Tahmasebi, Heidari et al. (2017) Tadesse, Suleiman et al. (2019)		Pinto, Lopes et al. (2016)			(Liu, Sukumaran et al. 2019)
7A	Bennett, Reynolds et al. (2012) Vijayalakshmi, Fritz et al. (2010) Pinto, Lopes et al. (2016) Liu et al. (2019a Hassan, Solouki et al. (2018)	Bennett, Reynolds et al. (2012) Esten Mason, Mondal et al. (2011) Pinto, Lopes et al. (2016) Li, Mao et al. (2019) Tadesse, Suleiman et al. (2019) Guan, Lu et al. (2018)	Guan, Lu et al. (2018) El Hassouni, Belkadi et al. (2019)			Vijayalakshmi, Fritz et al. (2010)	
7B	Paliwal, Röder et al. (2012)	Esten Mason, Mondal et al. (2011) Mohammadi, Zali et al. (2008) Paliwal, Röder et al. (2012) Tadesse, Suleiman et al. (2019) Li, Mao et al. (2019)	Liu et al. (2019a			Vijayalakshmi, Fritz et al. (2010) Shirdelmoghanloo (2014)	
7D	Esten Mason, Hays et al. (2013) Tahmasebi, Heidari et al. (2017)	Paliwal, Röder et al. (2012) Tahmasebi, Heidari et al. (2017) Pinto, Lopes et al. (2016) Tadesse, Suleiman et al. (2019)	Tahmasebi, Heidari et al. (2017)	Pinto, Lopes et al. (2016)		Vijayalakshmi, Fritz et al. (2010)	Paliwal, Röder et al. (2012) Liu, Sukumaran et al. (2019)

Table 2. Previous studies that have identified QTL for leaf chlorophyll content, canopy temperature, stay green, photosystem II efficiency (Fv/Mv), normalized difference vegetation index (NDVI) and membrane damage where an interaction with heat stress has been identified or has been identified under heat stress conditions. Studies that have previously identified QTL related to heat stress adaptation are organised by chromosome (CH) and trait.

CH	leaf chlorophyll content (SPAD)	Canopy temperature	Stay green	Fv/Mv	NDVI	Membrane damage
1A	Shirdelmoghanloo (2014)		Pinto, Lopes et al. (2016)			
1B	Ali, Ibrahim et al. (2013) Talukder, Babar et al. (2014) Tahmasebi, Heidari et al. (2017) Pinto, Reynolds et al. (2010) Pinto, Lopes et al. (2016)	Pinto, Reynolds et al. (2010) Pinto, Lopes et al. (2016)	Pinto, Lopes et al. (2016)		Pinto, Reynolds et al. (2010) Pinto, Lopes et al. (2016)	
1D	Talukder, Babar et al. (2014)	Pinto, Lopes et al. (2016)		Sharma, Torp et al. (2017) Hassan, Solouki et al. (2018)	Pinto, Lopes et al. (2016)	Talukder, Babar et al. (2014)
2A			Vijayalakshmi, Fritz et al. (2010) Pinto, Lopes et al. (2016)		Pinto, Lopes et al. (2016)	
2B	Pinto, Lopes et al. (2016) Hassan, Solouki et al. (2018) Maulana, Ayalew et al. (2018)	Ali, Ibrahim et al. (2013) Pinto, Reynolds et al. (2010) Pinto, Lopes et al. (2016) Liu, Sukumaran et al. (2019)	Pinto, Lopes et al. (2016)	Hassan, Solouki et al. (2018)	Pinto, Reynolds et al. (2010) Pinto, Lopes et al. (2016)	Talukder, Babar et al. (2014) Hassan, Solouki et al. (2018)
2D	Pinto, Lopes et al. (2016) Maulana, Ayalew et al. (2018) Tahmasebi, Heidari et al. (2017)		Pinto, Lopes et al. (2016)			
3A					Pinto, Lopes et al. (2016)	
3B	Shirdelmoghanloo (2014) Pinto, Lopes et al. (2016) Tahmasebi, Heidari et al. (2017)	Pinto, Reynolds et al. (2010) Pinto, Lopes et al. (2016) Bennett, Reynolds et al. (2012)		Sharma, Torp et al. (2017) Hassan, Solouki et al. (2018)	Pinto, Lopes et al. (2016)	Hassan, Solouki et al. (2018)
3D					Liu, Sukumaran et al. (2019)	
4A	Shirdelmoghanloo (2014) Maulana, Ayalew et al. (2018)	Pinto, Reynolds et al. (2010) Pinto, Lopes et al. (2016)	Vijayalakshmi, Fritz et al. (2010) Pinto, Lopes et al. (2016)		Pinto, Reynolds et al. (2010) Pinto, Lopes et al. (2016)	
4B	Shirdelmoghanloo (2014) Maulana, Ayalew et al. (2018) Pinto, Lopes et al. (2016)		Pinto, Lopes et al. (2016)		Pinto, Lopes et al. (2016)	Hassan, Solouki et al. (2018)
4D					Pinto, Lopes et al. (2016)	
5A		Pinto, Reynolds et al. (2010)	Vijayalakshmi, Fritz et al. (2010)		Liu, Sukumaran et al. (2019)	
5B	Maulana, Ayalew et al. (2018)	Bennett, Reynolds et al. (2012)	Pinto, Lopes et al. (2016)		Pinto, Lopes et al. (2016)	

CH	leaf chlorophyll content (SPAD)	Canopy temperature	Stay green	Fv/Mv	NDVI	Membrane damage
5D		Pinto, Lopes et al. (2016) Liu, Sukumaran et al. (2019)	Vijayalakshmi, Fritz et al. (2010)		Liu, Sukumaran et al. (2019)	
6A	Talukder, Babar et al. (2014) Pinto, Lopes et al. (2016)	Liu, Sukumaran et al. (2019)	Vijayalakshmi, Fritz et al. (2010)	Hassan, Solouki et al. (2018)	Pinto, Lopes et al. (2016)	
6B	Shirdelmoghanloo (2014)		Pinto, Lopes et al. (2016)	Vijayalakshmi, Fritz et al. (2010) Hassan, Solouki et al. (2018)		Talukder, Babar et al. (2014)
6D		Liu, Sukumaran et al. (2019)			Liu, Sukumaran et al. (2019)	
7A	Talukder, Babar et al. (2014) Vijayalakshmi, Fritz et al. (2010) Hassan, Solouki et al. (2018)	Pinto, Reynolds et al. (2010)	Pinto, Lopes et al. (2016)		Pinto, Lopes et al. (2016)	Talukder, Babar et al. (2014) Hassan, Solouki et al. (2018)
7B		Paliwal, Röder et al. (2012)	Vijayalakshmi, Fritz et al. (2010)		Pinto, Reynolds et al. (2010)	
7D	Vijayalakshmi, Fritz et al. (2010) Tahmasebi, Heidari et al. (2017)	Pinto, Lopes et al. (2016)	Vijayalakshmi, Fritz et al. (2010) Pinto, Lopes et al. (2016)		Pinto, Lopes et al. (2016) Liu, Sukumaran et al. (2019)	

1.6 Rationale for the Current Study to Improve the Adaptation of Wheat to the Heat Stress Conditions of the Southern Australian Environment

Heat stress is a key abiotic stress in the Mediterranean-type environments of southern Australian environment, accounting for considerable production losses within farming systems. Substantial research needs to be conducted to understand how plants respond to heat stress to understand what traits and genetics are responsible for improved adaptation to heat stress conditions. In addition to heat stress, many other factors limit production, and these, although research topics in their own right, need to be considered as confounding factors when investigating heat stress responses in plants.

The aims of the study are two-fold. Firstly, to achieve a greater understanding of how heat stress affects wheat production across the southern Australian grain belt, to better understand the genetic variation for adaptation to heat stress conditions currently available in Australian commercial germplasm. Secondly, achieve a greater understanding of the underlying genetics associated with tolerance to heat stress and identify QTL and markers associated with improved adaptation to heat stress conditions. To achieve this, numerous bi-parental doubled haploid populations based on diverse background parents (Mace, Gladius, and Scout),

representing key family groups within Australia wheat germplasm, along with other parents of interest, will be screened for adaptation to heat stress conditions in multiple representative field environments, and in a controlled environment heat stress facility. At the completion of this study, wheat breeders should have improved knowledge regarding the physiological and genetic basis of heat stress tolerance and the tools they need to manipulate it.

Chapter 2. A Field and Controlled Environment Evaluation of Wheat (*Triticum aestivum*) Adaptation to Heat Stress

The manuscript as published in Field Crops Research 229 (2018), doi:10.1016/j.fcr.2018.09.013

2.1. Contextual Statement

Heat stress has previously been found to be a key limitation of crop production in the Mediterranean-type environments of southern Australia. Various methods have been used to characterise adaptation to such stress conditions, including controlled environment assays due to their reliability and repeatability. However, there is limited knowledge on the relevance of such assays to real-world growing conditions in farmers' fields. Delayed sowing to expose sensitive growth stages to the higher incidence of high temperatures later in the growing season is a method that has been used to screen for adaptation to heat stress conditions in field conditions. However, the relevance of such methods is questioned due to the altered photoperiod and plant water availability compared to that of an optimum sowing time. This manuscript investigated if adaptation to heat stress conditions could be measured in relevant growing conditions by evaluating variety performance across a range of representative and relevant environments contrasting for terminal heat stress conditions, using conventional sowing times. Stress conditions were characterised by determining a range of temperature co-variates for each variety in each environment allowing the role of temperature stress in GxE interactions to be evaluated. Additionally, the same varieties were evaluated in controlled environment conditions targeting grain filling heat stress to understand the link between the controlled environment assay and field adaptation.

2.2. Statement of Authorship

Statement of Authorship

Title of Paper	A field and controlled environment evaluation of wheat (*Triticum aestivum*) adaptation to heat stress
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
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Principal Author

Name of Principal Author (Candidate)	Mr Paul Telfer
Contribution to the Paper	Designed and ran experiments, analysed the data collected, interpreted the data and wrote the manuscript.
Overall percentage (%)	85
Certification.	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date: 25/02/2022

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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A field and controlled environment evaluation of wheat (*Triticum aestivum*) adaptation to heat stress

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ABSTRACT

Heat stress is a major constraint on wheat (*Triticum aestivum* L.) production in many regions of the world. While research into heat stress tolerance has been conducted across many crop species, there are still significant gaps in our understanding of the impacts of heat stress on production, the level of genetic variation for heat stress tolerance in the field and the varying phenotypic responses to various yield components. Here, we report on the heat stress tolerance for 24 bread wheat (*Triticum aestivum* L.) genotypes which were evaluated across 13-environments over two growing seasons in the Mediterranean-type climate of southern Australia. Numerous climatic co-variates were measured to further understand interactions of temperature stress on crop performance. Not surprisingly, heat stress was found to have significant negative impacts on grain yield in field conditions, with reductions of 302 kg ha^{-1} °C^{-1} for each day with maximum temperature in excess of 30°C during anthesis and a reduction of 161 kg ha^{-1} °C^{-1} for each day with maximum temperature in excess of 30°C during grain fill. Genotype by environment interactions for grain yield performance under varying levels of heat stress were also observed in the field, suggesting that plant breeding selection strategies could be used to improve adaptation to heat stress. Additionally, all genotypes were phenotyped using a controlled environment assay (plants exposed to an air temperature of 36°C and a wind speed of 40 km h^{-1} for three consecutive, eight-hour days, 10 days post the end of anthesis). Significant differences in genotype performance for leaf senescence and leaf chlorophyll content in response to heat stress were identified under the controlled environment conditions. Further evaluation showed that some of the field genotype by environment interaction for heat stress tolerance could be explained by performance under controlled environment conditions. This suggests that detailed physiological studies in controlled environment conditions do relate to performance in field conditions.

1. Introduction

Abiotic constraints to bread wheat (*Triticum aestivum* L.) production, such as heat, drought and frost have negative effects on grain yield (Collins et al., 2008; Dolferus et al., 2011). Abiotic stresses are common throughout the Australian grain belt, particularly in southern Australia, where high temperatures at critical developmental stages significantly reduce grain yield potential (Zheng et al., 2012). Compounding this is the frequent co-occurrence of multiple types of abiotic stress particularly hot, windy and terminal-drought conditions (Machado and Paulsen, 2001; Shah and Paulsen, 2003). To minimise the effect of abiotic stress on crop production, winter cereals grown in environments with a Mediterranean-type climate are typically sown so that plants develop during the cooler winter months. Generally this results in anthesis occurring before the characteristic warmer and drier conditions of late spring and early summer. Mediterranean-type climates are typically prone to brief periods of high temperatures during late spring with maximum daily temperatures of 35°C or greater, accompanied by winds in excess of 40 km h^{-1} (Alexander et al., 2010; Talukder et al., 2013). Additionally, climate change predictions suggest that such events may become more frequent (Hayman et al., 2012).

Accumulated thermal units, are fundamental to plant growth and an important driver of plant growth and productivity. As discussed by Porter and Gawith (1999), there are optimal temperatures for plant growth and function that vary depending on the developmental stage of the plant. Associated with this are minimum temperatures and

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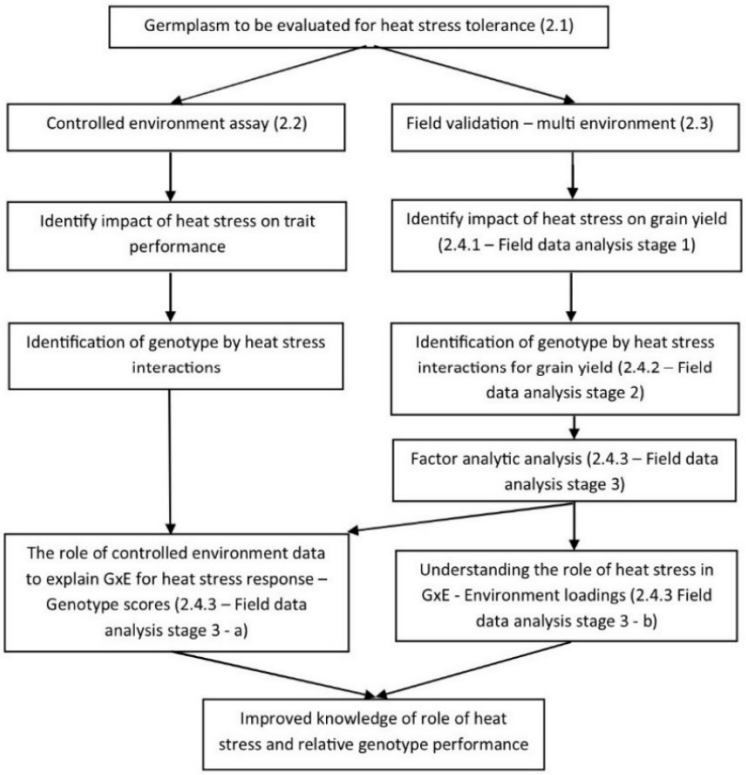

Fig. 1. Flowchart of the assays used to evaluate heat stress response and the analyses used to evaluate heat stress interactions and genotype performance (numbers in parentheses indicate the relevant section of the materials and methods).

maximum temperatures where it is understood that plant function virtually stops, with limited ability for plant function to recover. For wheat, Porter and Gawith (1999) reported this as being a minimum temperature of 9.5°C, optimal temperature of 21°C and maximum temperature of 31°C for anthesis. Minimum temperature of 9.2°C, optimal temperature of 20.7°C and maximum temperature of 35.4°C for grain fill. The focus of the current study is the impact of high temperatures (above optimum) on wheat productivity.

The interaction between high temperatures and wheat plants is complex with numerous physiological processes adversely affected (Wahid et al., 2007). Reproductive tissues in wheat are sensitive to heat stress as early as the young microspore stage of pollen development (Saini et al., 1999; Dolferus et al., 2011). However, in many environments (such as southern and western Australia), wheat is rarely exposed to heat stress at this growth stage but rather more frequently around anthesis and during grain fill (Alexander et al., 2010). During anthesis, heat stress reduces pollen viability and subsequently reduces fertilisation and seed set (Barnabas et al., 2008). During grain fill, temperature stress negatively effects starch and protein accumulation (Bhullar and Jenner, 1985; Zahedi et al., 2004). Plant development is accelerated by elevated temperatures, reducing grain fill duration and consequently grain size (Wardlaw, 1994; Stone and Nicolas, 1995b; Wollenweber et al., 2003; Sharma et al., 2008). Heat stress also accelerates leaf senescence, thus reducing plant photosynthetic capacity (Tewolde et al., 2006; Talukder et al., 2014). These factors all contribute to reduced grain yield. This has been demonstrated by Bennett et al. (2012a) and Kuchel et al. (2007a) who identified a reduction of up to 187 kg ha^{-1} for every one degree increase in average temperature during anthesis and grain fill in multi-year field experiments across southern Australia. However, genotypic variation for response to heat stress has been identified under controlled environment conditions (Stone and Nicolas, 1995a; Esten Mason et al., 2011), providing scope for improved performance under stress conditions.

Delayed sowing has commonly been used to identify heat stress tolerant phenotypes in the field in an effort to move sensitive reproductive stages to coincide with higher incidence of heat stress (Reynolds et al., 2007; Pinto et al., 2010; Bennett et al., 2012b; Esten Mason et al., 2013; Sadras et al., 2015). Although increased exposure to heat stress can be achieved, plants are also exposed to a range of abnormal environmental conditions during crop development including longer photoperiod and altered plant available water (Sadras et al., 2015). This can create an unrepresentative growing environment, particularly for a Mediterranean-type climate, so caution is required in comparing results from delayed-sowing experiments with those from agronomically recommended sowing dates. Alternative field methodologies, such as those used by Alexander et al. (2010) and Talukder et al. (2013), include using chambers in the field to limit confounding environmental factors while managing heat stress conditions. Such methods allow heat stress to be achieved within an agronomically representative environment. However, the physical encumbrance of handling chambers in field conditions limits capacity to screen large breeding populations to identify superior performing phenotypes.

Furthermore, identifying a true heat stress tolerant phenotype is difficult under field conditions due to other confounding abiotic stresses, such as drought and spring radiation frost occurring at similar developmental stages. The temperature, duration and timing of heat stress events can also differ across seasons, thus reducing the reliability and repeatability of the phenotypic screen. Variation in time-to-anthesis within the germplasm being evaluated may also mean that naturally occurring heat stress arises at different growth stages for each genotype studied. This may lead to selection for stress escape (through earlier/later maturity) rather than true genetic stress tolerance. To overcome these limitations, controlled environment phenotypic screens have been developed to manage these factors (Tashiro and Wardlaw, 1990; Stone and Nicolas, 1995a; Esten Mason et al., 2010, 2011; Maphosa et al., 2014). However, none of these reports have validated genotypic

response to heat stress in a controlled environment assay by evaluating the same germplasm for grain yield and physical grain quality response to heat stress in representative field environments.

The current study aimed to validate the phenotypic data collected from a novel controlled environment heat stress assay, using multi-environment field trials planted across locations (and seasons) with varying heat-stress events. The results provide further understanding of the implications of heat stress on production systems and also the adaptation patterns of key Australian germplasm to the heat stress conditions that are frequently encountered in Mediterranean regions.

2. Materials and methods

A combination of controlled environment assay and field studies along with a range of analyses were used to understand the impacts of heat stress on production and genotypic variation for response to heat stress conditions as summarised in Fig. 1.

2.1. Plant materials

In the 2013 and 2014 growing seasons, 24 wheat genotypes (Supplementary Table 1) were evaluated in the field and in a controlled environment assay, housed within a greenhouse situated at the Australian Grain Technologies (AGT) breeding facility, based at The University of Adelaide's Roseworthy Campus, South Australia. Genotypes were selected to represent the diversity of germplasm used in Australian wheat breeding. Exotic genotypes (3) were also included from a previous larger screen of heat-stress tolerance candidate germplasm (Telfer & Bennett, unpublished data).

2.2. Controlled environment testing

In the southern Australian environment, heat stress conditions are relatively common during the later stages of plant and crop development. With maximum daily temperatures of 35°C or greater accompanied by winds in excess of 40 km h^{-1}. These events last up to a few days in length (Alexander et al., 2010; Talukder et al., 2013), being relatively common and of increasing frequency during grain fill. Consequently, a controlled environment assay has been developed to expose plants to an air temperature of 36°C and a wind speed of 40 km h^{-1} for three consecutive, eight-hour days, using a purpose-built controlled environment chamber. At the end of each eight-hour treatment, the controlled-environment was allowed to cool to the ambient temperature (averaging 14°C). This equated to greenhouse growing conditions for the duration of the study of a minimum temperature of 6°C, maximum temperature of 34°C, average temperature of 18°C, minimum Vapour Pressure Deficit (VPD) of 0.04 kPa, maximum VPD of 3.36 kPa and average VPD of 0.80 kPa. During the stress treatment, the conditions experienced were; minimum temperature of 28°C, maximum temperature of 38°C, average temperature 36°C, minimum VPD 2.2 kPa, maximum VPD of 5.8 kPa and average VPD of 4.6 kPa. Conditions inside the heat assay when allowed to cool to ambient conditions at night were; minimum temperature 7°C, maximum temperature 30°C, average temperature 16°C, minimum VPD of 0.1 kPa, maximum VPD of 3.3 kPa and average VPD of 0.7 kPa. Deviations from the target temperature conditions for the heat assay were due to time required for heating and cooling of the assay chamber and temperature logging intervals. Additionally, there was a large range in baseline greenhouse temperatures, due to the influence of the external conditions. Temperature conditions were determined from temperature and relative humidity conditions logged half-hourly for the duration of the experiment both within the controlled environment assay and greater greenhouse. All plants were watered using an automated drip irrigation system to avoid the confounding effect of drought stress.

Heat stress conditions inside the chamber are produced by six thermostatically controlled heating elements (totalling 23 kW) in

Table 1
Description of traits measured in the controlled environment experiments.

Trait	Trait description	Trait calculation	Stage observed	Units	Reference
Leaf1	Visual leaf damage score of the primary tiller flag leaf (1-9)		Immediately prior to heat stress treatment	1-9 score	
Leaf2	Visual leaf damage score of the primary tiller flag leaf (1-9)		End of heat stress treatment	1-9 score	
Leaf3	Visual leaf damage score of the primary tiller flag leaf (1-9)		10 days post the start of heat stress treatment	1-9 score	
SPAD1	Leaf chlorophyll (SPAD) content (average of 10 measurements per main tiller flag leaf)		Immediately prior to heat stress treatment	SPAD units	Adapted from Pask et al. (2012)
SPAD2	Leaf chlorophyll (SPAD) content (average of 10 measurements per main tiller flag leaf)		End of heat stress treatment	SPAD units	Adapted from Pask et al. (2012)
SPAD3	Leaf chlorophyll (SPAD) content (average of 10 measurements per main tiller flag leaf)		10 days post the start of heat stress treatment	SPAD units	Adapted from Pask et al. (2012)
Spikelet number per spike	Number of spikelets per spike		Physiological maturity	Spikelets $spike^{-1}$	
Grain number per spike	Total number of grains in the mature spike		Physiological maturity	Grains $spike^{-1}$	
Spike weight	Total weight of spike		Physiological maturity	Grams	
Grain weight	Total weight of grain produced per spike		Physiological maturity	Grams	
Primary tiller weight	Total weight of primary tiller		Physiological maturity	Grams	
Thousand Kernel Weight (TKW)	Thousand grain weight	Grain weight / Grain number x 1000	Physiological maturity	Grams	Adapted from Pask et al. (2012)
Spikelet fertility	Average grain number per spikelet	Grain number / Spikelet number	Physiological maturity	Grains $Spikelet^{-1}$	
Harvest index (HI)	Proportion of primary tiller mass present as grain	Grain weight / Primary tiller weight	Physiological maturity	Ratio	Adapted from Pask et al. (2012)
Spike fertility index	Harvest index of spike only	Grain weight / Spike weight	Physiological maturity	Ratio	

Fig. 2. The controlled environment growth chamber used to expose plants to an average temperature of 36°C and a wind speed of 40 km h^{-1} for three consecutive eight hour days, 10 days after the main tiller has finished anthesis.

combination with a 7.5 kW, 100 cm diameter fan connected by 90 x 90 cm square ducting to recirculate air through the chamber. The chamber consists of a steel frame covered in 3 mm thick clear polycarbonate sheeting, with dimensions being 180 cm high, 150 cm wide and 322 cm long (Pictured in Fig. 2).

Plants were sown in 9 cm square by 25 cm high plastic pots, arranged in a two replicate randomised split-plot design with treatments randomised within adjacent split-plots. Two experimental treatments were used; i) an unstressed control, ii) heat stressed. All plants were grown under greenhouse conditions for the duration of their life cycle, monitored and managed for pests and diseases, watering and climate control. The main tiller of each plant was monitored for growth stage Z69 (Zadoks et al., 1974); the end of anthesis. Ten days after the end of anthesis plants were removed from the experimental arrangement, and the main tiller tagged with a barcode for laboratory identification. At Z69 a visual leaf damage score (Leaf1) was recorded for the flag leaf of the main tiller of each plant; the score being proportional to the viable leaf area present (1 – no damage, to 9 – leaf completely senesced). Plants to be subjected to the heat stress treatment were loosely tied to a supporting stake to avoid any physical damage to the plants. Heat-stressed plants were placed in the chamber for three consecutive days (and returned to their experimental layout thereafter). At the end of the three-day treatment, all plants (including controls) were scored again for leaf damage (Leaf2), and subsequently 10 days later (Leaf3). Leaf chlorophyll levels were observed at the same time (SPAD1, SPAD2 and SPAD3, respectively) and measured using a SPAD meter (Pask et al., 2012).

At physiological maturity, the tagged primary tiller was harvested by cutting at soil level, placed in a paper bag and dried in an oven at 40°C for 72 h. The following measurements were taken on the primary tiller; grain number per spike, grain weight per spike, spikelet number per spike, weight of the whole spike and the weight of the whole intact stem. These measurements allowed the calculation of thousand kernel weight (TKW), spikelet fertility (average grain number per spikelet), main tiller harvest index (HI) and spike fertility index. All phenotypic traits observed from controlled environment experiments along with trait calculations are summarised in Table 1.

Phenotypic data (Table 1) collected from the controlled environment assay were analysed using a REML analysis, using the statistical software R (R Core Team, 2018) and the ASREML-R (Butler et al., 2009) package following Equation 1 to explore the heat stress effect on wheat performance and its interaction with genotype. In brief this involved fitting fixed terms in the model of, genotype, heat treatment, genotype by treatment interaction. Where significant ($P < 0.05$), bloc, row and column effects were retained as random terms in the model.

Eq. (1). Controlled environment assay analysis

$$Y = X\tau + Zu + e \quad (1)$$

Where Y is the vector of phenotypic data (as listed in Table 1), τ is the vector of fixed effects which includes the genotype and heat treatment main effects, and the genotype by heat treatment interaction. X is the design matrix for the vector of fixed effects. u is the vector of random effects, consisting of the random experiment effects, block effects and other spatial modelling terms (Gilmour et al., 1997), with corresponding design matrix Z. e is the vector of residuals.

2.3. Field evaluation

The 24 genotypes used in the controlled-environment experiment were also included in field experiments conducted across two seasons (2013 and 2014). The field experiments were randomised complete block designs, with two replicates of plots, arranged in a rectangular grid. Each plot consisted of either five or six rows sown into 1.125 m wide by 5 m long plots, with plots reduced to a length of 3.2 m prior to anthesis using herbicide. Six locations were used in Southern Australia in 2013 (Angas Valley [34.69°S, 139.27°E], Booleroo Centre [32.83°S, 138.37°E], Minnipa [32.83°S, 135.16°E], Pinnaroo [35.35°S, 141.05°E], Roseworthy [34.50°S, 138.68°E] and Winulta [34.24°S, 137.86°E]). These same six locations were used in 2014 with the addition of Kaniva [36.41°S, 141.20°E], Victoria; thus giving 13 location by year combinations over the two years of the study. All field experiments were sown to achieve 200 seeds m^{-1}. Plot management, including fertiliser regimes, pest and weed management for each experiment followed best regional agronomic practice. At physiological maturity each plot was harvested to determine grain yield (kg ha^{-1}). This structure of experiments repeated over multiple year by location combinations, allowed for a multi-environment trial analysis to identify genotype trends for grain yield and understand underlying genotype by environment interactions for grain yield performance. In this study linear regression analysis was used to determine heat stress interactions across environments. Furthermore, two types of multi-environment trial analysis were conducted for grain yield to examine genotype by environment interactions; these being a mixed linear model as described by Freeman (Freeman, 1973) and a factor analytic analysis of grain yield to examine genotype by environment interactions (Smith et al., 2001).

At each of the locations, a single factory calibrated temperature logger (TinyTag™ Talk2), was situated adjacent to the experiment in the field and at approximately 1 m high to represent approximate mature crop height. These temperature loggers logged half-hourly temperatures throughout the growing season. The temperature data was used to develop degree-day maturity models for each experiment. In addition, temperature co-variates were calculated from collected data for each experimental plot (Table 2, as defined by (Dreccer et al., 2008)). Temperature parameters were calculated independently within each development period for each plot, and are summarised in Table 2.

Spike emergence dates (50% of spikes fully emerged from the flag leaf sheath) were collected for each genotype at Roseworthy in both 2013 and 2014 and used to estimate the timing of anthesis for all genotypes in other experiments. This was calculated by a degree day model (Sadras and Monzon, 2006) using mean daily temperature > 0°C as determined from the half-hourly temperatures logged throughout the growing season to interpolate daily mean temperature. This method, although missing elements of developmental drivers, does capture the major component of temperature (Slafer and Rawson, 1994; Bonhomme, 2000). At two other locations in each year (Winulta and Angas Valley) a Zadoks maturity score (Zadoks et al., 1974) was recorded to confirm the accuracy of the calculated anthesis dates.

2.4. Statistical analysis of field data

This study generated data from multi-environment field experiments, with the dataset providing a basis to understand the general impact of heat stress on crop performance across environments as well as specific genotype by heat stress interactions. This was achieved

Table 2
The climatic co-variates calculated within each developmental stage, independently for each field plot, to describe stress conditions experienced.

Developmental stage	Degree day range relative to anthesis
Anthesis	300 °Cd before anthesis to 100 °Cd post
Grain fill	100 °Cd to 600 °Cd post anthesis
Climatic covariates	**Description**
Growing season rainfall (mm)	May to October rainfall
Average maximum temperature (°C)	Calculated for anthesis and grain fill
Number of days > 30 °C	Calculated for anthesis and grain fill
Number of days > 35 °C	Calculated for anthesis and grain fill

through four analyses as detailed below:

Field data analysis stage 1

Grain yield and climatic co-variate data from the 13 field experiments were summarised into experiment means (see Table 5 later) and included in a linear regression analysis to understand the impact of the climatic co-variates (Table 2) on site mean grain yield performance. Independently for each climatic co-variate, a year by climatic co-variate interaction term was fitted to the liner regression model, if significant ($P < 0.05$) this was retained to differentiate the effect of changing climatic conditions across each year of the study.

Field data analysis stage 2

Grain yield data collected from the field was further analysed to explore the interaction between genotype grain yield performance and the genotype specific climatic co-variates. Field spatial trends (including; block, row and column effects) not accounted for by the genotype by climatic co-variate interaction were included in the model if significant ($P < 0.05$). Grain yield data was modelled with each of the climatic co-variates (Table 2) using the model of Freeman (1973), with genotype fitted as a random term and climatic co-variates main effects and climatic co-variate by genotype interactions fitted as fixed terms to investigate the role of the climatic co-variate on genotype by environment interaction on grain yield performance.

Field data analysis stage 3

Similar to that described by Dreccer et al. (2008) and Yan and Hunt (2001), a factor analytic analysis of grain yield was conducted to examine the underlying genotype by environment interactions. This allowed the field performance of the lines to be compared to their performance under heat stress in the controlled environment and to determine the impact of the climatic co-variates on genotype by environment interaction for grain yield performance. This was achieved by:

a

a Correlating the genotype scores from a field multi-environment trial factor analytic analysis (Smith et al., 2001) to the genotype performance data generated in the controlled environment assay, and

b Correlating the environmental loadings from the factor analytic model with the mean climatic co-variates (Table 2).

3. Results

3.1. Phenotypic response to controlled environment heat stress conditions

Experiments conducted in the controlled environment assay induced significant negative heat stress effects on performance for a number of traits (Table 3). In the case of TKW, the heat stress treatment reduced TKW by 5% relative to the control. Similar trends were evident for leaf senescence score immediately after the stress treatment was imposed (Leaf2) and leaf chlorophyll levels immediately after heat stress (SPAD2), as seen in Table 3.

In this study, there were no significant genotype by heat treatment interactions for the key yield component traits targeted; fertility and TKW. However, significant genotype interactions with heat stress treatment were identified for leaf senescence immediately after stress conditions (Leaf2) and leaf chlorophyll level immediately after heat stress conditions (SPAD2) (Table 4). For example, Halberd, an older Australian variety, had leaf chlorophyll (SPAD units) reduced by 10.5% immediately after the heat and wind stress was imposed. In contrast, Mace, a more recent Australian variety, had a 2.9% reduction of leaf chlorophyll. The average reduction of all genotypes was 2.8%.

3.2. Grain yield response in field experiments

A range of climatic co-variates were recorded at each of the locations in both years of the study (Table 5), with a large portion of locations experiencing temperature conditions above optimal for plant growth as reported by Porter and Gawith (1999). As shown in Table 6, a number of the climatic co-variates were correlated, including the negative correlation of most temperature co-variates with growing season rainfall. Regression analysis of site mean grain yield and site mean climatic co-variates was conducted to identify the trends of changing climatic conditions on crop performance (Fig. 3). Several climatic co-variates showed a significant interaction with year (when a year by

Table 3
Summary of statistical analysis (P-value) for traits measured in the controlled environment study, including treatment means for traits with a significant heat treatment effect.

Trait	Genotype		Treatment		Genotype treatment interaction		Control Mean	Heat Stress Mean	Unit
Fertility	< 0.001	***	ns		ns				
TKW	< 0.001	***	0.041	*	ns		47.4	44.9	g 1000-grains^{-1}
HI	< 0.001	***	ns		ns				
Spike fertility index	0.005	**	ns		ns				
Leaf2	< 0.001	***	< 0.001	***	0.003	**	1.2	1.6	1-9 score
Leaf3	< 0.001	***	ns		ns				
SPAD2	< 0.001	***	< 0.001	***	0.002	**	56.3	54.6	SPAD units
SPAD3	< 0.001	***	ns		ns				

*** = P-value < 0.001, ** = P-value < 0.01, & * = P-value < 0.05, ns = not significant at $P < 0.05$.

Table 4
Percentage change in trait performance for two significant ($P < 0.001$) leaf senescence measurements from the controlled environment assay. The mean presented is of all genotypes included in the study.

Genotype	SPAD2 % effect	Leaf2 % effect
AUS4683	7.5	−9.3
AUS4906	−5.8	26.6
AUS4926	−4.0	−20.3
AXE	−1.1	22.0
CORRELL	−4.0	88.6
EGA GREGORY	5.0	16.9
EMU ROCK	−1.1	−22.3
GLADIUS	−3.4	44.1
H45	2.0	−4.7
HALBERD	−10.5	188.8
JANZ	1.5	18.9
KENNEDY	−5.6	97.4
KUKRI	0.2	14.5
LIVINGSTON	−4.7	18.2
MACE	−2.9	−8.2
RAC1629	−5.4	−7.9
RAC1837	−0.8	7.0
RAC1859	−3.0	−28.8
RAC875	−7.1	69.9
SCOUT	−0.9	41.4
SUNSTATE	−0.6	89.5
SUNTOP	−5.7	27.9
WYALKATCHEM	−5.4	13.3
YITPI	−11.7	32.3
Standard error	0.9	9.9
Mean	−2.8	29.8

climatic co-variate term was fitted within the linear regression model). This was the case for Fig. 3A, 3D, 3E and 3 F, with a line of best fit indicated for each year of the study. A significant year interaction was not found for Fig. 3B and C, with a single line of best fit indicated across both years of the study. A single effect on grain yield was determined across both years of the study for each climatic co-variate with a significant ($P < 0.05$) interaction with grain yield. The variable having the largest significant ($P < 0.05$) effect on grain yield was average maximum temperature during grain fill, with every one degree increase in average maximum temperature during grain fill causing a reduction of 442 kg ha^{-1} °C^{-1} across the two years of the study (r^2 ranging from 0.98 to 0.25 for 2013 and 2014 respectively) (Fig. 3D). Every one degree increase in average maximum temperature during anthesis also identified a large effect, with a 389 kg ha^{-1} °C^{-1} ($r^2 = 0.52$ across both 2013 and 2014) reduction in grain yield (Fig. 3B). For the number of days experienced in excess of 30 °C and 35 °C, the effects on grain yield were larger during anthesis, with every one day over 30 °C during anthesis causing a yield reduction of 302 kg ha^{-1} day^{-1} ($r^2 = 0.40$ across both 2013 and 2014). During grain fill every day in excess of 30 °C and 35 °C caused a reduction in grain yield of 161 kg ha^{-1} day^{-1} (r^2 ranging from 0.67 to 0.19 for 2013 and 2014 respectively) and 182 kg ha^{-1} day^{-1} (r^2 ranging from 0.59 and 0.08 to 2013 and 2014 respectively), respectively.

3.3. Understanding differences in genotype response to heat stress in the field

The relative performance of genotypes included in this study were evaluated using linear mixed models for each of the climatic co-variates (Field data analysis stage 2), which allowed for the identification of significant genotype by climatic co-variate interactions (Table 7). Grain fill average maximum temperature, which had a significant ($P < 0.001$) negative impact on grain yield, but no climatic co-variate by genotype interactions, was excluded from this analysis. Effects on grain yield for all genotypes included in the study to the climatic co-variates found to be significant are displayed in Table 7. Genotype response to maximum temperature during anthesis, identified that two of the exotic lines had the smallest response to increasing heat stress conditions (AUS4683 and AUS4906). However, these were also the genotypes with the lowest average grain yield.

Some genotypes showed contrasting responses to heat stress conditions during anthesis and grain fill. AUS4683 and AUS4906 showed a relatively positive response to heat stress conditions during anthesis, and a relatively negative response to heat stress during grain fill (Table 7). In contrast Scout and RAC875 showed the inverse response. Additionally some genotypes including Mace and Axe showed relatively small responses to both anthesis and grain fill heat stress conditions.

3.4. Factor analytic analysis of grain yield across environments to dissect genotype by environment interactions

Genotype by environment interactions were examined using a factor analytic analysis of multi-site grain yield data collected across the 13 year by location combinations included in the study (Field data analysis stage 3). Experiment loadings for each factor from the factor analytic analysis were correlated with experiment mean climatic co-variates to determine if the climatic co-variates explained any of the experiment loadings. Genotype scores from the factor analytic analysis were correlated with phenotype data collected in the controlled environment assay to understand the link between genotype performance in the field and performance in the controlled environment assay, and therefore suitability of controlled environment screening as a surrogate selection environment for heat stress tolerance.

Table 5
Mean grain yield and mean climatic co-variates for each experiment, calculated from genotype specific climatic-covariates in each experiment.

Location	Year	Mean grain yield (kg ha^{-1})	Growing season rainfall (mm)	Anthesis average maximum temperature (°C)	Anthesis days > 30 °C (no.)	Grain fill average maximum temperature (°C)	Grain fill days > 30 °C (no.)	Grain fill days > 35 °C (no.)
Angas Valley	2013	1789	165.4	26.0	6.1	27.7	8.3	1.9
Booleroo	2013	3119	292.6	24.7	2.4	26.3	7.2	2.0
Minnipa	2013	2294	196.6	23.2	4.7	27.3	9.5	2.4
Pinnaroo	2013	2318	223.7	24.1	3.1	27.4	9.9	3.2
Roseworthy	2013	3489	302.2	21.9	2.0	26.3	8.7	2.5
Winulta	2013	5222	388.2	20.2	1.0	24.8	5.4	0.0
Angas Valley	2014	3440	138.8	23.6	0.1	28.5	12.4	5.8
Booleroo	2014	2969	187.8	25.9	4.7	31.5	17.3	8.2
Kaniva	2014	3180	170.2	24.9	4.5	29.6	13.8	5.5
Minnipa	2014	3434	227.4	21.7	0.5	26.7	6.0	0.8
Pinnaroo	2014	2383	103.8	23.5	1.7	29.3	13.5	4.0
Roseworthy	2014	4194	231.6	23.4	2.4	28.8	11.5	5.3
Winulta	2014	4098	192.6	22.0	0.3	27.2	11.1	2.4

Table 6
Correlation matrix of climatic co-variates measured in all experiments.

	Growing season rainfall		Anthesis average maximum temperature		Anthesis days > 30 °C		Grain fill average maximum temperature		Grain fill days > 30 °C		Grain fill days > 35 °C
Growing season rainfall	1.00										
Anthesis average maximum temperature	−0.56	*	1.00								
Anthesis days > 30°C	−0.22		0.74	**	1.00						
Grain fill average maximum temperature	−0.72	**	0.69	**	0.39		1.00				
Grain fill days > 30°C	−0.67	*	0.56	*	0.26		0.94	***	1.00		
Grain fill days > 35°C	−0.55		0.60	*	0.25		0.92	***	0.94	***	1.00

*** = P-value < 0.001, ** = P-value < 0.01, & * = P-value < 0.05.

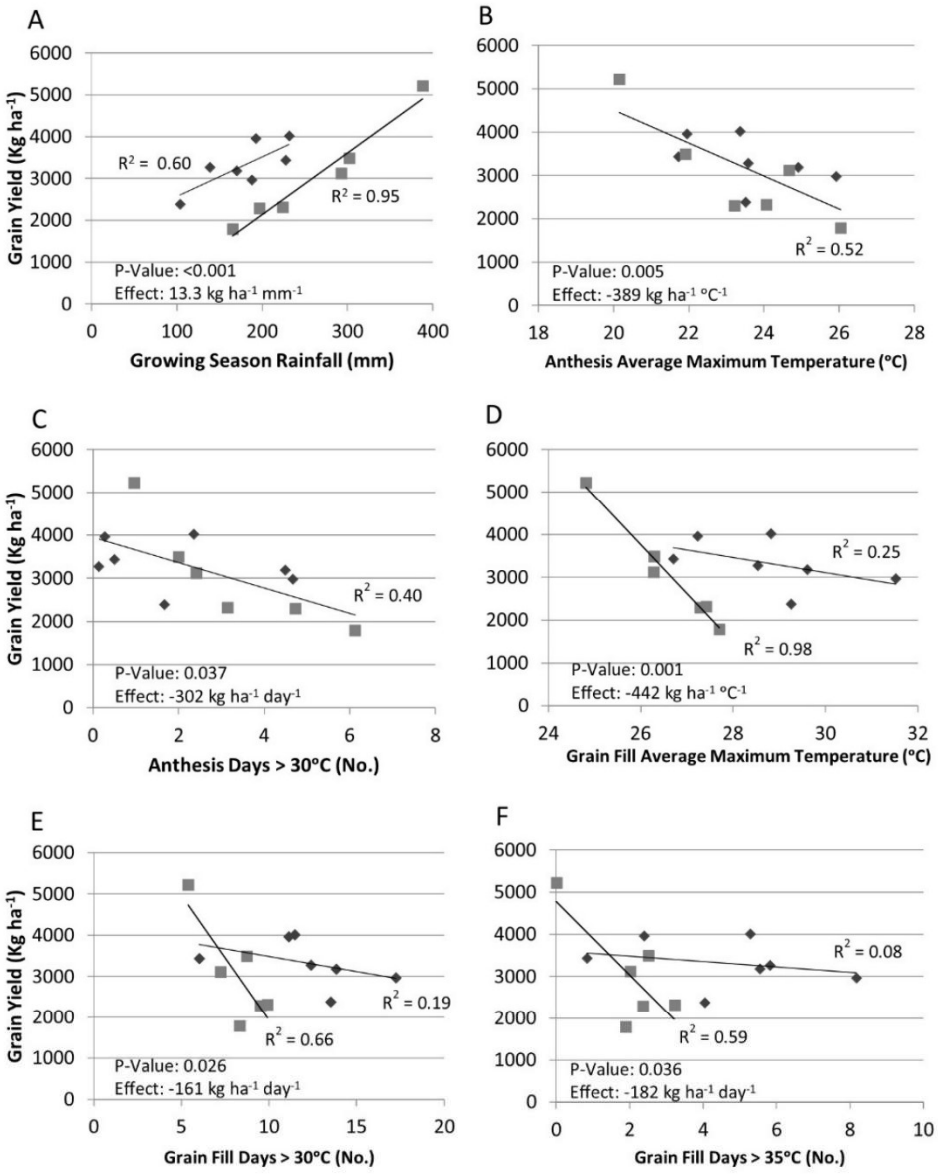

Fig. 3. The effect on site mean grain yield for climatic co-variates found to have a significant ($P < 0.05$) interaction with grain yield as determined through linear regression analysis. These being growing season rainfall (A), anthesis average maximum temperature (B), anthesis days > 30 °C (C), grain fill average maximum temperature (D), grain fill days > 30 °C (E) and grain fill days > 35 °C (F). For each there is an effect on grain yield attributed to the change in the climatic co-variate across all environments in the study and the associated P-value of that relationship. Additionally, some climatic co-variates had a significant ($P < 0.05$) interaction with year. Where this was the case, a year by climatic co-variate interaction term was retained within the linear regression model. This was the case for (A), (D), (E) and (F), with an r^2 produced for each year of the study. For (B) and (C) no significant year interaction was identified, with the r^2 indicating the strength of the relationship across both years of the study. Red squares represent 2013 data, and blue diamonds represent the 2014 season data.

3.4.1. The impact of heat stress on genotype by environment interaction

In this study six factors were fitted to the factor analytic model for grain yield, and accounted for a total of 98% of the genotype by environment variance for grain yield (Table 8). Experiment loadings from the factor analytic analysis were correlated with mean climatic co-variates measured in each experiment (Table 9). Temperature parameters during anthesis were found to be more highly correlated than temperature parameters during grain fill, particularly for factor 1 (FA1) (FA1 accounted for 85% of GxE for grain yield). Anthesis average maximum temperature had the largest correlation within FA1 (correlation of -0.69 with FA1). Rainfall and crop water availability is fundamental in determining crop production. However, in this study grain

Table 7
Summary of statistical analysis of grain yield data collected in the field to identify genotype by climatic co-variate interactions for grain yield (*Field data analysis stage 2*). The mean grain yield (BLUP) for each genotype across all environments, and the grain yield (kg ha^{-1}) response of each genotype to each of climatic co-variate (*Field data analysis stage 2.*).

Summary of statistics	Mean Grain yield	Growing season rainfall	Anthesis average maximum temperature	Anthesis days > 30°C	Grain fill days > 30°C	Grain fill days > 35°C
P-value co-variate effect		< 0.001	< 0.001	0.025	< 0.001	< 0.001
P-value genotype by climatic co-variate effect		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Genotype response						
Unit	kg ha^{-1}	kg ha^{-1} mm^{-1}	kg ha^{-1} °C^{-1}	kg ha^{-1} day^{-1}	kg ha^{-1} day^{-1}	kg ha^{-1} day^{-1}
AUS4683	2141	1.2	0.8	32.3	−72.0	−127.1
AUS4906	1822	0.3	−16.1	24.4	−75.6	−109.7
AUS4926	2327	2.0	−125.8	−71.4	−19.8	−29.7
AXE	3418	2.7	−29.6	14.4	−2.1	19.9
CORRELL	3360	5.9	−102.7	−83.3	−51.7	−75.4
EGA GREGORY	2924	4.1	−74.3	−31.5	−38.1	−43.0
EMU ROCK	3655	6.3	−207.4	42.2	−92.5	−192.2
GLADIUS	3517	6.6	−125.7	−3.8	−67.9	−86.7
H45	3329	5.9	−160.3	4.4	−48.4	−107.2
HALBERD	2932	5.2	−74.0	2.8	−72.8	−126.3
JANZ	3167	5.1	−83.5	−69.9	−36.5	−56.2
KENNEDY	3131	4.8	−57.2	−22.9	−35.8	−79.9
KUKRI	3199	5.5	−147.1	−45.6	−29.3	−63.3
LIVINGSTON	3270	5.7	−176.6	−47.9	−37.9	−55.7
MACE	3709	6.7	−98.0	2.8	−34.7	−52.9
RAC1629	3648	5.4	−143.7	−10.1	5.2	−8.8
RAC1837	3560	6.1	−170.8	−26.7	−31.1	−65.1
RAC1859	3417	4.8	−186.8	−29.7	−36.6	−89.2
RAC875	3650	5.7	−196.2	−106.3	−8.9	6.7
SCOUT	3358	3.5	−167.2	−84.2	−10.7	−15.6
SUNSTATE	2784	5.4	−66.7	73.4	−66.8	−91.6
SUNTOP	3400	5.9	−162.2	−35.3	−27.1	−46.3
WYALKATCHEM	3511	6.4	−214.9	−1.4	−27.7	−80.9
YITPI	3188	6.4	−150.5	−93.8	−88.7	−130.6
Standard Error	99.9	0.4	12.6	9.4	5.5	9.9
Mean	3184	4.9	−122.4	−23.6	−42.0	−71.1

yield had larger correlations with a number of the heat stress co-variates than with growing season rainfall.

3.4.2. Genotype heat stress tolerance and its influence on genotype by environment interaction

Under controlled environment conditions, significant genotype by stress treatment interactions were identified for Leaf senescence (Leaf2) and leaf chlorophyll (SPAD2) levels post stress. Genotype responses to heat stress observed under the controlled environment conditions were correlated with genotype scores from the factor analytic analysis of multi-site grain yield data (Table 10). Moderate correlations were identified between controlled environment traits and grain yield performance in the field, particularly for Leaf2 with FA1 and FA6, and SPAD2 with FA6.

4. Discussion

4.1. The response to heat stress of a diverse set of germplasm in controlled environment conditions

The negative impacts of heat stress on wheat physiology have been the focus of numerous studies (Tashiro and Wardlaw, 1990; Stone and Nicolas, 1995a; Esten Mason et al., 2010, 2011; Maphosa et al., 2014). However, there have been few reports examining the validity of using controlled-environment heat-stress assays to improve field based heat stress tolerance. In the current study, we used a controlled-environment assay to phenotype heat stress response during grain fill, and subsequently validated the genotype response against field performance across two seasons that represent 13 location by year combinations.

Table 8
The percentage variance accounted for by each factor (FA) in the factor analytic analysis of grain yield.

Factor	% Variance Explained
FA1	85.1
FA2	4.2
FA3	2.2
FA4	3.7
FA5	2.2
FA6	0.6
Total	98.0

Table 9
The correlation of experimental means for climatic co-variates and experiment loading as calculated from the factor analysis of grain yield data across all experiments.

	FA1	FA2	FA3	FA4	FA5	FA6
Growing Season Rainfall	0.45	0.15	−0.74**	−0.10	−0.18	−0.31
Anthesis average maximum temperature	−0.69**	−0.09	0.79**	0.09	0.27	−0.20
Anthesis days > 30°C	−0.59*	0.09	0.40	0.03	0.02	−0.21
Grain fill average maximum temperature	−0.37	−0.17	0.78**	0.44	−0.03	−0.03
Grain fill days > 30°C	−0.19	−0.25	0.68*	0.61*	−0.11	0.05
Grain fill days > 35°C	−0.14	−0.32	0.64*	0.55	0.01	−0.23

*** = P-value < 0.001, ** = P-value < 0.01, & * = P-value < 0.05.

Table 10
The correlation of genotype heat stress effect for the Leaf2 and SPAD2 measurements observed in the controlled environment assay, with the genotype scores produced in the factor analytic analysis of multi-site grain yield across all environments.

	FA1	FA2	FA3	FA4	FA5	FA6
Leaf2	−0.42*	0.07	−0.17	−0.28	0.14	−0.42*
SPAD2	0.10	−0.13	0.25	−0.14	0.21	0.47*

*** = P-value < 0.001, ** = P-value < 0.01, & * = P-value < 0.05.

A significant impact from the heat stress treatments imposed 10 days post anthesis were found; particularly on the grain yield components - grain number and grain size. However, the genotype by heat stress treatment interaction was not significant for grain yield and grain yield components. This is in contrast to previous studies (Telfer & Bennett, unpublished data) that used the same controlled environment assay as the current study, which identified negative impacts on yield components, as well as significant genotype differences in stress response. Similarly, Hays et al. (2007) identified significant differences in genotype performance, with susceptible genotype Karl 92 showing a decrease in kernel weight of up to 28.3% compared to a non-significant response in Halberd. Similarly, Maphosa et al. (2014) and Stone and Nicolas (1995a) demonstrated a reduction in single kernel mass as well as differences between the response of genotypes to heat stress. These three studies, as well as the current study, focused on post-anthesis heat stress – which is the most common time to encounter heat stress in Mediterranean-type environments. Studies investigating pre-anthesis heat stress have identified negative impacts on grain number resulting from abnormal pollen and pollen-tube development (Saini et al., 1983; Wardlaw, 1994), in addition to Dawson and Wardlaw (1989) who identified contrasting responses between genotypes to high temperature conditions during booting.

The reasons to why the controlled environment assay evaluation of heat stress response in this study did not produce the significant genotype by heat stress treatment interactions as previously seen, are not fully understood. It may be a case of high experimental error, or that a smaller number and range of genotypes (to allow field comparisons) has reduced the genetic variance being tested.

Significant differences in the genotype response to heat stress conditions were found in the controlled environment assay in this study for traits related to leaf senescence and leaf chlorophyll content. This is consistent with previous studies that have found leaf chlorophyll levels to be reduced and leaf senescence to be accelerated as a result of heat stress conditions (Maphosa et al., 2014; Talukder et al., 2014).

4.2. Impacts of heat related climatic co-variates on grain yield

The negative impact of heat stress on grain yield was demonstrated across the 13 field experiments. Negative grain yield responses to increasingly severe heat stress conditions were shown to be significant, with negative impacts (Fig. 3) evident for most of the temperature related climatic co-variates (Table 2). The effect on grain yield was found to be generally larger during flowering when compared to grain fill, which is not surprising given previous studies have identified grain number per unit area to be the yield component most significantly correlated with grain yield (Kuchel et al., 2007b; Bennett et al., 2012a; Esten Mason et al., 2013). Ferris et al. (1998) reported similar findings with larger negative impacts on grain yield with stress during anthesis. This is likely a reflection of the physiological processes impacted during each developmental stage, with pollen viability and fertility negatively affected during flowering, thereby subsequently causing a reduction in grain number (Ferris et al., 1998; Barnabas et al., 2008). Previous studies have additionally found heat stress during grain fill resulted in increased leaf senescence rates and reduced grain fill duration (Tewolde et al., 2006; Talukder et al., 2013), thus reducing final grain size.

The importance of evaluating heat stress tolerance in the field over multiple years has also been highlighted by this study. A number of the climatic co-variates measured herein showed a different magnitude of effect in each year of the study. In 2013 there was a higher mean growing season rainfall across the experiments conducted compared to 2014 (Table 5), as well a larger range in rainfall between sites. Conversely, in the 2014 season temperatures were generally higher, indicating a negative year based correlation between growing season rainfall and temperature stress conditions. This aligns with the common co-occurrence of multiple abiotic stress conditions previously discussed by (Machado and Paulsen, 2001; Shah and Paulsen, 2003). However, despite the lower levels of temperature stress evident in the 2013 growing season, the rate of response to increasing temperature conditions was greater in 2013 compared to 2014 if a significant year interaction was present (Fig. 3). Perhaps an indicator of acclimation, with a 'softer' environment evident in the 2013 season making plants more prone to heat stress.

More data, with additional environments sampled across extra growing seasons is required to fully understand the complex interactions discussed. Other year anomalies present in the data collected in this study should not be completely ignored, and the impacts that they may have on the data and subsequent interpretation. In 2013 the experiment at Winulta experienced a relatively high rainfall compared to all other experiments in the study and may have had a disproportionality large impact on the 2013 data. The reduced range of climatic co-variates observed and therefore smaller differences between environments in 2014, as well as a smaller range in grain yields achieved compared to 2013, is likely reducing the ability of the model to identify grain yield trends in response to temperature stress.

Heat stress, as measured by various temperature co-variates was found to be of particular importance for this study and is similar to the findings of Kuchel et al. (2007a). The large dataset captured across multiple locations and years in this study has provided a basis for dissecting heat stress related genotype by environment interactions and assessment of genotype response to heat stress conditions, and confirmed that temperature is a key driver of genotype by environment interactions and crop performance. However, further data would help to explain how other environmental factors influence the impact of heat stress on plant performance.

4.3. Contrasting genotype performance in response to heat stress and developmental stage

The performance of each genotype in the field was examined with respect to climatic co-variates (Table 7). Many of the genotypes included in the current study have previously been described as being tolerant to abiotic stresses. Halberd (Hays et al., 2007), RAC875 (Izanloo et al., 2008; Fleury et al., 2010) and Gladius (Fleury et al., 2010; Talukder et al., 2014) have been described as heat stress tolerant, as well as RAC875 and Gladius being described as drought tolerant (Izanloo et al., 2008; Fleury et al., 2010; Bennett et al., 2012a). In this study, Halberd was found to be relatively tolerant to heat stress conditions experienced in the field during anthesis. While RAC875 was shown to be less tolerant than the mean of genotypes included, and Gladius similar to the mean. The opposite was true for heat stress conditions experienced during grain fill, with Gladius and RAC875 both performing better than Halberd, and RAC875 performing better than the mean of all genotypes included in the study. This is likely a reflection of tolerance mechanisms important for adaptation to anthesis and grain fill heat stress conditions, respectively. Promisingly, genotypes such as Axe and Mace showed superior performance to both anthesis and grain fill heat stress conditions, suggesting that tolerance mechanisms for both developmental stages may be combined.

4.4. Exotic sources of heat stress tolerance identified

Three exotic genotypes selected from a previous heat stress tolerance screen (Telfer & Bennett, unpublished data) were included in the study. The relative heat stress tolerance of these genotype was confirmed in this study, with AUS4683 and AUS4906 having the smallest response to increasing average maximum temperature during anthesis, while AUS4926 had a heat stress response less than the mean of the genotypes included in the study for heat stress co-variates during grain fill (Table 7). However, any advantages for adaptation to heat stress conditions identified may be of reduced relevance due to significantly lower grain yields achieved by these genotypes relative to genotypes with a more adapted background. Further work is required to identify if the genetic basis to the heat stress tolerance of these lines can be exploited independent of their low grain yield.

4.5. Using climatic co-variates to understand heat stress environments

To evaluate the performance of genotypes under varying heat stress conditions, a range of climatic co-variates were used (Table 2). As well as growing season rainfall, the temperature conditions were quantified to evaluate both gradually increasing temperatures across a large duration of time (average maximum temperature) as well as short extremes of temperature (number of days above 30°C or 35°C degrees). As was noted in this study, Campbell et al. (2004) reported the frequent collinearity of climatic co-variates, and an understanding of this is needed in analysis and interpretation of results. Additionally, growing season rainfall was negatively correlated with all of the temperature climatic co-varies, supporting the notion that abiotic stress conditions often co-occur (Machado and Paulsen, 2001; Shah and Paulsen, 2003)

This study found that heat stress conditions during anthesis and grain fill adversely impacted on grain yield production. This relationship was confirmed through the factor analytic analysis of genotype by environment interaction for grain yield. Correlations between mean climatic co-variates with environmental loadings calculated in the factor analytic analysis (Table 9) showed that many of the climatic co-variates measured were important in influencing genotype by environment interactions for a number of the factors, particularly FA1, FA3 and FA4. This is similar to that described by Kuchel et al. (2007a) and Dreccer et al. (2008) who showed that various climatic co-variates were important drivers of genotype by environment interactions and crop performance. In the current study FA1 accounted for approximately 85% of the genotype by environment interaction present, with the co-variates describing temperature during anthesis showing the highest correlations, thus indicating a strong influence on genotype by environment interaction for grain yield. Interestingly, correlations with some anthesis heat stress co-variates were higher than growing season rainfall (Table 9). FA2, FA3 and FA4, although accounting for much less of the variance of genotype by environment interaction (Table 8), did show stronger correlations with grain fill heat stress co-variates. This demonstrates that heat stress during both grain fill and anthesis are important in determining the adaptation of genotypes for the southern Australian Mediterranean-type environments included in this study.

4.6. Controlled environment and field testing to understand genotype response to heat stress

Using the controlled-environment assay in this study did not produce the significant genotype by heat stress treatment interactions for grain number and size observed in previous experiments using this assay (Telfer & Bennett, unpublished data) as well as other studies (Stone and Nicolas, 1995a; Hays et al., 2007; Maphosa et al., 2014). However differential genotype responses to the heat stress conditions imposed were identified for leaf senesces (Leaf2) and leaf chlorophyll content (SPAD2) (Table 3 and Table 4). The genotype response to the heat stress treatment for these traits was correlated with the genotype score produced from the factor analytic analysis of grain yield (Table 10), allowing the phenotype observed in the controlled environment to be linked to performance in the field. This process illustrated that traits measured in the controlled environment, that also identified differential genotype performance, appear to be related to genotype performance in the field (Table 10). This was more evident for Leaf2 which had a significant ($P < 0.05$) correlation of -0.42 with FA1. Suggesting that selection for maintenance of green leaf area under heat stress conditions will in turn select for grain yield maintenance under heat stress conditions.

Conducting evaluation of adaption to heat stress conditions in a controlled environment has numerous advantages, namely consistency of stress conditions over all genotypes and being independent of other confounding factors. This study, in part, confirms the relevance of the controlled environment assay phenotypic screen in determining adaption to heat stress in the field, with traits found to be significant in the controlled environment assay correlating to genotype by environment interactions for grain yield performance in the field. This study also demonstrated that evaluation of relative heat stress tolerance of genotypes was possible by using multiple representative field environments and modelling climatic co-variates to determine genotype response to heat stress conditions (Table 7). A breeding program selecting in field environments prone to heat stress is likely to make genetic gain for adaptation to heat stress conditions. Additionally, targeted evaluation such as that discussed and demonstrated here is likely to accelerate genetic gain for adaptation to such conditions. Although the relationship wasn't strong, the controlled environment and field methods used in this study were found to be complementary and informative for assessing heat stress tolerance. Assessing large scale populations for genetic studies may not always be feasible across large multi-environment trials, in which case controlled environment studies may provide an opportunity to reliably phenotype germplasm with minimal confounding factors (Wahid et al., 2007).

5. Conclusions

This study demonstrated that multi-environment field trials could be used to characterise genotypes for heat stress tolerance. The results herein demonstrated that evaluating adaptation to heat stress conditions in the field where stress conditions may be more complex and impact on multiple physiological processes and growth stages is valuable. With an appropriate design and selection of experimental locations, heat stress evaluation is possible even in the presence of other confounding environmental factors. Such results provide the justification and opportunity to incorporate evaluating adaptation to heat stress conditions as a routine component in standard multi-environment field studies. Breeders will subsequently have improved knowledge of variation for heat stress tolerance in their germplasm, as well as being in a better position to select for improved tolerance in their breeding materials. This will ultimately improve the base-level heat tolerance in parents used for variety development. In this study the results from a controlled environment study were correlated with grain yield results in the field. This demonstrates that the controlled environment assay used in this study can also be used, in part, to understand heat stress tolerance expressed in field conditions. A key finding of this study was that green leaf area retention, post heat stress in a controlled environment, can be used to improve heat stress tolerance in the field.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.fcr.2018.09.013.

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Chapter 3. Genetic Analysis of Wheat (*Triticum aestivum*) Adaptation to Heat Stress

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3.1. Contextual Statement

Adaptation to stress conditions, usually termed 'tolerance', is poorly defined. Previous definitions have not necessarily aligned with breeding objectives to improve crop productivity as well as adaptation to stressed conditions. This manuscript presents a new framework to assess adaptation to heat stress conditions that have greater relevance to breeders' selection objectives. This framework considers adaptation as the combination of responsiveness (positive response to stressed conditions) and performance (stable performance advantage across stressed and unstressed treatments). This manuscript presents the genetic analysis of seven doubled haploid genetic mapping populations screened in a controlled environment assay targeting grain-filling heat stress conditions (as described in Chapter 2). QTL for stable performance across the stress and control treatments, as well as QTL with a positive responsiveness to stress conditions, are discussed for their potential role in adaptation to stress conditions.

3.2. Statement of Authorship

Statement of Authorship

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Principal Author

Name of Principal Author (Candidate)	Mr Paul Telfer		
Contribution to the Paper	Designed and ran experiments, analysed and interpreted the data, and prepared the manuscript.		
Overall percentage (%)	85		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	25/02/2022

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Dr James Edwards		
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ORIGINAL ARTICLE

Genetic analysis of wheat (*Triticum aestivum*) adaptation to heat stress

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Abstract

Key message **Adaptation to abiotic stresses such as high-temperature conditions should be considered as its independent components of total performance and responsiveness.**

Abstract Understanding and identifying improved adaptation to abiotic stresses such as heat stress has been the focus of a number of studies in recent decades. However, confusing and potentially misleading terminology has made progress difficult and hard to apply within breeding programs selecting for improved adaption to heat stress conditions. This study proposes that adaption to heat stress (and other abiotic stresses) be considered as the combination of total performance and responsiveness to heat stress. In this study, 1413 doubled haploid lines from seven populations were screened through a controlled environment assay, subjecting plants to three consecutive eight hour days of an air temperature of 36 °C and a wind speed of 40 km h^{-1}, 10 days after the end of anthesis. QTL mapping identified a total of 96 QTL for grain yield determining traits and anthesis date with nine correlating to responsiveness, 72 for total performance and 15 for anthesis date. Responsiveness QTL were found both collocated with other performance QTL as well as independently. A sound understanding of genomic regions associated with total performance and responsiveness will be important for breeders. Genomic regions of total performance, those that show higher performance that is stable under both stressed and non-stressed conditions, potentially offer significant opportunities to breeders. We propose this as a definition and selection target that has not previously been defined for heat stress adaptation.

Introduction

Bread wheat (*Triticum aestivum* L.) is grown in many regions throughout the world and contributes significantly to human nutrition. Many of the wheat-growing regions are classed as arid or semi-arid environments, including southern Australia (Zheng et al. 2012) where co-occurring stresses are often experienced, including terminal drought, high temperatures and high wind (Machado and Paulsen 2001; Shah and Paulsen 2003). Additionally, periods of elevated temperatures, with maximum daily temperatures in excess of 35 °C accompanied with winds in excess of 40 km h^{-1}, are common during post-anthesis periods in late spring in southern Australia (Alexander et al. 2010; Talukder et al. 2013).

Heat stress conditions are most likely to occur later in the season, often coinciding with grain-filling (Alexander et al. 2010). High temperature stress at this stage is associated with reduced starch and protein accumulation (Bhullar and Jenner 1985; Zahedi et al. 2004), accelerated plant development and leaf senescence and reduced photosynthetic rate, grain yield and grain size (Sharma et al. 2008; Stone and Nicolas 1995; Talukder et al. 2014a; Tewolde et al. 2006; Wardlaw 1994; Wollenweber et al. 2003). Kuchel et al. (2007) and Bennett et al. (2012) reported grain yield reductions in field experiments conducted across southern Australia of up to 187 kg ha^{-1} for every one degree increase in

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average temperature during anthesis and grain fill. Similarly, Telfer et al. (2018) identified a reduction of 161 kg ha^{-1} for each day with a maximum temperature in excess of 30 °C during grain fill.

Although stress generally occurs less frequently during reproductive developmental stages of wheat, plants are acutely sensitive. Heat stress during pollen development at the young microspore stage can result in reduced pollen viability and reduced grain number being set (Dolferus et al. 2011; Saini et al. 1999). Telfer et al. (2018) identified a grain yield reduction of 302 kg ha^{-1} for each day with a maximum temperature in excess of 30 °C during anthesis.

Heat stress tolerance in plants has previously been investigated using a number of different approaches. Many studies have been undertaken using controlled environment facilities, allowing for reliable and repeatable experimental conditions to be imposed (Esten Mason et al. 2010, 2011; Maphosa et al. 2014; Stone and Nicolas 1995; Tashiro and Wardlaw 1990; Telfer et al. 2018). In contrast, other research has been performed using small scale semi-controlled environment chambers within field plot experiments (Alexander et al. 2010; Talukder et al. 2013). Delayed sowing of field nurseries has also been used as a strategy to increase plant exposure to higher temperature conditions later in the growing season (Bennett et al. 2012; Esten Mason et al. 2013; Pinto et al. 2010; Reynolds et al. 2007; Sadras et al. 2015). With this strategy, although quite successful in increasing terminal heat stress, plants are also exposed to a range of growing conditions that are abnormal, including longer photoperiod and altered plant available water (Sadras et al. 2015). Telfer et al. (2018) investigated genotype-by-environment interactions (GxE) in wheat using a range of representative growing environments contrasting for temperature conditions during the anthesis and grain fill periods to evaluate adaptation to heat stress conditions.

Previous studies focused on the underlying genetics have found numerous QTL for performance of wheat under heat stress conditions. Such studies have used the various evaluation methods discussed above, including controlled environment assays where QTL have been identified for grain number (Esten Mason et al. 2010, 2011; Mohammadi et al. 2008; Shirdelmoghanloo 2014), grain size (Esten Mason et al. 2010, 2011), leaf senescence and stay-green (Shirdelmoghanloo 2014; Talukder et al. 2014b; Vijayalakshmi et al. 2010) and grain fill duration (Shirdelmoghanloo 2014). Field-based studies evaluating performance under heat have identified QTL for grain yield (Bennett et al. 2012; Bhusal et al. 2017; Bonneau et al. 2013; Esten Mason et al. 2013; Pinto et al. 2016, 2010), grain size (Bennett et al. 2012; Bhusal et al. 2017; Paliwal et al. 2012; Pinto et al. 2016, 2010), grain number (Bhusal et al. 2017; Pinto et al. 2016, 2010), grain fill duration (Bhusal et al. 2017; Paliwal et al. 2012; Pinto et al. 2016), along with leaf senescence and stay-green phenotype (Pinto et al. 2016, 2010). While most studies have focused on investigating QTL for performance under heat stress, some work has acknowledged the important genotype by environment interactions of abiotic stress adaptation including that of Parent et al. (2017) who looked to understand the interactions of heat and drought on QTL performance across multiple field environments.

Breeders use a range of representative and relevant environments to evaluate and select material for improved adaptation within their breeding programs, often referred to as the target population environment (TPE) (Basford and Cooper 1998; Comstock 1977). Within each of these environments, various components will influence and interact to produce the growing environment including rainfall, temperature, frost, soil nutrition, plant pests and diseases. Further variation of these environmental factors across seasons, years and locations and how these factors interact with differential varietal performance is referred to as GxE (Basford and Cooper 1998). An understanding of the factors influencing GxE and varietal adaptation is an important process of targeted breeding and selection for improved adaptation of new varieties. A number of methods have been used to understand and measure differences in varietal adaptation to abiotic stress conditions. Examples include Fischer and Maurer (1978), who defined a susceptibility index comparing performance of varieties under both water limited and non-stressed growing conditions. Alternatively, Rosielle and Hamblin (1981) discussed comparing mean performance of varieties under stressed and non-stressed environments. These computational methods have been applied to studies of adaptation to heat stress conditions, with Esten Mason et al. (2010) and Esten Mason et al. (2013) using a susceptibility index as defined by Fischer and Maurer (1978) for controlled environment and field-based studies, respectively, on response to heat stress. Bennett et al. (2012) used mean performance when looking at impacts of heat stress resulting from delayed sowing in wheat, similar to that defined and discussed by Rosielle and Hamblin (1981). Studies by Sio-Se Mardeh et al. (2006) and Mohammadi et al. (2010) evaluated the effectiveness and potential unintended implications of various selection indices when evaluating relative tolerance to drought conditions, including that defined by Fischer and Maurer (1978) and Rosielle and Hamblin (1981), finding that different outcomes were achieved from different indices and potential interactions with the severity of stress conditions present. This included Mohammadi et al. (2010) who demonstrated that a susceptibility index, in favouring lines that have a low penalty in performance under stress conditions relative to unstressed conditions, can inadvertently favour lines that have an inherently low performance ability, a combination not favourable for overall performance and therefore not favoured by breeders.

Past research has often used confusing and potentially misleading terminology to describe improved adaptation to stress conditions including that of heat stress, using terms like tolerance, resistance and susceptibility. It is not always clear what these terms mean, as demonstrated by the range of definitions as well as a lack of understanding on adaptation in a GxE sense. This was recognised by Lemerle et al. (2006), who noted the limitations of comparing the difference in performance between a stressed and control treatment, as the performance of varieties under stressed and non-stressed conditions was frequently highly correlated. To overcome this limitation, Lemerle et al. (2006) and Dolferus et al. (2019) further defined tolerance as a positive deviation from the response expected under stress conditions relative to non-stressed conditions. This definition identifies tolerance in terms of responsiveness of a variety or gene to changing stress conditions. Breeders, who are selecting for performance in a heterogeneous set of environments, may need to consider trait performance and responsiveness separately. A similar notion is discussed by Blum (2005), who recognised that independent selection for yield potential and abiotic stress resistance is required to maximise the value of breeding efforts. Furthermore, we propose that the use of the word tolerance should not be used to infer desirability. Rather, a particular combination of performance and responsiveness that best delivers a positive economic return to farmers should be considered the desirable objective of breeding, and for the purposes of this manuscript will be referred to as adaptation to heat stress. Herein we propose and evaluate a framework for exploring the uncorrelated traits of performance and responsiveness to stress, for both lines and QTL, in this case for adaptation to heat stress in wheat.

Although a number of QTL associated with adaptation to heat stress conditions have been reported, their role in responsiveness is not well known and to the knowledge of the authors there are no examples of molecular markers being used in wheat breeding to improve heat stress adaptation. This study uses a series of wheat doubled haploid (DH) populations, tested within a controlled environment heat chamber, to dissect the genetic basis of performance under high temperatures as well as responsiveness to heat stress conditions in wheat.

Methods and materials

Germplasm

For this study, seven DH bi-parental populations were used (Table 1) that represent the major genetic backgrounds of wheat varieties grown in southern Australia, and potential novel sources of adaptation to heat stress conditions.

The putative sources of novel heat stress adaptation used in the creation of these mapping populations were identified in a previous heat stress screens, using the method of Telfer et al. (2018), and were performed on 222 Focused Identification of Germplasm Strategy (FIGS) lines (Mackay et al. 2004). These FIGS lines were sourced from wheat regions (primarily within and adjacent to the Fertile Crescent) where heat stress conditions are similar to that experienced in southern Australia.

Genotyping of germplasm and genetic map construction

Linkage map construction for the mapping populations used in this study is described in detail by Norman et al. (2017). In brief, DNA was isolated from seed for all lines in each population and genotyped using a custom Axiom™ Affymetrix array containing 18,101 SNP markers. Linkage maps of the SNP markers were created for each bi-parental DH population using a combination of the R/qtl (Broman and Sen 2009; Broman and Wu 2015) and R/ASMap (Taylor and Butler 2015) packages within the R statistical environment (R Core Team 2018). The resulting genetic linkage maps for

Table 1 The populations screened for heat stress tolerance in the controlled environment assay, along with additional population and map information

Population	Pedigree	No. lines	# Polymorphic SNP markers	# Unique positions	Genetic length (cM)	Mean interval*
MG	MACE/GLADIUS	176	5047	1429	3009	2.1
SM	SCOUT/MACE	226	4950	1360	3030	2.2
SG	SCOUT/GLADIUS	369	5143	1761	2998	1.7
RG	RAC1548/GLADIUS	132	5133	1183	3055	2.6
L1G	AUS17750/GLADIUS	116	6159	1272	3102	2.4
L2G	AUS17840/GLADIUS	124	5514	1132	3144	2.8
HK	HALBERD/KENNEDY	121	6296	1365	3342	2.5

*Mean interval (cM) between unique map positions

each population (Supplementary Material Table 1) had in excess of 5000 polymorphic markers (Table 1).

Heat stress assay protocol

The controlled environment assay used to screen the germplasm described for adaptation to heat stress conditions in this study is described in Telfer et al. (2018). Seeds were planted individually into pots (7 cm × 7 cm × 16 cm) within a greenhouse and laid out in a rectangular array indexed by rows and columns on irrigation mats equipped with timers to ensure adequate plant available moisture. A whole nutrient solution was applied on a weekly basis, pests and diseases were managed as required. When plants reached the end of anthesis (Z69) (Zadoks et al. 1974), they were removed from their experimental layout and a barcoded identity tag applied to the primary tiller. Plants designated as control plants were immediately returned to their original experimental grid layout location. Plants designated to be heat-stressed were then loosely tied to a stake to support the plant to prevent physical damage by wind during the stressing treatment before being placed in the heat stress chamber, where they experienced three consecutive days of eight hours under an air temperature of 36 °C and a wind speed of 40 km h^{-1}. Conditions in the greenhouse for the duration of the experiments averaged 18 °C with vapour pressure deficit (VPD) averaging 0.8 kPa. Conditions in the heat stress chamber averaged 36 °C with VPD averaging 4.6 kPa, with stress chamber night-time ambient conditions averaging 16 °C and VPD averaging 0.7 kPa. At the end of day three the heat-stressed plants were removed from the heat stress chamber and returned to their original experimental grid layout location, where plants remained until they matured. All plants were harvested by cutting off at ground level, then dried in an oven (40 °C for 72 h), and then processed for final measurements (Table 2) in the lab.

Experimental design

Populations were assessed in five experiments that are summarised in Supplementary Table 2. Experiment 1 was conducted in 2013 and the remainder in 2014. The first four experiments were carried out on four single independent bi-parental populations (HK, RG, L2G and L1G), while the fifth experiment included three interrelated populations derived from Gladius, LRPB Scout and Mace (encompassing populations MG, SM and SG, hereafter collectively called GSM). In all experiments, the experimental units were single plant pots which were laid out in the greenhouse in a rectangular array indexed by rows and columns. The treatments comprised the factorial combinations of lines (DH lines and parents) and heat stress treatments (stressed and control). The design of all experiments involved a split-plot structure in which the main plots comprised pairs of adjacent pots, where the adjacency was in the row dimension, and the sub plots were the individual pots within main plots. Lines were randomised to the main plots, and the heat stress treatments to the sub plots. Supplementary Table 2 shows the total number of lines and main plots used in each experiment.

The manner in which lines were randomised to main plots differed between experiments. The design for experiment 1 (evaluating the HK population) employed two replicates. Within each replicate, there were three additional main plots of each of four lines, namely the parent's Halberd and Kennedy and the check varieties Wyalkatchem and Gladius. In order to manage the workload and heat chamber capacity most effectively, experiments 2, 3, 4 and 5 were divided into blocks with a different sowing time allocated to each block. In experiments 2, 3 and 4, there were three blocks that were aligned with rows (1 to 8, 9 to 16 and 17 to 24). The sowing dates for each block were at weekly intervals. The designs for these experiments employed partial replication of lines (Cullis et al. 2006) so that some lines were allocated to two main plots and the remainder to a single main plot only. The percentage of DH lines with two replicates is shown in Supplementary Table 2. DH lines with two replicates were

Table 2 Traits measured or observed as a part of the controlled environment heat stress assay

Trait	Abbreviation of traits used for QTL analysis	Trait description	Stage observed	Units
Spikelet number per spike	Spn	Number of spikelets per spike	Post-harvest	Spikelets $spike^{-1}$
Grain number per spike	Gne	Total number of grains in the mature spike	Post-harvest	Grains $spike^{-1}$
Grain yield per spike	Gwe	Total weight of grain produced per spike	Post-harvest	Grams
Thousand grain weight	Tgw	Thousand grain weight—derived from grain number per spike and grain yield per spike	Post-harvest	Grams
Spikelet fertility	Sfi	Average number of grains per spikelet	Post-harvest	Grains $Spikelet^{-1}$
Anthesis date	Flt	Zadoks growth stage 69 (the end of anthesis for the primary tiller)	End of anthesis	Days from planting

spread over different blocks. Parental lines had additional replicates and were typically allocated in at least two main plots in each block. Experiment 5 comprised a total of 1087 DH lines across three populations. Seed for the experiment was sourced from two different locations: the first seed source (denoted "A") comprised 460 DH lines (including lines from each population) and the second ("B") the remaining 627 DH lines. All pots from a single source were located together to form a physical block in the greenhouse. The complete experiment comprised 2496 pots arranged as 156 rows by 16 columns. Within this layout, the block for seed source "A" occupied 66 rows and "B" occupied 90 rows. A separate design was constructed for each seed source block using the same approach for experiments 2, 3 and 4. Thus, there were three sowing date blocks for each seed source block with the dates for source "A" preceding those for "B". The parental lines were allocated to main plots in both seed source blocks.

QTL analysis

In the current study, the treatment structure involved a two-level factor that reflects a control and stress treatment. Cullis and Smith (2016) developed a one-stage linear mixed model (LMM) approach for QTL experiments of this nature. In their approach, the two treatments are thought of as representing two traits so are modelled using a bi-variate genetic variance structure. Importantly, this structure allows the derivation of a third trait of interest, namely responsiveness. A one-stage analysis was not feasible in the current study, so the most efficient possible two-stage approximation was adopted. The first stage analysis was identical to the baseline LMM (that is, the LMM without the inclusion of marker data) in the Cullis and Smith (2016) approach. This provided data for the second stage QTL analysis which was conducted using the WGAIM package (Taylor and Verbyla 2011).

The data for any given experiment and response variable were analysed using a LMM in which both genetic and non-genetic effects were included. The latter were included to encapsulate the plot structure of the experimental design as described above. In terms of genetic effects, we let $u_{g_{i+}}$ and $u_{g_{i-}}$ denote the true (total) genetic effect for the ith DH line for the stress and control treatments, respectively. These effects may be regarded as representing two traits so were accommodated in the model using a bivariate genetic variance structure. This involved a genetic variance for each set of effects, denoted σ^2_{g+} and σ^2_{g-}, for the stress and control treatments, respectively, and a covariance between them, denoted $\sigma_{g\pm}$. Thus the genetic correlation between the two traits was given by $\rho_{g\pm} = \sigma_{g\pm}/\sqrt{\sigma^2_{g+}\sigma^2_{g-}}$. The bi-variate genetic variance structure can also be viewed as arising from a regression of the true genetic effects associated with the stress treatment on the effects associated with the control treatment. This can be written, for the ith DH line, as

$$u_{g_{i+}} = \beta_g u_{g_{i-}} + \delta_{g_i}$$

where β_g is the slope of the regression and is given by $\sigma_{g\pm}/\sigma^2_{g-}$. Typically, the slope of this regression line would be positive, reflecting the fact that, on average, DH lines with higher genetic effects under the control treatment also have higher genetic effects under the stress treatment. Departures from this average response are represented by δ_{g_i} since this is the deviation (or residual) from the regression line for the ith DH line. These deviations define the third trait of responsiveness. A DH line with a positive response would have a large positive deviation, reflecting the fact that when subjected to the heat stress treatment the line has a higher genetic effect than would be expected given its genetic effect in the absence of stress.

All stage 1 analyses were fitted using ASReml-R (Butler et al. 2009) which provided residual maximum likelihood (REML) estimates of variance parameters and empirical best linear unbiased predictions (EBLUPs) of random effects, together with their associated prediction error variances (PEVs). The EBLUPs of the genetic effects for the DH lines for the control and deviation traits were then de-regressed (Garrick et al. 2009) for use as data in the second stage QTL analysis, and from here on are denoted as performance and responsiveness, respectively. For example, the EBLUP of the genetic effect for the ith DH line performance is denoted by $\tilde{u}_{g_{i+}}$. The reliability of this prediction is defined as the squared correlation between $\tilde{u}_{g_{i+}}$ and the true genetic value $\left(u_{g_{i+}}\right)$. This can be estimated as $r^2_{i+} = 1 - PEV_{i+}/\hat{\sigma}^2_{g+}$, where PEV_{i+} is the prediction error variance for $\tilde{u}_{g_{i+}}$ and $\hat{\sigma}^2_{g+}$ is the REML estimate of the genetic variance for the stress treatment. The de-regressed data for the ith DH line were then given by $\tilde{u}_{g_{i+}}/r^2_{i+}$. De-regressed data for the responsiveness trait were obtained in an analogous manner.

Note that if the estimated genetic correlation between the stress and control treatments was unity then there were no deviations about the regression line and the estimated genetic variance for the responsiveness trait was zero. In this case, there was no QTL analysis for responsiveness, and a QTL analysis occurred for performance only. Given that the totality of genetic effects within the experiments can be defined using the uncorrelated traits performance and responsiveness, no further investigation of the EBLUP of the genetic effect under heat stress is required.

The de-regressed data from the performance and responsiveness datasets were then analysed to identify QTL for each trait measured using the WGAIM package (Taylor and Verbyla 2011).

Additionally, due to anthesis date being recorded prior to the stress treatment being applied to the experimental

design, the QTL analysis of anthesis date data was instead conducted on the raw data (control treatment data), using WGAIM (Taylor and Verbyla 2011).

In each instance, QTL analysis was conducted on unique markers, not the total number of markers available for each population. The number of unique markers available for each population is detailed in Table 1. While QTL analysis was conducted using the maps of individual populations as required by WGAIM, the position of the identified QTL is reported with their consensus map position as published by Norman et al. (2017) and shown in Supplementary Table 1. A suitable marker from within the identified interval for each QTL was used to identify the RefSeq physical position (Alaux et al. 2018), with the base pair position of the start of the candidate marker sequence reported for each QTL.

Results

Assessing line adaptation to heat stress

The relationship between control and heat treatments for spikelet number per spike EBLUPS is represented in Fig. 1a. The deviations (responsiveness) in line performance away from the regression between these traits (as illustrated by HX0004*109 and HX0004*007 in Fig. 1a) is also displayed in respect to relative performance of the control (Fig. 1b). A proposed framework to individually assess components' trait performance and responsiveness to stress conditions is shown in Fig. 1c. Using this framework, genetic variance was observed for performance for each of the traits measured in each of the populations, while responsiveness to heat stress was observed for 21 of the 35 trait-by-population datasets (Table 3). In general, the variance attributable to performance was substantially larger than responsiveness.

Using the example for demonstation of spikelets per spike in the RG population (Fig. 1), with a threshold of plus and minus one from the mean for trait performance and plus and minus 0.5 from the mean for trait responsiveness, we can see that the majority of lines broadly fell into the description of mean performance and non-responsive to heat stress. There were, however, twelve lines showing a positive response to heat stress (including HX0004*109) and eleven lines that showed a negative response to heat (including HX0004*007). One line (HX0004*109) demonstrated a positive response to heat stress with a high performance under unstressed control conditions, which is the most favourable combination.

QTL analysis

Our QTL analysis identified 96 QTL across all populations included in the study (Table 1) and traits measured (Table 2). The consensus map position (cM) and percentage variance accounted for by each QTL identified for each phenotypic trait measured and population is shown in Fig. 2. Pearson correlation coefficients comparing the data collected for each trait described in Table 2 are shown in Supplementary Table 3.

The timing of anthesis and grain filling, as a result of a line's relative maturity, can be a large determinant of heat stress severity. Often in Australia, later maturing lines in field conditions will be exposed to higher temperatures. As this study was conducted under a semi-controlled environment, minimising changes in temperature as the season progresses, anthesis date per se should not have had a large impact on heat stress severity. However, mapping of anthesis date QTL was conducted given the fundamental influence that phenology genes have on yield and yield-related traits in wheat (Chen et al. 2018; Groos et al. 2003; Huang et al. 2006; Maphosa et al. 2014). A total of 15 QTL were identified for anthesis date across all seven populations (Table 4), with many appearing in similar positions to the well-known *Ppd-B1*, *Ppd-D1*, *Vrn-A1* and *Vrn-D1* loci. Of these QTL, eight were found to be located near QTL for other plant performance traits.

A total of 49 performance QTL were identified for spikelet number per spike, grain number per spike and spikelet fertility (Table 4). QTL for grain number determining traits were found in six populations (none were identified in the RG population) across 16 different chromosomes ranging in genetic variance accounted for from 1.7 to 20.8%. A further 23 performance QTL were identified for grain yield per spike and thousand grain weight (Table 4). These QTL were found in four populations (HK, MG, SG and SM), across 12 chromosomes ranging in contribution to genetic variance from 1.4 to 24%.

A total of nine QTL for responsiveness (Table 4) were identified representing nine different loci on eight different chromosomes. All but one of the responsiveness QTL were identified in the RG population, including a single QTL for grain yield per spike (*QGwe.agt-RG.2A*) on chromosome 2A (69.0–71.1 cM) with Gladius being the source of the favourable allele, and seven QTL for spikelet number per spike on chromosomes 3A, 4B, 4D, 5A, 6A and 6B with both the Gladius and RAC1548 parents being the source of the favourable alleles. The last QTL for responsiveness to heat stress was found in the SG population for spikelet number per spike (*QSpn.agt-SG.1D*) on chromosome 1D (23.4–24.6 cM) with the favourable allele inherited from Scout.

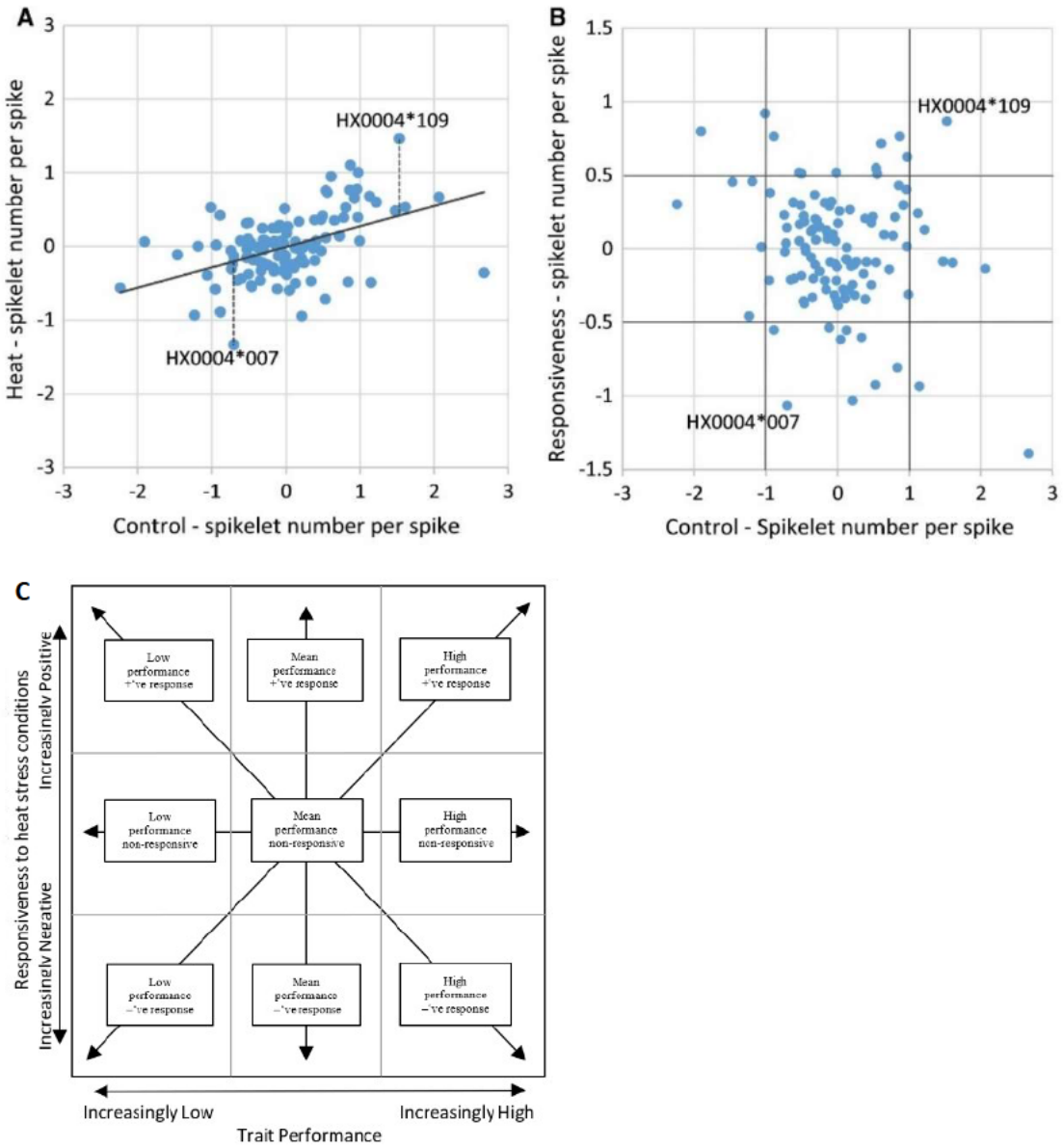

Fig. 1 A scatter plot of line EBLUPS for spikelet number per spike in the RG population under control and heat stress conditions in (**a**) allowed for the comparison of line performance under the two treatment conditions where a significant interaction effect was identified. In this case, the deviation from the mean or responsiveness was identified (as demonstrated by the dashed lines between the mean response line and lines showing a large deviation). A scatter plot of performance under control conditions and responsiveness (**b**) allowed the individual line performance and responsiveness to heat stress conditions to be evaluated as per the framework (**c**), allowing assessment of total trait performance separately but in concurrence with responsiveness to stress conditions

Key QTL regions

Clusters of collocated or close proximity QTL (< 10 cM) comprise up to seven QTL and include both performance and responsiveness QTL from all the traits and populations (Table 4). The largest cluster identified (cluster 2B-2) was on chromosome 2B with seven QTL positioned between 50.9 and 70.8 cM, consisting of performance QTL for spikelet fertility, grain number per spike, grain yield per spike, spikelet number per spike and thousand grain weight. On chromosome 2D between 41.7 and 45.8 cM, a performance QTL for spikelet fertility (*QSfi.agt-HK.2D*) was found to be collocated (cluster 2D-1) with anthesis date QTL (*QFlt.agt-SG.2D*, *QFlt.agt-HK.2D* and *QFlt.agt-MG.2D*) in three populations (SG, HK and MG). A further cluster of five performance QTL were identified on chromosome

Table 3 The genetic variance and reliability (average reliability across DH lines) for each population for performance and responsiveness for each trait

Trait	Variance components and reliabilities	MG	SM	SG	RG	L1G	L2G	HK
Spikelet fertility	Performance genetic variance	0.54	0.33	0.15	0.25	0.23	0.20	0.39
	Performance reliability	0.80	0.73	0.50	0.41	0.50	0.68	0.57
	Responsiveness genetic variance	0.02	0.01	0.02	0.06	–	0.02	0.09
	Responsiveness reliability	0.07	0.04	0.07	0.14	–	0.09	0.21
Grain number per spike	Performance genetic variance	216.14	201.96	94.51	53.71	96.83	105.11	195.66
	Performance reliability	0.76	0.73	0.50	0.30	0.48	0.71	0.59
	Responsiveness genetic variance	–	8.52	–	–	–	–	74.13
	Responsiveness reliability	–	0.05	–	–	–	–	0.34
Grain yield per spike	Performance genetic variance	0.51	0.72	0.28	0.11	0.23	0.34	0.32
	Performance reliability	0.74	0.78	0.59	0.29	0.60	0.53	0.53
	Responsiveness genetic variance	–	–	–	0.01	–	0.01	0.18
	Responsiveness reliability	–	–	–	0.03	–	0.03	0.38
Spikelet number per spike	Performance genetic variance	3.25	7.21	2.96	1.83	2.66	5.20	5.01
	Performance reliability	0.69	0.80	0.62	0.29	0.54	0.79	0.72
	Responsiveness genetic variance	–	1.91	0.78	0.78	0.72	0.52	0.98
	Responsiveness reliability	–	0.38	0.23	0.21	0.28	0.16	0.22
Thousand grain weight	Performance genetic variance	107.11	117.92	45.34	44.40	32.87	50.46	50.54
	Performance reliability	0.73	0.73	0.52	0.36	0.46	0.53	0.52
	Responsiveness genetic variance	–	16.58	–	6.72	18.18	9.08	13.82
	Responsiveness reliability	–	0.26	–	0.08	0.34	0.16	0.17

Blank cells indicate caseswhere there was no responsiveness (the estimated genetic correlation between controland heat stress conditions was one)

5B (cluster 5B-1) in the SM population between 50.4 and 63.3 cM for the traits: grain yield per spike, thousand grain weight, spikelet fertility, grain number per spike and spikelet number per spike, accounting for 10.2 to13.2% of genetic variance. Another cluster was identified on chromosome 7A (cluster 7A-3) consisting of four QTL between 152.3 and 164.1 cM in the SG population (*QSfi.agt-SG.7A.2, QGne.agt-SG.7A.2 and QGwe.agt-SG.7A.2*) and SM population (*QSpn.agt-SM.7A*).

Three responsiveness QTL were found to collocate or occur in close proximity to other performance QTL. On chromosome 1D (cluster 1D-1), responsiveness QTL for spikelet number per spike (*QSpn.agt-SG.1D*) identified in the SG population occurred in close proximity to the performance QTL for spikelet fertility (*QSfi.agt-HK.1D.1*) in the HK population. On chromosome 2A (cluster 2A-1), the responsiveness QTL for grain yield per spike (*QGwe.agt-RG.2A*) in the RG population occurred in proximity to performance QTL for spikelet fertility (*QSfi.agt-SG.2A.1*) and grain number per spike (*QGne.agt-SG.2A.1*), both of which were identified in the SG population. A responsiveness QTL for spikelet number per spike (*QSpn.agt-RG.3A.1*) on chromosome 3A in the RG population was identified in proximity (cluster 3A-1) to a thousand grain weight performance QTL identified in the HK population (*QTgw.agt-HK.3A*) and also in the SG population (*QTgw.agt-SG.3A*). The remaining responsiveness QTL occurred in isolation.

Discussion

Assessing adaptation to high-temperature conditions using a framework to assess total performance value and responsiveness to heat stress

We propose in this study that adaptation to heat stress conditions should be assessed as the combination of total performance value of a variety as well as responsiveness to high-temperature conditions. Previous definitions of tolerance (Fischer and Maurer 1978; Rosielle and Hamblin 1981) may not fully define what breeders need to select for when

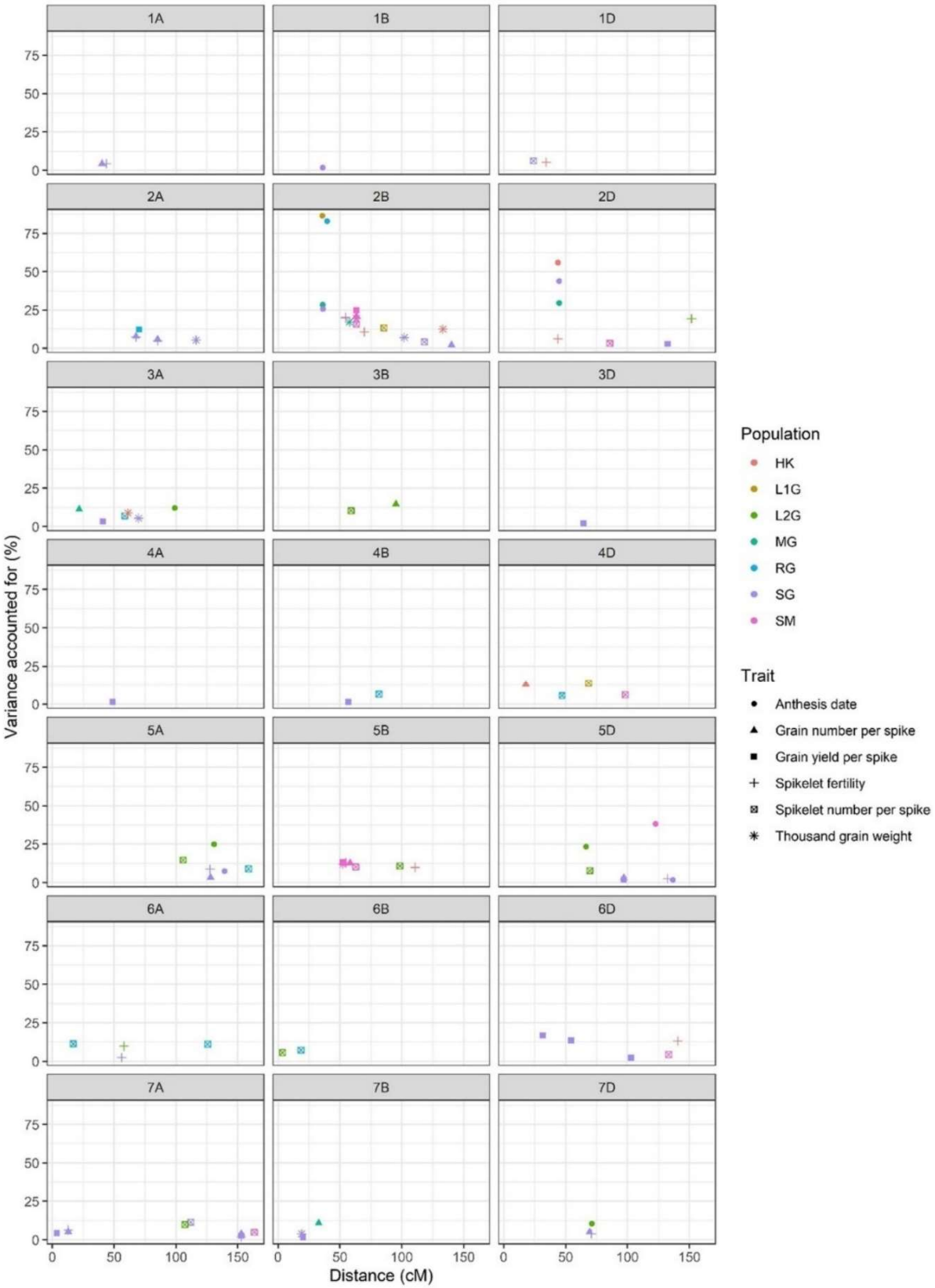

Fig. 2 The position (cM) and percentage (%) variance accounted for QTL identified for each population and phenotypic trait measured

Table 4 All QTL identified in the performance and responsiveness datasets for traits measured to quantify grain yield determining traits, as well as anthesis date QTL produced from non de-regressed control treatment data

Chromosome	Population	Trait	Unit	Data-set	Left–Right marker interval (cM)	Effect	Variance accounted for (%)	LOD	QTL Name	Favourable allele	RerfSeq physical position (Chromosome-base pair)	Cluster
1A	SG	Grain number per spike	Grains spike^{-1}	Performance	40.1–40.2	−3.10	4.4	3.964	*QGne.agt-SG.1A*	GLADIUS	1A-149868075	1A-1
1A	SG	Spikelet fertility	Grains spikelet^{-1}	Performance	43.3–43.8	−0.13	4.4	4.069	*QSfi.agt-SG.1A*	GLADIUS	1A-461048293	
1B	SG	Anthesis date	Days from planting	Control	33.7–38.4	1.95	1.6	2.21	*QFlt.agt-SG.1B*	SCOUT	1B-50777948	
1D	SG	Spikelet number per spike	Spikelets spike^{-1}	Responsiveness	23.4–24.6	0.48	6.2	3.411	*QSpn.agt-SG.1D*	SCOUT	1D-10613578	1D-1
1D	HK	Spikelet fertility	Grains spikelet^{-1}	Performance	32.3–36.2	−0.23	5.4	1.976	*QSfi.agt-HK.1D.1*	KENNEDY	1D-15335256	
2A	SG	Spikelet fertility	Grains spikelet^{-1}	Performance	67.4–67.7	−0.16	7.4	4.517	*QSfi.agt-SG.2A.1*	GLADIUS	2A-672427795	2A-1
2A	SG	Grain number per spike	Grains spike^{-1}	Performance	67.4–67.7	−4.14	7.8	4.737	*QGne.agt-SG.2A.1*	GLADIUS	2A-672427795	
2A	RG	Grain yield per spike	Grams	Responsiveness	69–71.1	−0.16	12.3	2.76	*QGwe.agt-RG.2A*	GLADIUS	2A-699439896	
2A	SG	Spikelet fertility	Grains spikelet^{-1}	Performance	83.9–86.2	0.13	4.9	2.838	*QSfi.agt-SG.2A.2*	SCOUT	2A-731377737	2A-2
2A	SG	Grain number per spike	Grains spike^{-1}	Performance	83.9–86.2	3.61	5.9	3.334	*QGne.agt-SG.2A.2*	SCOUT	2A-731377737	
2A	SG	Thousand grain weight	Grams	Performance	115.9–116.3	−2.21	5.4	3.574	*QTgw.agt-SG.2A*	GLADIUS	2A-758123477	
2B	SG	Anthesis date	Days from planting	Control	34.3–38	7.73	25.6	34.27	*QFlt.agt-SG.2B*	SCOUT	2B-58325043	2B-1
2B	L1G	Anthesis date	Days from planting	Control	34.3–37.3	11.86	86.5	35.952	*QFlt.agt-L1G.2B*	AUS17750	2B-49979399	
2B	MG	Anthesis date	Days from planting	Control	34.3–37.7	8.11	28.4	10.561	*QFlt.agt-MG.2B*	MACE	2B-49384102	
2B	RG	Anthesis date	Days from planting	Control	37.3–42	21.12	83.0	43.729	*QFlt.agt-RG.2B*	RAC1548	2B-65316281	

Table 4 (continued)

Chromosome	Population	Trait	Unit	Data-set	Left–Right marker interval (cM)	Effect	Variance accounted for (%)	LOD	QTL Name	Favourable allele	RefSeq physical position (Chromosome-base pair)	Cluster
2B	SM	Spikelet fertility	Grains spikelet^{-1}	Performance	50.9–58.4	0.32	20.0	10.166	*QSfi.agt-SM.2B*	SCOUT	2B-154838642	2B-2
2B	MG	Thousand grain weight	Grams	Performance	57.7–57.7	−5.12	17.2	4.487	*QTgw.agt-MG.2B*	GLADIUS	2B-154527560	
2B	SM	Grain number per spike	Grains spike^{-1}	Performance	62.5–64.1	8.19	20.8	10.557	*QGne.agt-SM.2B*	SCOUT	2B-211958955	
2B	SM	Grain yield per spike	Grams	Performance	62.5–64.1	0.53	24.9	13.476	*QGwe.agt-SM.2B*	SCOUT	2B-211958955	
2B	SM	Spikelet number per spike	Spikelets spike^{-1}	Performance	62.5–64.1	1.36	15.8	9.168	*QSpn.agt-SM.2B*	SCOUT	2B-211958955	
2B	SM	Thousand grain weight	Grams	Performance	62.5–64.1	6.13	19.6	9.598	*QTgw.agt-SM.2B.2*	SCOUT	2B-211958955	
2B	HK	Spikelet fertility	Grains spikelet^{-1}	Performance	68.7–70.8	−0.32	10.8	3.977	*QSfi.agt-HK.2B*	KENNEDY	2B-494376558	
2B	L1G	Spikelet number per spike	Spikelets spike^{-1}	Performance	84.8–86.2	0.85	13.4	3.058	*QSpn.agt-L1G.2B*	AUS17750	2B-680597254	
2B	SG	Thousand grain weight	Grams	Performance	101.7–102.3	2.55	7.1	4.453	*QTgw.agt-SG.2B*	SCOUT	2B-742527939	
2B	SG	Spikelet number per spike	Spikelets spike^{-1}	Performance	118–118.3	0.46	4.2	2.573	*QSpn.agt-SG.2B*	SCOUT	2B-763840907	
2B	HK	Thousand grain weight	Grams	Performance	132.3–134.1	3.71	12.6	2.579	*QTgw.agt-HK.2B*	HALBERD	2B-778463800	2B-4
2B	SG	Grain number per spike	Grains spike^{-1}	Performance	139.8–140.5	2.21	2.2	2.046	*QGne.agt-SG.2B*	SCOUT	2B-785231658	
2D	HK	Spikelet fertility	Grains spikelet^{-1}	Performance	41.7–45.8	0.24	6.2	2.046	*QSfi.agt-HK.2D*	HALBERD	2D-35488158	2D-1
2D	HK	Anthesis date	Days from planting	Control	41.7–45.8	25.56	55.9	23.143	*QFlt.agt-HK.2D*	HALBERD	2D-35488158	
2D	SG	Anthesis date	Days from planting	Control	43.7–45.8	−10.12	43.9	60.438	*QFlt.agt-SG.2D*	GLADIUS	2D-35488158	
2D	MG	Anthesis date	Days from planting	Control	43.7–45.8	−8.28	29.6	11.465	*QFlt.agt-MG.2D*	GLADIUS	2D-35488158	

Table 4 (continued)

Chromosome	Population	Trait	Unit	Data-set	Left–Right marker interval (cM)	Effect	Variance accounted for (%)	LOD	QTL Name	Favourable allele	RefSeq physical position (Chromosome-base pair)	Cluster
2D	SM	Spikelet number per spike	Spikelets spike^{-1}	Performance	85.2–86.1	0.62	3.3	2.043	*QSpn.agt-SM.2D.1*	SCOUT	2D-411408155	
2D	SG	Grain yield per spike	Grams	Performance	131.7–133.2	0.15	3.0	3.367	*QGwe.agt-SG.2D*	SCOUT	2D-623502747	
2D	L2G	Spikelet fertility	Grains spikelet^{-1}	Performance	144.6–158.9	0.25	19.3	2.765	*QSfi.agt-L2G.2D*	AUS17840	2D-640208540	
3A	MG	Grain number per spike	Grains spike^{-1}	Performance	21.1–22.1	−6.06	11.2	3.007	*QGne.agt-MG.3A*	GLADIUS	3A-19512715	
3A	SG	Grain yield per spike	Grams	Performance	40.3–41.3	− 0.15	3.3	3.499	*QGwe.agt-SG.3A.2*	GLADIUS	3A-35409442	

Table 4 (continued)

Chromosome	Population	Trait	Unit	Data-set	Left–Right marker interval (cM)	Effect	Variance accounted for (%)	LOD	QTL Name	Favourable allele	RefSeq physical position (Chromosome-base pair)	Cluster
3A	RG	Spikelet number per spike	Spikelets spike^{-1}	Responsiveness	58.1–58.8	−0.54	7.0	2.815	*QSpn.agt-RG.3A.1*	GLADIUS	3A-393420046	3A-1
3A	HK	Thousand grain weight	Grams	Performance	60.6–61.6	3.10	8.8	2.06	*QTgw.agt-HK.3A*	HALBERD	3A-482063383	
3A	SG	Thousand grain weight	Grams	Performance	64.8–74.7	−2.21	5.4	3.221	*QTgw.agt-SG.3A*	GLADIUS	3A-534162732	
3A	L2G	Anthesis date	Days from planting	Control	98–100.2	49.77	12.0	3.618	*QFlt.agt-L2G.3A*	AUS17840	3A-690461041	
3B	L2G	Spikelet number per spike	Spikelets spike^{-1}	Performance	57.9–60	0.89	10.3	3.602	*QSpn.agt-L2G.3B.1*	AUS17840	3B-80545396	
3B	L2G	Grain number per spike	Grains spike^{-1}	Performance	94.9–95.8	4.87	14.6	2.767	*QGne.agt-L2G.3B.2*	AUS17840	3B-740374047	
3D	SG	Grain yield per spike	Grams	Performance	62.9–66	−0.12	1.9	2.196	*QGwe.agt-SG.3D*	GLADIUS	3D-307569329	
4A	SG	Grain yield per spike	Grams	Performance	48.1–49.3	0.10	1.4	1.571	*QGwe.agt-SG.4A*	SCOUT	4A-45874882	
4B	SG	Grain yield per spike	Grams	Performance	57.1–56.6	−0.10	1.5	1.616	*QGwe.agt-SG.4B*	GLADIUS	4B-552032837	
4B	RG	Spikelet number per spike	Spikelets spike^{-1}	Responsiveness	80.9–82.2	0.52	6.5	2.959	*QSpn.agt-RG.4B*	RAC1548	4B-653320867	
4D	HK	Grain number per spike	Grains spike^{-1}	Performance	16.4–18.9	7.06	12.8	2.998	*QGne.agt-HK.4D*	HALBERD	4D-6515044	
4D	RG	Spikelet number per spike	Spikelets spike^{-1}	Responsiveness	47.8–46.2	−0.48	5.6	2.612	*QSpn.agt-RG.4D*	GLADIUS	4D-31640720	
4D	L1G	Spikelet number per spike	Spikelets spike^{-1}	Performance	66.3–70.8	0.86	13.9	3.073	*QSpn.agt-L1G.4D*	AUS17750	4D-459054013	
4D	SM	Spikelet number per spike	Spikelets spike^{-1}	Performance	96.2–100.3	−0.84	6.1	3.53	*QSpn.agt-SM.4D.1*	MACE	4D-497661850	
5A	L2G	Spikelet number per spike	Spikelets spike^{-1}	Performance	104–107.6	1.06	14.7	5.551	*QSpn.agt-L2G.5A.1*	AUS17840	5A-562792889	

Table 4 (continued)

Chromosome	Population	Trait	Unit	Data-set	Left–Right marker interval (cM)	Effect	Variance accounted for (%)	LOD	QTL Name	Favourable allele	RefSeq physical position (Chromosome-base pair)	Cluster
5A	SG	Spikelet fertility	Grains spikelet^{-1}	Performance	127.3–127.6	−0.18	8.7	7.579	*QSfi.agt-SG.5A*	GLADIUS	5A-585431407	5A-1
5A	SG	Grain number per spike	Grains spike^{-1}	Performance	127.6–128.2	−2.75	3.4	2.909	*QGne.agt-SG.5A*	GLADIUS	5A-586605813	
5A	L2G	Anthesis date	Days from planting	Control	130.6–131.1	71.77	24.9	8.405	*QFlt.agt-L2G.5A*	AUS17840	5A-594576720	
5A	SG	Anthesis date	Days from planting	Control	139.2–139.3	4.15	7.4	10.598	*QFlt.agt-SG.5A*	SCOUT	5A-618864168	
5A	RG	Spikelet number per spike	Spikelets spike^{-1}	Responsiveness	154.8–162.5	−0.61	9.0	3.464	*QSpn.agt-RG.5A.3*	GLADIUS	5A-662522522	
5B	SM	Grain yield per spike	Grams	Performance	50.4–54	−0.38	13.2	7.116	*QGwe.agt-SM.5B*	MACE	5B-378070602	5B-1
5B	SM	Thousand grain weight	Grams	Performance	50.4–54	−4.79	12.0	5.845	*QTgw.agt-SM.5B.1*	MACE	5B-378070602	
5B	SM	Spikelet fertility	Grains spikelet^{-1}	Performance	54–55.4	−0.26	13.0	6.499	*QSfi.agt-SM.5B*	MACE	5B-399226535	
5B	SM	Grain number per spike	Grains spike^{-1}	Performance	57.1–59.1	−6.40	12.7	6.414	*QGne.agt-SM.5B*	MACE	5B-424354708	
5B	SM	Spikelet number per spike	Spikelets spike^{-1}	Performance	62.2–63.3	−1.09	10.2	5.846	*QSpn.agt-SM.5B*	MACE	5B-447563617	
5B	L2G	Spikelet number per spike	Spikelets spike^{-1}	Performance	98–98.9	0.91	10.8	4.207	*QSpn.agt-L2G.5B*	AUS17840	5B-573554956	
5B	HK	Spikelet fertility	Grains spikelet^{-1}	Performance	110.1–111.6	−0.30	9.9	3.058	*QSfi.agt-HK.5B*	KENNEDY	5B-587465697	
5D	L2G	Anthesis date	Days from planting	Control	65.3–67.9	−69.51	23.4	7.356	*QFlt.agt-L2G.5D*	GLADIUS	5D-376541710	5D-1
5D	L2G	Spikelet number per spike	Spikelets spike^{-1}	Performance	67.9–71.2	−0.77	7.8	2.965	*QSpn.agt-L2G.5D*	GLADIUS	5D-376541710	
5D	SG	Grain number per spike	Grains spike^{-1}	Performance	90.1–103.8	2.61	3.1	1.942	*QGne.agt-SG.5D*	SCOUT	5D-441011633	5D-2
5D	SG	Grain yield per spike	Grams	Performance	90.1–103.8	0.12	2.1	1.664	*QGwe.agt-SG.5D*	SCOUT	5D-441011633	
5D	SM	Anthesis date	Days from planting	Control	103.8–141.8	−4.34	38.2	8.19	*QFlt.agt-SM.5D*	MACE	5D-441011633	

Table 4 (continued)

Chromosome	Population	Trait	Unit	Data-set	Left–Right marker interval (cM)	Effect	Variance accounted for (%)	LOD	QTL Name	Favourable allele	RerfSeq physical position (Chromosome-base pair)	Cluster
5D	SG	Spikelet fertility	Grains spikelet^{-1}	Performance	131.2–133.3	0.10	2.6	2.266	*QSfi.agt-SG.5D*	SCOUT	5D-470039360	5D-3
5D	SG	Anthesis date	Days from planting	Control	134.8–138.7	−2.01	1.7	2.289	*QFlt.agt-SG.5D*	GLADIUS	5D-480184692	
6A	RG	Spikelet number per spike	Spikelets spike^{-1}	Responsiveness	14.9–18.7	−0.68	11.3	4.266	*QSpn.agt-RG.6A.1*	GLADIUS	6A-19159190	
6A	SG	Spikelet fertility	Grains spikelet^{-1}	Performance	55.3–56.5	0.10	2.5	2.268	*QSfi.agt-SG.6A.2*	SCOUT	6A-178074344	6A-1
6A	L2G	Spikelet fertility	Grains spikelet-^{1}	Performance	56.5–59.3	0.18	9.8	2.342	*QSfi.agt-L2G.6A*	AUS17840	6A-454646910	
6A	RG	Spikelet number per spike	Spikelets spike^{-1}	Responsiveness	125.1–126	0.67	11.1	4.713	*QSpn.agt-RG.6A.2*	RAC1548	6A-614445685	
6B	L2G	Spikelet number per spike	Spikelets spike^{-1}	Performance	2.6–4.4	0.66	5.6	2.203	*QSpn.agt-L2G.6B*	AUS17840	6B-11318688	
6B	RG	Spikelet number per spike	Spikelets spike^{-1}	Responsiveness	17.8–19.2	−0.54	7.2	3.197	*QSpn.agt-RG.6B*	GLADIUS	6B-26512972	
6D	SG	Grain yield per spike	Grams	Performance	25.1–37.6	−0.34	16.8	7.694	*QGwe.agt-SG.6D.1*	GLADIUS	6D-16115822	6D-2
6D	SG	Grain yield per spike	Grams	Performance	40.6–68.5	0.31	13.6	4.576	*QGwe.agt-SG.6D.2*	SCOUT	6D-291963877	
6D	SG	Grain yield per spike	Grams	Performance	99.2–106.4	−0.13	2.3	1.883	*QGwe.agt-SG.6D.3*	GLADIUS	6D-444594388	
6D	SM	Spikelet number per spike	Spikelets spike^{-1}	Performance	133.2–133.2	−0.71	4.3	2.484	*QSpn.agt-SM.6D.2*	MACE	6D-464347256	6D-3
6D	HK	Spikelet fertility	Grains spikelet^{-1}	Performance	140.3–140.8	−0.35	13.2	4.772	*QSfi.agt-HK.6D.2*	KENNEDY	6D-470969377	
7A	SG	Grain yield per spike	Grams	Performance	3.5–3.7	0.17	4.2	4.66	*QGwe.agt-SG.7A.1*	SCOUT	7A-6776918	7A-1
7A	SG	Spikelet fertility	Grains spikelet^{-1}	Performance	12.6–12.9	0.15	6.3	5.609	*QSfi.agt-SG.7A.1*	SCOUT	7A-23388854	
7A	SG	Grain number per spike	Grains spike^{-1}	Performance	12.6–12.9	3.38	5.2	4.544	*QGne.agt-SG.7A.1*	SCOUT	7A-23388854	
7A	L2G	Spikelet number per spike	Spikelets spike^{-1}	Performance	106.7–107.5	0.87	9.8	3.604	*QSpn.agt-L2G.7A.1*	AUS17840	7A-673132616	7A-2
7A	SG	Spikelet number per spike	Spikelets spike^{-1}	Performance	111.8–112	0.76	11.5	6.658	*QSpn.agt-SG.7A.2*	SCOUT	7A-679763265	

Table 4 (continued)

Chromosome	Population	Trait	Unit	Data-set	Left–Right marker interval (cM)	Effect	Variance accounted for (%)	LOD	QTL Name	Favourable allele	RefSeq physical position (Chromosome-base pair)	Cluster
7A	SG	Spikelet fertility	Grains spikelet^{-1}	Performance	152.3–153.2	0.08	1.7	1.541	*QSfi.agt-SG.7A.2*	SCOUT	7A-720347357	7A-3
7A	SG	Grain number per spike	Grains spike^{-1}	Performance	152.3–153.2	2.91	3.9	3.397	*QGne.agt-SG.7A.2*	SCOUT	7A-720347357	
7A	SG	Grain yield per spike	Grams	Performance	152.3–153.2	0.13	2.6	2.836	*QGwe.agt-SG.7A.2*	SCOUT	7A-720347357	
7A	SM	Spikelet number per spike	Spikelets spike^{-1}	Performance	162.6–164.1	0.75	4.9	2.864	*QSpn.agt-SM.7A*	SCOUT	7A-735502508	
7B	SG	Thousand grain weight	Grams	Performance	18.3–19.6	1.88	3.9	2.68	*QTgw.agt-SG.7B*	SCOUT	7B-11370906	7B-1
7B	SG	Grain yield per spike	Grams	Performance	19.6–20.7	0.11	1.6	1.799	*QGwe.agt-SG.7B*	SCOUT	7B-11370906	
7B	MG	Grain number per spike	Grains spike^{-1}	Performance	26.9–38.8	5.94	10.8	2.82	*QGne.agt-MG.7B*	MACE	7B-40439568	
7D	SG	Grain number per spike	Grains spike^{-1}	Performance	68.8–69.7	3.32	5.0	4.442	*QGne.agt-SG.7D*	SCOUT	7D-88190365	7D-1
7D	SG	Spikelet fertility	Grains spikelet^{-1}	Performance	70.4–71	0.12	3.9	3.577	*QSfi.agt-SG.7D.1*	SCOUT	7D-90599277	
7D	L2G	Anthesis date	Days from planting	Control	70.4–71.8	− 46.69	10.5	3.615	*QFlt.agt-L2G.7D*	GLADIUS	7D-90599277	

The position of QTL is reported as the consensus map position (Supplementary Table 1) and as a RefSeq physical position. The cluster column indicates groups of QTL that occur within 10 cM of neighbouring QTL

developing new varieties with adaptation to stressed growing conditions. When breeders evaluate lines for adaptation they have a myriad of factors to consider, including identifying if lines have broad or narrow adaptation profiles to their target population of environments, and how varieties adapt to different growing conditions within these environments (Basford and Cooper 1998; Finlay and Wilkinson 1963). Often broad adaptation is preferred, with stable performance over a range of growing conditions (Finlay and Wilkinson 1963). In this situation it may be preferable for non-responsiveness, as lines that have a high performance value that are non-responsive to changing heat stress conditions (or even small negative responses) maintain their relative performance advantage across both heat stress and non-stress conditions. Therefore selecting for overall performance value may be of greater benefit to breeders than selecting for responsiveness. This is supported by Richards (1996) who discussed selection of grain yield under favourable conditions, which also proved effective in identifying varieties with good relative performance under stressed conditions. However, an understanding of the two, and their interaction, when selecting for improved adaptation to the target population of environments would be of benefit to breeders.

This framework can also be applied to assessing the role of QTL or individual genes in adaptation to heat stress conditions as shown in this study.

Performance QTL that also show responsiveness to heat stress conditions

Three of the nine responsiveness QTL identified were found to be collocated with performance QTL (Table 4). On chromosome 1D, a spikelet fertility QTL (*QSfi.agt-HK.1D.1*) was identified between 32.3 and 36.2 cM in the HK population with Kennedy conferring the favourable allele. This is close (cluster 1D-1) to the responsiveness QTL (approximately 8 cM or 4.7×10^6 bp) for spikelet number per spike (*QSpn.agt-SG.1D*) located between 23.4 and 24.6 cM in the SG population with the Scout parent providing the favourable allele. Given that the QTL are from different parents and different populations, it is hard to fully understand the relationship between allelic variation and impacts on adaptation, and would require further investigation with the understanding that there are performance and responsiveness interactions present for traits impacting on grain number in this region across genotypes. These QTL occur in similar regions to QTL reported by Talukder et al. (2014b) for leaf chlorophyll content and plasma membrane damage post-heat stress at 11.21 cM, and of a QTL reported by Pinto et al. (2016) for canopy temperature at 41.8 cM.

On chromosome 2A a responsiveness QTL for grain yield per spike (*QGwe.agt-RG.2A*) was identified between 69.0 and 71.1 cM in the RG population with Gladius providing the favourable allele. This was close to (cluster 2A-1) QTL for spikelet fertility (*QSfi.agt-SG.2A.1*) and grain number per spike (*QGne.agt-SG.2A.1*), both identified in the SG population at the same interval between 67.4 and 67.7 cM, with the Gladius parent providing the favourable allele. These QTL are approximately 1 cM apart based on their consensus map position and are likely coincident. This is supported by the observation that Gladius is the common parent in both of the populations and the common source of the favourable allele for each QTL. This region appears to be important for grain number determination, as well as being responsive to heat stress. No other QTL have been reported in this region in relation to adaptation to high temperatures, although the photoperiod gene *Ppd-A1* is proximal and known to occur centrally on the short arm of chromosome 2A (Wilhelm et al. 2009). *Ppd-A1* is also known to play a role in grain number determination (Arjona et al. 2018; Nadolska-Orczyk et al. 2017). There was, however, no association between this region and anthesis date in any of the populations studied.

In a third region on chromosome 3A (cluster 3A-1), a responsiveness QTL (*QSpn.agt-RG.3A.1*) was identified at 58.1–58.8 cM for spikelet number per spike in the RG population with Gladius conferring the favourable allele. Two other QTL were identified in this region for thousand grain weight; *QTgw.agt-SG.3A* identified in the SG population at 64.8–74.7 cM with Gladius providing the favourable allele, and *QTgw.agt-HK.3A* in the HK population at 60.6–61.6 cM with Halberd providing the favourable allele. This region has been highlighted previously as a region of potential importance for adaptation to heat stress conditions with Bennett et al. (2012) identifying QTL in this region for grain yield and thousand grain weight, and Vijayalakshmi et al. (2010) identifying QTL for senescence rate. The identification of QTL for thousand grain weight in the current study align and confirm the results of Bennett et al. (2012). Interestingly this area has also been identified as being responsive to heat, with Gladius providing the favourable allele for spikelet number per spike. Generally, grain number and grain size traits are negatively correlated with a trade-off between two traits in response to resource availability (Griffiths et al. 2015; Quintero et al. 2018; Sadras 2007); however, the opposite is observed here. This is possibly a result of the controlled environment conditions, where plants have near ideal conditions for the duration of their growth cycle (excluding the experimentally imposed heat stress treatment). Potentially, this alters the source–sink relationships in the absence of terminal stress, which often occur in field conditions. Indeed, Quintero et al. (2018) reported that the trade-off between grain number and grain size depends on environmental conditions during flowering and grain fill by changing the source–sink relationships, meaning that in some favourable conditions the trade-off can be reduced to

a minimal level potentially resulting in an increase in both grain weight and number.

Heat stress responsiveness QTL independent of total performance

Six of the nine responsiveness QTL were found to occur in isolation of performance QTL (*QSpn.agt-RG.4B*, *QSpn.agt-RG.4D*, *QSpn.agt-RG.5A.3*, *QSpn.agt-RG.6A.1*, *QSpn.agt-RG.6A.2* and *QSpn.agt-RG.6B*), inferring that they did not significantly impact on trait value in the absence of heat, but were shown to be responsive to changing temperature conditions. The responsiveness QTL for spikelet number per spike on 4B (*QSpn.agt-RG.4B*) appears in a similar location to a 'grain fill duration' QTL reported by Shirdelmoghanloo (2014) confirming that this locus plays a role in heat stress adaptation. Similarly, the spikelet number QTL identified on chromosome 6A (*QSpn.agt-RG.6A.1*) was in a similar region to a QTL for leaf chlorophyll content (SPAD) at 28.4 cM identified by Pinto et al. (2016) when grown under high-temperature irrigated conditions in Mexico. Furthermore, a responsiveness QTL for spikelet number on chromosome 6B (*QSpn.agt-RG.6B*) occurred in a similar location to previously reported QTL for senescence rate (Vijayalakshmi et al. 2010), single grain weight (Shirdelmoghanloo 2014) and leaf chlorophyll content (Shirdelmoghanloo 2014), confirming the potential role of this locus in adaptation to heat stress conditions. The QTL *QSpn.agt-RG.4D, QSpn.agt-RG.5A.3* and *QSpn.agt-RG.6A.2* occurred in regions not previously described in relation to adaptation to high temperatures and present novel loci for further investigation.

Key regions, parents and traits for performance independent of heat stress

A large number of QTL were identified across the whole wheat genome for traits relevant to grain yield determination under controlled environment conditions (Table 4). Of these, a number do not collocate with responsiveness QTL, inferring that these QTL may play a role in overall performance, with stable performance across both high-temperature conditions and unstressed conditions.

Within these performance QTL, there are a number of regions with multiple QTL influencing grain number, grain size, or both (Table 4). This includes two QTL identified on chromosome 1A (cluster 1A-1) in the SG population for grain number per spike (*QGne.agt-SG.1A*) and spikelet number per spike (*QSfi.agt-SG.1A*), occurring in a similar region to QTL identified previously for heat stress adaptation by Esten Mason et al. (2010) and Pinto et al. (2016) for single kernel weight and thousand grain weight, respectively.

On chromosome 2B a cluster of QTL (cluster 2B-2) was identified for grain number traits (*QSfi.agt-SM.2B*, *QGne.agt-SM.2B*, *QSpn.agt-SM.2B* and *QSfi.agt-HK.2B*) and grain weight traits (*QGwe.agt-SM.2B*, *QTgw.agt-SM.2B.2* and *QTgw.agt-MG.2B*). This is a region that has been discussed previously in relation to adaptation to high temperatures with Paliwal et al. (2012) and Pinto et al. (2010) finding various QTL for thousand grain weight, grain fill duration and stay green, and Esten Mason et al. (2010) identifying QTL for grain number per spike in this region. The results of this study confirm the potential role of this region in adaptation to heat stress conditions, with stable performance under both control and hot conditions. Although curiously here, Scout was shown to provide alleles favourable for both higher grain number and thousand grain weight, traits normally negatively correlated as previously discussed. A similar region was identified on chromosome 5B (50.4–63.3 cM) in cluster 5B-1, with multiple QTL for grain number traits (*QGwe.agt-SM.5B*, *QSfi.agt-SM.5B*, *QGne.agt-SM.5B* & *QSpn.agt-SM.5B*) collocating with a thousand grain weight QTL (*QTgw.agt-SM.5B.1*) identified in the SM populations, with all QTL accounting for > 10% of the genetic variance, and the parent Mace providing the favourable allele for all QTL. This is another example of grain number and grain weight traits that are normally negatively correlated being positively correlated, a potential result of controlled environment conditions altering source–sink relationships as previously discussed (Quintero et al. 2018). In this region no association was found with anthesis date, indicating a role in adaptation without affecting plant maturity. This region has also been highlighted in previous research in relation to adaptation to heat stress conditions with Bennett et al. (2012) identifying QTL for canopy temperature and grain yield in this region, and Esten Mason et al. (2011) identifying QTL for kernel number.

Of the other regions and significant QTL described in Table 4, regions were identified that have not been previously reported as being important for performance under high-temperature conditions. This includes a region on 6D between 131.7 and 133.2 cM (cluster 6D-3), where two novel QTL were identified, a spikelet fertility QTL (*QSfi.agt-HK.6D.2*) in the HK population with the Kennedy parent the donor of the favourable allele and accounting for 13.2% of genetic variance. Collocated was a QTL for spikelet number per spike QTL (*QSpn.agt-SM.6D.2*) in the SM population with Mace providing the favourable allele.

Sources of heat stress adaptation from exotic germplasm

Two doubled haploid populations used in this study were developed using FIGS lines identified using the same assay used in this study and described by Telfer et al. (2018), for further investigation of potentially novel sources of heat stress adaptation. A total of 11 QTL were identified within

these two populations for performance traits measured as a part of the heat stress experiment and an additional five QTL for anthesis date (Table 4). Ten of the 11 QTL identified for performance traits sourced their favourable allele from the exotic parent. A number of these QTL occur in regions not previously described in literature as relating to adaptation to high-temperature conditions, including *QSpn.agt-L1G.2B*, *QSfi.agt-L2G.2D*, *Spn.agt-L1G.4*D and *QSpn.agt-L2G.5A.1*. These QTL present opportunities as potentially novel loci from an exotic germplasm source, with no other QTL identified in previous research or other populations used in the current study. Further validation of these regions will elucidate any potential advantages of these loci in relevant growing conditions and for future breeding strategies.

Five QTL with the exotic parent providing the favourable allele were identified as occurring in similar regions to QTL identified in previous studies. Further research would be required to confirm if these alleles from the exotic parent represent novel sources of adaptation or are already captured by breeding germplasm. *QSpn.agt-L2G.3B.1*, a QTL for spikelet number per spike aligns with various QTL identified for grain yield, thousand grain weight and stay green (Esten Mason et al. 2011, 2013; Pinto et al. 2016; Sharma et al. 2017). *QGne.agt-L2G.3B.2*, a QTL for grain number per spike also identified on chromosome 3B, aligns with previous QTL identified for grain yield, thousand grain weight and kernel number (Bennett et al. 2012; Esten Mason et al. 2010; Pinto et al. 2016). *QSpn.agt-L2G.5B,* a spikelet number per spike QTL identified on chromosome 5B, occurs in a similar region to QTL for grain weight reported by Mohammadi et al. (2008). *QSfi.agt-L2G.6A*, a QTL for spikelet fertility on chromosome 6A, occurs adjacent to *QSfi.agt-SG.6A.2* a spikelet fertility QTL identified in the SG population in cluster 6A-1. These QTL occur in a similar region to a senescence rate QTL reported by Vijayalakshmi et al. (2010). *QSpn.agt-L2G.6B*, a QTL for spikelet number per spike identified on chromosome 6B, occurs in a similar region to QTL for senescence rate, single grain weight and stay green (Shirdelmoghanloo 2014; Vijayalakshmi et al. 2010). Finally, *QSpn.agt-L2G.7A.1*, a spikelet number per spike QTL on chromosome 7A in cluster 7A-2 approximately 4 cM and or 6.6×10^6 bp away from *QSpn.agt-SG.7A.2*, a spikelet number per spike QTL identified in the SG population, occur in a region previously reported as being important for grain yield and thousand grain weight under heat stress conditions (Bennett et al. 2012; Esten Mason et al. 2013).

Conclusion

This study proposes and confirms a new framework and new terminology for traditional stress tolerance studies where adaptation to stress is considered as the combination of total performance value of a variety or genotype and the responsiveness to stressed conditions. Breeders will need to carefully consider their breeding objectives to weigh up the opportunities and risks within their breeding programs with respect to total performance and selecting for a positive response to stress conditions. QTL that offer stable performance regardless of stress conditions may offer greater opportunities to breeders for crop improvement than selecting for responsiveness to stress conditions. In this study, there were relatively few QTL combinations of responsiveness collocating with performance. Additionally, there were very few examples when considered at the line level, of a line exhibiting a combination of both high performance and positive responsiveness. This may indicate that such a combination is of very low frequency and potentially difficult to achieve within a breeding program. As this is a new framework to assess adaptation to heat stress conditions, and likely other abiotic stresses, there is still further research to be conducted to fully understand the physiology of heat stress adaptation and for appropriate selection methodologies within breeding programs. One possible outcome may be that breeders can use their evaluation environments to screen for stable performance and use targeted environments to test for responsiveness to heat stress conditions to understand the responsiveness of their germplasm independently.

Supplementary information The online version of this article (https://doi.org/10.1007/s00122-021-03778-2) contains supplementary material, which is available to authorised users.

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Data availability The datasets generated during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Chapter 4. A Multi-Environment Framework to Evaluate the Adaptation of Wheat (*Triticum aestivum*) to Heat Stress

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4.1. Contextual Statement

Evaluation of adaptation to heat stress is best done in representative growing conditions rather than in surrogate assays such as a controlled environment assay, as discussed in Chapter 2. The multi-environment approach presented in Chapter 2 presents opportunities to understand adaptation to stress conditions and the role of stress conditions in GxE interactions. This manuscript applies the multi-environment approach to assessing genetic mapping populations to identify QTL associated with improved performance in heat stress conditions. Presented is the genetic analysis of heat stress adaptation of five doubled haploid mapping populations (a subset of the populations included in Chapter 3) evaluated across six representative environments (three locations in two consecutive years) in southern Australia. Adaptation is examined as the combination of responsiveness to changing stress conditions and stable performance across all environments, using the framework presented in Chapter 3, allowing identified QTL to be evaluated for their role in heats tress adaptation and specific relevance to breeders' selection objective to be explored.

4.2. Statement of Authorship

Statement of Authorship

Title of Paper	Genetic analysis of wheat (Triticum aestivum) adaptation to heat stress
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Name of Principal Author (Candidate)	Mr Paul Telfer
Contribution to the Paper	Designed and ran experiments, analysed and interpreted the data, and prepared the manuscript.
Overall percentage (%)	85
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date: 25/02/2022

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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ORIGINAL ARTICLE

A multi-environment framework to evaluate the adaptation of wheat (*Triticum aestivum*) to heat stress

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Abstract

Key message **Assessing adaptation to abiotic stresses such as high temperature conditions across multiple environments presents opportunities for breeders to target selection for broad adaptation and specific adaptation.**

Abstract Adaptation of wheat to heat stress is an important component of adaptation in variable climates such as the cereal producing areas of Australia. However, in variable climates stress conditions may not be present in every season or are present to varying degrees, at different times during the season. Such conditions complicate plant breeders' ability to select for adaptation to abiotic stress. This study presents a framework for the assessment of the genetic basis of adaptation to heat stress conditions with improved relevance to breeders' selection objectives. The framework was applied here with the evaluation of 1225 doubled haploid lines from five populations across six environments (three environments selected for contrasting temperature stress conditions during anthesis and grain fill periods, over two consecutive seasons), using regionally best practice planting times to evaluate the role of heat stress conditions in genotype adaptation. Temperature co-variates were determined for each genotype, in each environment, for the anthesis and grain fill periods. Genome-wide QTL analysis identified performance QTL for stable effects across all environments, and QTL that illustrated responsiveness to heat stress conditions across the sampled environments. A total of 199 QTL were identified, including 60 performance QTL, and 139 responsiveness QTL. Of the identified QTL, 99 occurred independent of the 21 anthesis date QTL identified. Assessing adaptation to heat stress conditions as the combination of performance and responsiveness offers breeders opportunities to select for grain yield stability across a range of environments, as well as genotypes with higher relative yield in stress conditions.

Introduction

Many regions throughout the world experience heat, drought, and frost stress that limit crop production. In the Mediterranean-type climates of southern Australia, heat stress conditions during the sensitive crop development stages of anthesis and early grain filling are common (Zheng et al. 2012), often co-occurring with other abiotic stresses such as drought and high wind (Machado and Paulsen 2001; Shah and Paulsen 2003). In southern Australia, heat stress conditions during anthesis and early grain fill are typically short periods, of up to a few days in length, with daily maximum temperatures in excess of 35 °C accompanied with winds in excess of 40 km h^{-1} (Alexander et al. 2010; Talukder et al. 2013). The impacts of such stress events can be significant, with Kuchel et al. (2007a) and Bennett et al. (2012b) reporting yield loss of up to 187 kg ha^{-1} for every one-degree increase in average temperature during anthesis and grain fill in field experiments conducted across southern Australia. This was confirmed by Telfer et al. (2018), who identified a reduction in grain yield of 161 kg ha^{-1} for each day with a maximum temperature in excess of 30 °C during grain fill, and a reduction of 302 kg ha^{-1} for each day with maximum temperature in excess of 30 °C during anthesis.

Stress conditions, like those described, impact negatively on a range of developmental stages and physiological processes (Wahid et al. 2007). Plant mechanisms that manage antioxidants, heat shock proteins, maintenance of cell membrane stability, and maintenance of protein stability and function often underpin a plant's ability to cope with stress

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(Dolferus et al. 2011; Wahid et al. 2007). In the field, heat stress results in reduced pollen viability and reduced seed set when it occurs during anthesis (Dolferus et al. 2011; Saini et al. 1999). Stress during grain filling leads to reduced starch and protein accumulation (Bhullar and Jenner 1985; Zahedi et al. 2004), accelerated plant development, premature leaf senescence, and reduced photosynthetic rate and capacity (Stone and Nicolas 1995; Tewolde et al. 2006), which ultimately reduces grain size (Sharma et al. 2008; Stone and Nicolas 1995; Talukder et al. 2014a; Wardlaw 1994) and grain yield (Talukder et al. 2014a; Tewolde et al. 2006).

Tolerance to heat stress in wheat has been previously reported. This includes the Australian wheat varieties Halberd (Hays et al. 2007) and Gladius (Fleury et al. 2010; Talukder et al. 2014a), as well as breeding and research line, RAC875 (Bennett et al. 2012a; Izanloo et al. 2008). This was also confirmed by Telfer et al. (2018) who showed that Halberd expressed tolerance to heat stress during flowering, and Gladius and RAC875 expressed tolerance to heat stress during grain filling. Additionally, Gladius and RAC875 have been reported as drought-tolerant (Bennett et al. 2012a; Fleury et al. 2010; Izanloo et al. 2008; Shirdelmoghanloo et al. 2016a).

Various methods have been used to evaluate performance under heat stress conditions, using controlled environments or in-field conditions, including Telfer et al. (2018) who compared a controlled environment assay and a field assay for the evaluation of adaption to heat stress conditions. Controlled environment conditions offer many advantages, including ensuring a consistent and repeatable stress environment and being able to manage a range of confounding factors that may be present in field conditions. The oftentimes confounding effects of maturity can be managed using controlled environments, in contrast to field experiments where material that develops at a different rate may be exposed to different temperature stress conditions making comparisons challenging. Despite the advantages of controlled environments, validation is required under representative field conditions. To ensure a high incidence of heat stress conditions, delayed sowing has been a commonly used methodology to ensure that sensitive developmental stages coincide with heat stress conditions typically experienced later in the season (Bennett et al. 2012b; Esten Mason et al. 2013; Pinto et al. 2010; Reynolds et al. 2007; Sadras et al. 2015). Unfortunately, in this system plants are exposed to growing conditions that are not representative of agronomic practices employed by grain producers, such as longer photoperiod and altered plant available water (Sadras et al. 2015). With phenological development, a primary driver of adaptation, and development rate interacting strongly with sowing date, assessments of heat stress adaptation using sowing date variation are consequently confounded, precluding clear conclusions being drawn from such studies.

Field evaluation of heat stress adaptation has also been carried out using portable heat chambers in the field to induce heat stress treatments (Alexander et al. 2010; Talukder et al. 2013; Thistlethwaite et al. 2020). Such methodology provides the relevance of a field trial, being managed to agronomic best practice, with the added benefit of controlled environment evaluation through consistent heat stress treatments and fewer confounding factors arising from variation in phenology. The physical encumbrance of handling heat chambers in the field, however, largely limits such systems to small exploratory experiments with few genotypes.

Telfer et al. (2018) previously discussed an alternative approach whereby the heat stress tolerance of wheat germplasm was evaluated across a multi-environment (contrasting for heat stress) study. Such a system enables the material to be grown in representative growing environments using regional best practice agronomy and using the natural variation in temperature across the environments to evaluate genotype response to heat stress. This provides the opportunity to evaluate adaptation to heat stress conditions in large-scale breeding trials across a range of environments, subsequently enabling the identification and validation of genetic loci using genetic mapping populations. Kuchel et al. (2007a) also reported similar results, having evaluated doubled haploid bi-parental material across multiple representative environments. In that study, variable expression of QTL for grain yield, and associated traits, were attributable to environmental factors such as temperature and rainfall conditions varying across the environments sampled. Similarly, Tura et al. (2020) was able to identify QTL-by-environment interactions for grain yield and related traits.

A number of studies have identified QTL for adaptation to heat stress conditions that potentially confer improved grain yield (Bennett et al. 2012b; Bhusal et al. 2017; El Hassouni et al. 2019; Esten Mason et al. 2013; Hassan et al. 2018; Liu et al. 2019; Paliwal et al. 2012; Pinto et al. 2010, 2016; Tadesse et al. 2019; Tahmasebi et al. 2017; Vijayalakshmi et al. 2010), grain size (Ali et al. 2013; Bennett et al. 2012b; Bhusal et al. 2017; Esten Mason et al. 2010; Guan et al. 2018; Liu et al. 2019; Mason et al. 2011; Mohammadi et al. 2008; Paliwal et al. 2012; Pinto et al. 2010, 2016; Shirdelmoghanloo 2014; Shirdelmoghanloo et al. 2016b; Tadesse et al. 2019; Tahmasebi et al. 2017), grain number (Bhusal et al. 2017; El Hassouni et al. 2019; Esten Mason et al. 2010; Guan et al. 2018; Liu et al. 2019; Mason et al. 2011; Pinto et al. 2010, 2016; Sharma et al. 2016; Tahmasebi et al. 2017; Telfer et al. 2021), grain fill rate (Paliwal et al. 2012; Pinto et al. 2016; Sharma et al. 2016; Shirdelmoghanloo 2014), harvest index (El Hassouni et al. 2019; Shirdelmoghanloo 2014), senescence rate (Pinto et al. 2016; Shirdelmoghanloo 2014; Vijayalakshmi et al. 2010) and maturation rate (Bhusal et al. 2017; Paliwal et al. 2012; Shirdelmoghanloo 2014). QTL for physiological traits potentially related to

heat stress tolerance have also been identified, including leaf chlorophyll content (Ali et al. 2013; Liu et al. 2019; Maulana et al. 2018; Pinto et al. 2010, 2016; Shirdelmoghanloo 2014; Tahmasebi et al. 2017; Talukder et al. 2014b; Vijayalakshmi et al. 2010), canopy temperature (Ali et al. 2013; Bennett et al. 2012b; Liu et al. 2019; Paliwal et al. 2012; Pinto et al. 2010, 2016), photosystem II efficiency (Fv/Mv) (Hassan et al. 2018; Sharma et al. 2017; Vijayalakshmi et al. 2010), NDVI (Liu et al. 2019; Pinto et al. 2010, 2016) and membrane damage (Hassan et al. 2018; Talukder et al. 2014b). These studies have primarily been undertaken using delayed sowing systems in the field (Bennett et al. 2012b; Bhusal et al. 2017; Esten Mason et al. 2013; Hassan et al. 2018; Liu et al. 2019; Paliwal et al. 2012; Pinto et al. 2010, 2016; Sharma et al. 2016; Tahmasebi et al. 2017) or in controlled environment assays (Mason et al. 2011; Maulana et al. 2018; Mohammadi et al. 2008; Shirdelmoghanloo 2014; Talukder et al. 2014b; Telfer et al. 2021; Vijayalakshmi et al. 2010).

As discussed by Lemerle et al. (2006), tolerance has previously been defined as the difference in trait expression between an unstressed control and a stressed treatment. Usually, there is a strong correlation between trait expression in both stressed and unstressed conditions, making this a potentially misleading definition. Lemerle et al. (2006) and Dolferus et al. (2019) further defined tolerance as a positive deviation from the expected response between the stressed and unstressed treatments. As discussed by Telfer et al. (2021), this deviation from the expected response under stress conditions is more accurately interpreted as responsiveness to stress conditions and should be considered as an independent factor in adaptation in combination with performance value across all conditions.

This study builds on the research carried out by Telfer et al. (2021), by applying a framework for the evaluation of the genetic basis of adaptation to heat stress conditions in representative field conditions, and considers adaptation as the combination of performance and responsiveness. Telfer et al. (2021) identified QTL for performance and heat stress responsiveness in controlled environment conditions. This study uses the same populations to explore adaptation to heat stress conditions in representative field conditions. Here, QTL are discussed in relation to their role in adaptation to heat stress conditions as experienced in Mediterranean-type environments of southern Australia, and their relevance to breeding objectives.

Materials and methods

Germplasm and genotyping

Eight doubled haploid populations were used to evaluate adaptation to heat stress conditions in Mediterranean-type environments of southern Australia, and to identify QTL for grain yield and related traits across multiple representative environments. The populations used are described by Telfer et al. (2021) and are summarised in Table 1. In brief, these were developed to encompass historical germplasm pools representative of the Australian breeding germplasm pool, as well as to evaluate potential novel sources of heat stress tolerance identified in a heat stress focused identification of germplasm strategy (FIGS) set as mentioned by Telfer et al. (2021). All lines evaluated in this study from each population were genotyped using a custom Axiom™ Affymetrix array containing 18,101 SNP markers as described by Norman et al. (2017). Further, the linkage maps used for QTL analysis (Supplementary Table 1) were created through the R statistical environment (R Core Team 2018) using a combination of the R/qtl (Broman and Sen 2009; Broman and Wu 2015) and R/ASMap (Taylor and Butler 2017) package (Norman et al. 2017), resulting in excess of 5000 polymorphic markers for each population (exact numbers shown in Table 1). To prepare the linkage maps for analysis, the functionality of the WGAIM R package (Taylor and Verbyla 2011) was used to numerically encode the alleles (AA = 1, BB = − 1), impute missing values using the rules of Martínez and Curnow (1994) and generate unique interval markers using Verbyla et al. (2007).

Table 1 The DH populations evaluated in this study, summarising population parents and genetic map details

Population name	Pedigree	No. lines in Map	No. polymorphic SNP markers	No. Unique positions	Genetic length (cM)	Mean interval*
MG	Mace/Gladius	176	5047	1429	3009	2.1
SM	Scout/Mace	226	4950	1360	3030	2.2
SG	Scout/Gladius	369	5143	1761	2998	1.7
RG	RAC1548/Gladius	132	5133	1183	3055	2.6
L2G	AUS17840/Gladius	124	5514	1132	3144	2.8

*Mean interval (cM) between unique map positions

Experimental design and plot management

The methodology described by Telfer et al. (2018) to evaluate adaptation to heat stress conditions under representative field conditions across multiple environments was used as the basis for this study. Genetic material was evaluated at three cereal producing locations in South Australia; Angas Valley, Roseworthy, and Winulta, across two seasons in 2015 and 2016, totalling six environments (the details of each experiment are shown in Table 2). These environments were targeted to achieve a range in heat stress conditions, with Winulta having a maritime climate with relatively mild conditions during anthesis and grain filling compared to the inland site of Angas Valley which typically has warmer conditions. Roseworthy historically is intermediate in anthesis and grain fill conditions as is demonstrated by the temperature conditions during anthesis and grain filling in Table 2.

Populations were assigned to separate experiments except for the Mace/Gladius (MG), Scout/Mace (SM), and Scout/Gladius (SG) populations, which due to common parentage across the populations, were grouped and referred to as the GSM populations. Fewer lines were evaluated in 2016 compared to 2015. For 2016, entries were a subset of the 2015 entries selected to remove extreme maturity types as identified in the 2015 season. Lines with other extreme adverse phenotypes such as those with very tall plant heights were also removed. No direct selection occurred for grain yield and physical grain quality attributes which were the focus of this study. Field plots within experiments were sown in a grid format, and the experiment details are shown in Table 2. The experimental design used the principles of partially replicated design as discussed by Cullis et al. (2006), with each doubled haploid line in each population present in each environment and each doubled haploid line replicated in one of the three environments of that year. Consequently, doubled haploid lines were replicated on average 1.33 times at each environment, with check varieties (doubled haploid parents and locally adapted varieties) fully replicated in each environment (Table 2).

Within each experiment, plots were sown 1.32 m wide by 5 m long, with plots reduced to a length of 3.2 m before anthesis using herbicide. Each experiment was sown to achieve 200 seeds m^{-2}. Field experiments were managed using regional best practice agronomy, encompassing sowing and harvest times, crop nutrition, and pest (weed, insect, and fungal) management.

Phenotyping

At Roseworthy, in each year of the study, each plot was assessed to determine spike emergence date (50% of spikes fully emerged from the flag leaf sheath) of each plot. Along with temperature data collected at each environment (explained in further detail later), a degree-day model (Sadras and Monzon 2006) was used to estimate the anthesis date of each line in each environment, using daily mean temperature > 0 °C as the base temperature for plant development. Relative maturity observations were also made at each location in each year of the study to confirm the accuracy of the modelled anthesis date as described by Telfer et al. (2018).

All experiments were harvested and weighed after reaching physiological maturity, to determine grain yield (kg ha^{-1}). The grain was further evaluated for screenings percentage assessed by determining the weight of matter that passes through a 2 mm slotted sieve (Grain Trade Australia 2020), and test weight (TWT, kg hl^{-1}), assessed by weighing a 500 mL sample measured using a chrondrometer (Grain Trade Australia 2020).

Climatic co-variates to describe crop stress conditions

Temperature data were collected in each environment for the duration of the growing season using a single factory-calibrated temperature logger (TinyTag™ Talk2), logging on half-hourly intervals. The temperature logger was situated immediately adjacent to the block of experiments all situated adjacent to each other in each location, at 1 m in height to replicate approximate crop height at anthesis and grain fill.

Temperature observations were used to calculate climatic co-variates to describe the temperature conditions experienced by each line in each environment during the key developmental periods, anthesis (300°Cd before anthesis to 100°Cd post-anthesis) and grain filling (100°Cd to 600°Cd post anthesis). Climatic co-variates calculated for each line in each environment included average maximum temperature (°C), number of days > 30 °C and number of days > 35 °C, during both anthesis and grain filling (Dreccer et al. 2008; Telfer et al. 2018). Growing season rainfall (total cumulative rainfall (mm) from the start of May to the end of October) was also recorded in each environment. In the 2015 season, this was taken from the nearest Australian Bureau of Meteorology (BOM) weather station; Angas Valley sourced from Cambrai BOM station (No. 024513), Roseworthy sourced from Roseworthy BOM station (No. 023021) and Winulta sourced from Ardrossan BOM station (No. 022021)). In the 2016 season, this was measured on-site using a factory-calibrated Davis^{R} Vantage Vue Weather Station situated adjacent to the experiments logging at 15-min intervals for the duration of the experiment in each environment. The mean climatic co-variate observed across all lines in each experiment is shown in Table 2. The length of the anthesis period ranged from 29 to 36 days, and the grain fill period ranged from 24 to 36 days depending on the population and the environment in which they were grown.

Table 2 The field experiments conducted as a part of the study. Summarised by location, the populations and number of lines included in each experiment, experiment dimensions, sowing date, mean anthesis date for each experiment, and mean maximum daily temperature, number of days > 30 °C, number of days > 35 °C during anthesis and grain fill

Experiment	Year	Population	Location	GPS position	Plots	Columns	Rows	Reps	DH Genotypes	Check Genotypes	Sowing Date	Mean Anthesis Date (Degree days sowing to anthesis)	Mean Grain Yield kgha^{-1}	Mean Growing Season (May—Oct) Rainfall (mm)	Mean Anthesis Average Maximum Temperature (°C)	Mean Anthesis Number of Days > 30 °C	Mean Grain Fill Average Maximum Temperature (°C)	Mean Grain Fill Number of Days > 30 °C	Mean Grain Fill Number of Days > 35 °C
ANHGSM151	2015	MG, SM, SG	Angas Valley	− 34.75, 139.27	1296	12	108	1.4	922	7	15 May 2015	1451	2277	102	24.3	5.0	31.9	16.7	7.9
ANHGSM161	2016	MG, SM, SG	Angas Valley	− 34.70, 139.25	336	24	14	1.37	245	7	1 June 2016	1511	3379	221	20.8	2.0	26.0	8.8	0.9
RSHGSM152	2015	MG, SM, SG	Roseworthy	− 34.51, 138.68	1296	12	108	1.4	922	7	21 May 2015	1453	2843	190	24.2	4.2	30.6	10.8	6.0
RSHGSM162	2016	MG, SM, SG	Roseworthy	− 34.50, 138.68	336	24	14	1.37	245	7	15 May 2016	1511	6203	480	20.9	0.0	23.1	1.8	0.0
WTHGSM153	2015	MG, SM, SG	Winulta	− 34.30, 137.90	1296	12	108	1.4	922	7	12 May 2015	1458	2591	208	22.8	3.0	28.1	11.1	4.5
WTHGSM163	2016	MG, SM, SG	Winulta	− 34.26, 137.90	336	24	14	1.37	245	7	18 May 2016	1510	6972	381	19.5	0.0	23.2	2.9	0.0
ANHX32151	2015	L2G	Angas Valley	− 34.75, 139.27	216	12	18	1.52	142	8	15 May 2015	1379	1644	102	22.5	3.0	31.9	16.8	7.6
ANHX32161	2016	L2G	Angas Valley	− 34.70, 139.25	144	24	6	1.67	86	9	1 June 2016	1555	3298	221	21.5	2.6	26.5	9.3	1.4
RSHX32152	2015	L2G	Roseworthy	− 34.51, 138.68	216	12	18	1.52	142	8	22 May 2015	1387	2489	190	22.8	3.0	30.8	12.2	7.2
RSHX32162	2016	L2G	Roseworthy	− 34.50, 138.68	144	24	6	1.67	86	9	15 May 2016	1556	6226	480	20.8	0.0	23.6	2.5	0.0
WTHX32153	2015	L2G	Winulta	− 34.30, 137.90	216	12	18	1.52	142	8	12 May 2015	1382	2447	208	21.5	1.3	28.0	11.5	5.1
WTHX32163	2016	L2G	Winulta	− 34.26, 137. 90	144	24	6	1.67	86	9	18 May 2016	1552	6734	381	19.6	0.0	23.5	3.3	0.3
ANHX4151	2015	RG	Angas Valley	− 34.75, 139.27	240	12	20	1.49	161	8	15 May 2015	1380	2181	102	22.4	2.8	32.2	17.1	7.8
ANHX4161	2016	RG	Angas Valley	− 34.70, 139.25	192	24	8	1.62	117	8	1 June 2016	1565	3540	221	21.6	2.7	26.6	9.3	1.5
RSHX4152	2015	RG	Roseworthy	− 34.51, 138.68	240	12	20	1.49	161	8	22 May 2015	1379	2738	190	22.5	2.5	31.0	12.4	7.5
RSHX4162	2016	RG	Roseworthy	− 34.50, 138.68	192	24	8	1.62	117	8	15 May 2016	1566	7202	480	20.7	0.0	23.8	2.6	0.0

Table 2 (continued)

Experiment	Year	Population	Location	GPS position	Plots	Columns	Rows	Reps	DH Genotypes	Check Genotypes	Sowing Date	Mean Anthesis Date (Degree days sowing to anthesis)	Mean Grain Yield kgha^{-1}	Mean Growing Season (May—Oct) Rainfall (mm)	Mean Anthesis Average Maximum Temperature (°C)	Mean Anthesis Number of Days > 30 °C	Mean Grain Fill Average Maximum Temperature (°C)	Mean Grain Fill Number of Days > 30 °C	Mean Grain Fill Number of Days > 35 °C
WTHX4153	2015	RG	Winulta	− 34.30, 137.90	240	12	20	1.49	161	8	12 May 2015	1381	3043	208	21.4	1.1	27.9	11.7	5.1
WTHX4163	2016	RG	Winulta	− 34.26, 137.90	192	24	8	1.62	117	8	18 May 2016	1567	7903	381	19.6	0.0	23.7	3.4	0.4

The 2015 GSM experiments consisted of 154 MG, 203 SM and 360 SG lines. In 2016, there were 42 MG, 44 SM and 92 SG lines. In each year, the remaining plots in each experiment were made up of material not used in the analysis for this study

Statistical methods—Baseline model

For each of the populations, assume a set of r progeny varieties were sown in t environments and let $\boldsymbol{y} = \left(\boldsymbol{y}_1^T \ldots \boldsymbol{y}_t^T\right)^T$ be a vector of observed responses such as grain yield, screenings or TWT. The response was then analysed using a baseline multi-environment linear mixed model (ME-LMM) of the form

$$\boldsymbol{y} = X\tau + \boldsymbol{Z}\boldsymbol{u} + \boldsymbol{Z}_g\boldsymbol{u}_g + \boldsymbol{e} \quad (1)$$

where $X\tau$ was a fixed component containing a vector of fixed effects for estimating experiment means as well as an overall mean for the population, parents, and controls specific to each environment. $\boldsymbol{Z}\boldsymbol{u}$ was a random component consisting of effects $\boldsymbol{u}$ that were conformably partitioned to account for environment specific variation such as aspects of the experimental designs including blocks or replicates, and also variation due to potential extraneous nonlinear trends potentially existing across the row or column of the experiment. To further improve the flexibility of the ME-LMM to model local spatial variability, the residuals were partitioned to $\boldsymbol{e} = \left(\boldsymbol{e}_1^T \ldots \boldsymbol{e}_t^T\right)^T$ where the residuals within the j th environment were assumed to be distributed $\boldsymbol{e}_j \sim N\left(0, \sigma_j^2\boldsymbol{R}_j\right)$ with $\boldsymbol{R}_j$ parameterised as a separable auto-regressive structure of order one in the Row and Column direction. The important focus of this ME-LMM is the random genotype-by-environment interaction term $\boldsymbol{Z}_g\boldsymbol{u}_g$, with effects $\boldsymbol{u}_g$ of length $r \times t$ and indicator matrix $\boldsymbol{Z}_g$ that maps the genotypes to the plots within each experiment. These effects were assumed to be distributed $\boldsymbol{u}_g \sim N\left(0, \boldsymbol{\Delta} \otimes \boldsymbol{I}_r\right)$ where $\boldsymbol{\Delta}$ is a $t \times t$ covariance matrix consisting of environment-specific variances on its diagonal to capture genetic variation of the progeny lines within each environment and covariances on its off diagonals to model the genetic relatedness of the progeny between each pair of environments. When t was moderately large the estimation of $\boldsymbol{\Delta}$ became problematic and we sought the use of a parsimonious approximation by defining the genotype by environment effects to have a Factor Analytic (FA_k) model (Smith et al. 2001, 2005) with variance $\boldsymbol{\Lambda}\boldsymbol{\Lambda}^T + \boldsymbol{\Psi} \approx \boldsymbol{\Delta}$ where $\boldsymbol{\Lambda}$ is $t \times k$ matrix of environment loadings and $\boldsymbol{\Psi}$ is a diagonal matrix containing environment specific variances. The number of factors k used in the FA model depended on the number of environments in which the population progeny were sown.

Statistical methods-QTL analysis

For a given population, let $\boldsymbol{M}$ be an $r \times p$ matrix of unique interval markers. For each trait from this population, a QTL analysis was conducted using a whole genome scanning approach with two separate runs. In the first run, the focus

was to obtain overall performance QTL across environments. Let $\boldsymbol{m}_{ij}$ be the j th interval marker in the i th chromosome, then the performance QTL model was an extension of the ME-LMM defined in (1) where the total genetic effects were partitioned as

$$\boldsymbol{u}_g = \boldsymbol{m}_{ij}a_{ij} + \boldsymbol{u}_a + \boldsymbol{u}_p \tag{2}$$

In this extended genetic model, a_{ij} represents the main or overall performance effect of the interval marker $\boldsymbol{m}_{ij}$ across the t environments. We used the strategy of Rincent et al. (2014) for improving the power of detecting significant marker or marker by climatic interaction effects by including additional genomic and residual genetic terms in (2). Specifically, we include $\boldsymbol{u}_a$, a vector of random marker based additive genotype by environment effects that are assumed to be distributed $\boldsymbol{u}_a \sim N\left(0, \boldsymbol{\Delta}_a \otimes \boldsymbol{G}_{-i}\right)$ where $\boldsymbol{G}_{-i} = \boldsymbol{M}_{-i}\boldsymbol{M}_{-i}^T$ is an $r \times r$ genomic relationship matrix with the interval markers from the i th chromosome removed and $\boldsymbol{\Delta}_a$ is an $t \times t$ additive genetic covariance matrix. We also include $\boldsymbol{u}_p$, a vector of random polygenic residual genotype by environment effects that are assumed to be distributed $\boldsymbol{u}_p \sim N\left(0, \boldsymbol{\Delta}_p \otimes \boldsymbol{I}_r\right)$ where $\boldsymbol{\Delta}_p$ is a $t \times t$ residual genetic covariance matrix. Similar to the previous sections, where necessary, the marker-based additive and residual genotype by environment effects were both parsimoniously approximated by a Factor Analytic model.

In the second run, the focus was on detecting QTL displaying significant responsiveness across the numerical range of the climatic covariate. If $\boldsymbol{c}$ is the climatic covariate, then the climate covariate QTL model used an extended ME-LMM with $\boldsymbol{c}\boldsymbol{m}_{ij}b_{ij}$ replacing the first term on the right-hand side of (2).

In this new term b_{ij} represents the interval marker by climatic covariate interaction effect or responsiveness effect of the interval marker across environments.

The two QTL models were then used to scan the complete p interval markers across the 21 chromosomes of the wheat genome, and Wald statistics were calculated for each performance and responsiveness effect. Initial effect significance was determined using the thresholding technique of Li and Ji (2005) outlined for QTL ME-LMMs in Bonneau et al. (2013). Interval marker effects above the significance threshold were omitted from further analysis if they were within a window of 30 cM adjacent to an interval marker effect with greater significance. The remaining set of n_s significant interval markers were then considered linked to putative QTL and additively included in a final QTL model, using the example of significant performance QTL, was

$$\boldsymbol{u}_g = \sum_{s=1}^{n_s} \boldsymbol{m}_s a_s + \boldsymbol{u}_a^* + \boldsymbol{u}_p \tag{3}$$

where $\boldsymbol{u}_a^* \sim N\left(0, \boldsymbol{\Delta}_a \otimes \boldsymbol{G}_{-s}\right)$ and $\boldsymbol{G}_{-s} = \boldsymbol{M}_{-s}\boldsymbol{M}_{-s}^T$ is the genomic relationship matrix with interval markers excluded if they were within a 30 cM window of the interval markers in the final model. The significant performance and responsiveness QTL were then summarised with their chromosome, genetic distance, LOD score, and additive effect size. To aid in the interpretation of the size of the additive effect for responsiveness, a normalisation was conducted that multiplied each additive effect by the numerical range of the covariate over the environments. The normalised additive effect size was also provided. For each of the QTL, a linked marker was used to identify the RefSeq physical position (Alaux et al. 2018), with the base pair position at the start of the candidate marker sequence. Analysis to identify QTL for anthesis data was only conducted using the described methodology to identify performance QTL, as these data were only collected in the experiments collected at Roseworthy in each year of the study.

Statistical methods-Computations

Baseline ME-LMMs were fitted using the flexible linear mixed modelling software package ASReml-R (Butler et al. 2018) available in the R statistical computing environment and downloadable through VSNi website from https://www.vsni.co.uk/software/asreml. The ASReml-R package contains a suite of functionality for fitting and diagnosing complex linear mixed models and uses the REML algorithm of Patterson and Thompson (1971) to estimate model parameters. Diagnostic assessment of models was conducted using ASReml-R functions as well as functions from the post-processing package ASExtras available for download from https://mmade.org/. QTL analyses were conducted using the GWASReml R software package available from https://github.com/DrJ001/GWASReml. The package provides functionality for conducting one-stage QTL and GWAS analyses through extensions of the baseline ASReml-R model.

Results

QTL identified for performance

In total, 199 QTL were found in this study, of which 60 were found to be important for performance, with a stable effect across all environments sampled. QTL were spread across all chromosomes except for chromosome 3D, as shown in Fig. 1 (additional details for each QTL identified are shown in Supplementary Table 2). Genetic correlations for each population for each trait across each environment are shown in Supplementary Table 3. Of the 60 QTL for trait performance, 21 were identified for anthesis date, 14 for grain yield, 18 for TWT, and seven for screenings. Performance QTL were identified in all populations, although not for each

Fig. 1 QTL identified for performance and responsiveness to the climatic co-variates measured in each environment mapped against their position (cM) on the consensus map. Colours indicate QTL type and to which climatic co-variate the responsiveness QTL interact

trait in each population. Grain yield QTL were not identified in the RG population, TWT QTL were not identified in the SG population, while screenings QTL were not identified in the MG and SM populations. Anthesis date QTL were identified in all populations.

QTL identified for responsiveness to climatic co-variates

A further 139 QTL (Fig. 1 and Supplementary Table 2) were identified that showed a significant interaction with the climatic co-variates measured for each genotype in each environment. These QTL are termed 'responsive', indicating that there is variable trait expression in response to the climatic co-variates from each environment. Grain yield accounted for 44 of the QTL for responsiveness identified across all populations and climatic co-variates. For screenings, 43 responsiveness QTL were identified in the MG, SM, RG, and AUS17840/Gladius (L2G) populations, while none were identified in the SG population. A further 52 responsiveness QTL were found for TWT across all populations and all climatic co-variates, except for the MG population where only one responsiveness QTL was found for grain fill days > 35 °C.

Clusters of QTL

Of the 199 QTL identified, 18 occurred independently of any other QTL identified. The remaining 181 QTL occurred in clusters with one or more other QTL (within 10 cM of the interval associated with other QTL), with up to 27 QTL clustering together. QTL clusters included those characterised as performance QTL as well as responsiveness QTL.

There were 99 QTL that occurred in combination with each other, but which were not associated with QTL for anthesis date. These clusters in many cases combined QTL for performance and responsiveness for a range of climatic co-variates, and a range of traits. However, there were other regions identified where performance QTL only clustered with performance QTL and responsiveness QTL only clustered with responsiveness QTL. The QTL found to not be associated with anthesis date will be discussed in more depth as they offer opportunities to understand adaptation to heat stress conditions independently from anthesis date, which although important, are often considered differently by plant breeders as anthesis date is also an important selection target.

Of the 21 QTL identified for anthesis date, all except one (*QFlt.agt-SM.7B* on chromosome 7B) clustered with other QTL. This included a genomic region on chromosome 2B where anthesis date QTL were identified between 34.2 cM and 38.8 cM at the same position in the RG, MG, and SG populations, and these clustered with 24 other QTL between 29.8 cM and 43.5 cM. Although not specifically mapped in this study, this region likely aligns with the *Ppd-B1* photoperiod gene found on chromosome 2B (Scarth and Law 1983). QTL associated with this cluster were encompassed by each of the populations evaluated, each trait assessed, and each climatic co-variate measured. Similar but smaller clusters were found on chromosome 2D (likely associated with *Ppd-D1* (Law et al. 1978)), chromosome 5B (likely associated with *Vrn-B1* (Iwaki et al. 2002)), chromosome 5D (likely associated with *Vrn-D1* (Law et al. 1976)), as well as chromosomes 7A, 7B and 7D likely associated with the *FT* gene (Bonnin et al. 2008). On chromosome 5A, three anthesis date QTL were found to be clustered with one TWT QTL (*QTwt.agt-RG.5A*), which likely aligns to *Vrn-A1* (Law et al. 1976). These regions will not be discussed in depth as there are well understood associations with crop performance and phenology genes, as well as a strong confounding influence on adaptation to heat stress effect because anthesis date will influence the level of stress experienced.

Interpreting QTL with performance and responsiveness effects

Of the QTL identified, there are examples of performance QTL and responsiveness QTL occurring independently from other QTL, as well as performance QTL and responsiveness QTL occurring at the same position. Adding an additional layer of complexity, these regions also encompassed QTL for various phenotypic traits and response to a range of climatic co-variates. This is demonstrated in Fig. 2a–e, where responsiveness QTL collocating with a performance QTL for TWT on chromosome 4A (*QTwt.agt-L2G.4A*) shows the effect of each responsiveness QTL plotted against the range of each climatic co-variate experienced. Figure 2a–d shows the positive effect of the responsiveness QTL on TWT to increasing anthesis average maximum temperature, grain fill average maximum temperature, number of days > 30 °C, and number of days > 35 °C, as well as TWT decreasing in response to increasing growing season rainfall (Fig. 2e). However, given that these QTL collocated with a performance QTL (*QTwt.agt-L2G.4A*), they need to be considered simultaneously. Figure 2f shows the result of selecting either the high or low-performance allele at this locus and the resulting responsiveness QTL allele that would be selected. In this situation, the increased performance selected by targeting the Gladius allele of the *QTwt.agt-L2G.4A* locus would be associated with a negative response to increasing anthesis average maximum temperature, grain fill average maximum temperature, number of days > 30 °C, and number of days > 35 °C, and a positive response in TWT to increasing growing season rainfall. In other words, selecting for the higher performance allele would also lead to greater sensitivity to hot and dry conditions.

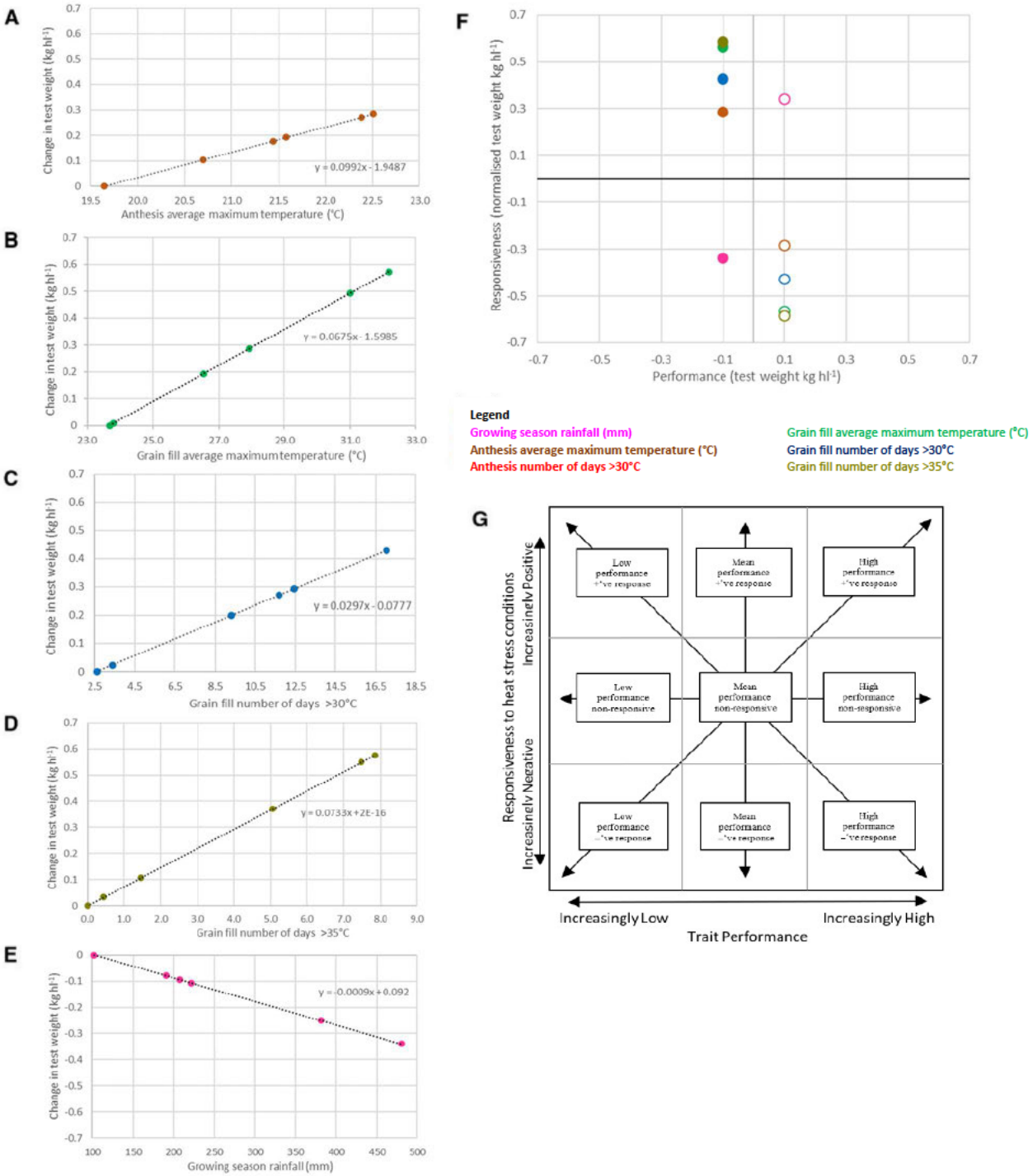

Fig. 2 To demonstrate the relationship between performance and responsiveness QTL, an example of TWT responsiveness QTL for various co-variates found to cluster with the TWT performance QTL QTwt.agt-L2G.4A, on chromosome 4A, are shown. For each responsiveness QTL, the additive effect on TWT for the RAC1548 allele is shown for the range observed for each climatic co-variate for; **a** QTwt.agt-RG.4A-1, **b** QTwt.agt-RG.4A-2, **c** QTwt.agt-RG.4A-3, **d** QTwt.agt-RG.4A-4 and **e** QTwt.agt-RG.4A-5. **f** Illustrates the impact on responsiveness by selecting for either the favourable performance allele (Gladius shown by open dots) or the alternative allele (RAC1548 allele represented by closed dots). **f** Can be interpreted within the framework proposed by Telfer et al. (2021) shown in (**g**)

Discussion

Assessing the contribution of performance and responsiveness QTL to heat stress adaptation

In the first approach to genetic analysis of heat stress adaptation used in this study, performance QTL were found that had consistent effects across a range of environments in the cereal growing areas of South Australia. In the second approach, regions were identified that interacted with climatic co-variates in their effects on the yield related traits researched herein. This dual approach to genetic analysis allows the framework proposed by Telfer et al. (2021) to be applied to the results of a multi-environment field QTL study. In this framework, the authors propose that attempting to characterise a QTL as tolerant fails to completely describe the nature of the genetic control being exerted by the locus. Telfer et al. (2021) suggested that adaptation to heat stress conditions (and likely other abiotic stresses) is better described as the combination of overall performance and responsiveness to stress conditions. Armed with this understanding, the optimal genetic combination can be selected by breeders for the specific heat stress environment being targeted. Alternatively, selection for QTL with high-performance value and no responsiveness may offer stable adaptation across a broad range of environments.

In this study, several of the performance and responsiveness QTL identified were found to occur collocated or closely located (< 10 cM), to anthesis date QTL identified in this study (Fig. 1 and Supplementary Table 2). Anthesis date is a complex trait determined by several mechanisms: photoperiod, vernalisation, and earliness per se, each controlled by several genes, and is a key component of variety adaptation and crop performance (Eagles et al. 2014; Kuchel et al. 2006). Favourable alleles at these loci are selected by breeders to ensure anthesis occurs at favourable times to minimise exposure to stresses such as frost, drought, and heat while maximising grain yield (Flohr et al. 2017). While this is an important topic, here the discussion is restricted to loci (totalling 116 QTL and described in Supplementary Table 2) that can be selected to improve adaptation to heat stress without affecting time to anthesis.

It was frequently observed that QTL alleles with a negative performance effect responded positively to increasing temperature and negatively to increasing rainfall (the reverse being true, logically, for the alternate allele). This response type is not rare (Telfer et al. 2018) and represents the classic genotype-by-environment response of heterogeneity of variance (Finlay and Wilkinson 1963; Malosetti et al. 2013). This was demonstrated by *QTwt.agt-L2G.4A* a performance QTL for TWT on chromosome 4A (Fig. 2), that collocated with responsiveness QTL for temperature co-variates and growing season rainfall (*QTwt.agt-RG.4A-5*). When considering the parental source of the allele conferring a positive trait effect for a performance QTL effect, it was the opposite allele that conferred positive responsiveness to increasing temperature conditions (and negative response to increasing rainfall). The negative association at the 4A locus between responsiveness to increasing temperature and increasing rainfall is not surprising as high temperature conditions and low rainfall are often associated with stressed environments (Lobell et al. 2015). In this case, breeders can select for either high overall performance, or adaptation to heat and drought stress. When considering the framework proposed in Fig. 2h, this would be classified as a positive response to stressed conditions and low performance value. Additionally, this highlights the correlated nature of climatic co-variates used to describe temperature and water stress as discussed by Telfer et al. (2018). However, this also reinforces the importance of considering adaptation as a whole and considering adaptation to stress conditions during different developmental periods as well as other stress factors such as water stress (considered in this study). The importance of this region for TWT has been highlighted previously by Huang et al. (2006), but also for grain yield (Tura et al. 2020; Yu et al. 2018; Zhang et al. 2018) and grain weight traits (Cui et al. 2014; McCartney et al. 2005; Tahmasebi et al. 2017; Tura et al. 2020; Zhang et al. 2018). Furthermore, studies targeting heat stress adaption have also reported QTL for grain yield in this region (Pinto et al. 2010; Tadesse et al. 2019). The region is likely to be TaCWI (Jiang et al. 2015), located in the centromeric region of chromosome 4A. The commercial varieties and breeding line parents used for the mapping populations have been screened for this gene and Gladius carries the large grain allele, while RAC1548 carries the small grain allele. The results of this study align the large grain allele of Gladius with improved TWT but a negative response to stressed conditions. Interestingly, a screenings performance QTL identified in the L2G population attributed the grain size advantage to AUS17840 rather than Gladius, something that will require further investigation.

An example identified in this study where a performance QTL was positively associated with responsiveness QTL for rainfall and temperature conditions under stressed conditions was found on chromosome 5B in the SM and MG populations. Here a performance QTL for grain yield (*QYld.agt-SM.5B-1*) was identified collocated with a range of responsiveness QTL for temperature co-variates during both anthesis and grain fill, with the Mace parent providing the preferred allele for both performance and responsiveness to increasing temperature. Similarly, Mace also provided the favourable drought (low rainfall) responsive

allele at collocated QTL (*QScn.agt-SM.5B-2* and *QScn.agt-MG.5B-3*) associated with screenings. When considering the framework posed in Fig. 2h, this would be classified as positive response with high-performance value. This is a rare combination, but one eagerly sought after by breeders as an opportunity to improve both overall performance and relative performance under heat and drought stress. This region was also described by Bennett et al. (2012b) for grain yield under delayed sowing conditions. Additionally, this region has previously been described for grain yield (Yu et al. 2018) and grain weight traits (Cui et al. 2014; Huang et al. 2003; Ramya et al. 2010; Tsilo et al. 2010; Yu et al. 2018; Zhang et al. 2018).

Alternatively, QTL for responsiveness to increasingly hot conditions not associated with rainfall would allow for the selection of genotypes that can cope with increasing terminal temperature stress conditions and retain performance under a range of rainfall environments. An example of this occurred on chromosome 7A, identified in the SM population, where a performance QTL for grain yield (*QYld.agt-SM.7A*) occurred in a similar location to heat responsive QTL for TWT (*QTwt.agt-SM.7A-1, QTwt.agt-SM.7A-2, and QTwt.agt-SM.7A-3).* Here the Mace parent provided the allele conferring both positive performance and positive responsive to stress. This region appears to align with a grain yield QTL identified in delayed sowing conditions by Bennett et al. (2012b), Pinto et al. (2016) and Hassan et al. (2018), grain number and grain weight per spike by Guan et al. (2018), as well as a leaf chlorophyll content QTL by Talukder et al. (2014b). Additionally, this region has been described in non-heat stress literature for its role in grain yield (Narjesi et al. 2015; Tura et al. 2020; Yu et al. 2018) and thousand grain weight (Cui et al. 2014; Groos et al. 2003; Huang et al. 2004).

Regions of performance and their role in adaptation

Genetic regions conferring a performance advantage in the absence of responsiveness to climatic conditions may provide breeders opportunities to select for broad adaptation to a range of stressed and unstressed environments. When considered within the framework described in Fig. 2h, this would be classified as having high-performance value and being non-responsive. This would be an advantage as many environments do not experience stressed conditions every season, or to the same level each season. A performance QTL that is independent of responsiveness would allow a lower risk alternative that would allow elite crop performance under both stressed and unstressed conditions as discussed by Telfer et al. (2021) and fits with the broad adaptation model proposed by Finlay and Wilkinson (1963).

Ten performance QTL were identified for grain yield, screenings, and TWT independent of any collocated responsiveness QTL (Fig. 1 and Supplementary Table 2). Grain yield QTL were identified on chromosome 2A (*QYld.agt-SG.2A*) and 7A (*QYld.agt-L2G.7A and QYld.agt-SG.7A*), with the Gladius parent providing the higher performing allele in each instance. *QYld.agt-SG.2A* on chromosome 2A occurs in region previously described for grain yield and thousand grain weight QTL by Tura et al. (2020) and Tsilo et al. (2010). On 7A, *QYld.agt-L2G.7A* occurs in a region where QTL for grain yield, kernel weight and TWT QTL have been previously reported (Cabral et al. 2018; Huang et al. 2003; Maphosa et al. 2014; Tura et al. 2020; Yu et al. 2018) including in heat stress studies (Bennett et al. 2012b; Guan et al. 2018; Mason et al. 2013; Pinto et al. 2016). Also, on 7A, *QYld.agt-SG.7A* occurred adjacent to a TWT performance QTL (*QTwt.agt-RG.7A*) identified in the RG population, with the Gladius parent being the source of the higher performance allele in both populations and for both traits. This is a region previously described by Pinto et al. (2016) and Mason et al. (2013) for thousand grain weight and grain yield, respectively, under late sown heat stress conditions. This region additionally has also previously been associated with thousand kernel weight (Huang et al. 2003) and test weight (Cabral et al. 2018) in studies investigating adaptation other than to heat stress conditions.

Regions of responsiveness and their role in adaptation

Regions associated with responsiveness were identified separate from performance QTL, accounting for 42 of the QTL identified (Fig. 1 and Supplementary Table 2). Regions of responsiveness independent of performance QTL may provide opportunities to breed specific adaptation but would be more limited in their application and situations in which they confer an advantage. Under extreme stress conditions, such genomic regions may offer opportunities for improvements in adaption, especially if combined with other QTL for performance. When considering the framework proposed in Fig. 2h, this would be classified as a positive response and mean performance value.

To demonstrate, a region identified on chromosome 5A in the SG population where a grain yield QTL for responsiveness to grain fill number of days > 30 °C (*QYld.agt-SG.5A-2*) and grain fill number of days > 35 °C (*QYld.agt-SG.5A-1*) were found to collocate with the grain yield QTL responsive to growing season rainfall (*QYld.agt-SG.5A-3*) with the Scout parent providing the stress-adapted allele in each instance. This is a similar result to previous work by Tura et al. (2020), Zhang et al. (2018) and Tadesse et al. (2019) who reported QTL for grain yield and thousand grain weight in the same region. Additionally, Sharma et al. (2016) described this region for grain filling duration in heat stress conditions induced by delayed sowing.

In most instances where clusters of responsive QTL were found, there were QTL responsive to temperature and QTL responsive to growing season rainfall collocated. However, one example of collocated responsiveness QTL independent of growing season rainfall was identified for grain yield on chromosome 5B in the SM population (grain fill average maximum temperature—*QYld.agt-SM.5B-3* and grain fill number of days > 35 °C—*QYld.agt-SM.5B-4*). This is a region not previously described for adaptation to heat stress conditions during grain fill but has been associated with grain yield (Tura et al. 2020; Yu et al. 2018) and thousand grain weight (Tura et al. 2020). Each of the QTL reported by Tura et al. (2020) was found to have QTL-by-environment interactions, potentially identifying similar qualities for adaptation that have been determined to be responsive to changing temperature conditions in the current study. However, this region has been found to be associated with the number of leaves per seedling when heat stress conditions were imposed during the seedling stage (Maulana et al. 2018).

Comparing field results to controlled environment evaluation of heat stress adaptation

The populations evaluated across multiple environments in this study to investigate adaptation to heat stress conditions were also evaluated previously in controlled environmental conditions by Telfer et al. (2021). In that study, adaptation to heat stress was investigated during grain filling by exposing plants to three consecutive eight hour days with an air temperature of 36 °C and a wind speed of 40 km h^{-1}. QTL analysis was conducted to identify performance and responsiveness in a similar framework to the current study. When comparing the results of Telfer et al. (2021), 86 QTL from the current study were found to be collocated with 49 QTL identified in controlled environment conditions (excluding anthesis date QTL).

Key regions of crossover between that discussed by Telfer et al. (2021) and the current study include five performance QTL identified by Telfer et al. (2021) between 50.4 and 63.3 cM on chromosome 5B in the SM population, for thousand kernel weight, grain yield per spike, spikelet fertility, grain number per spike, and spikelet number per spike, where Mace was the source of the high-performance allele. In the current study, this is a region where numerous QTL were identified in the L2G, SM, MG, and SG populations. A grain yield performance QTL (*QYld.agt-SM.5B-1*) identified in the SM population clustered with screenings QTL responsive to numerous climatic co-variates and growing season rainfall. As with Telfer et al. (2021), Mace was the source of adaptation for stressed environments at this locus. Associations with grain yield have been previously identified in this region (Bennett et al. 2012b) in heat stress conditions, as well as thousand grain weight (Huang et al. 2003; Tsilo et al. 2010; Zhang et al. 2018).

In the current study, a grain yield performance QTL (*QYld.agt-SM.2B-2*) and TWT performance QTL (*QTwt.agt-SM.2B*) clustered with QTL for screenings and TWT responsive to various temperature co-variates and growing season rainfall. These QTL mapped similarly to six yield-related QTL identified in the SM and MG populations identified by Telfer et al. (2021). In both studies, Scout was responsible for the stress-adapted allele in the SM population and Gladius in the MG population where it was associated with increased thousand grain weight. This occurs in a region described by Hassan et al. (2018) for cytoplasmic membrane stability and grain number per spike (Sharma et al. 2016), both in heat stress conditions induced by delayed sowing, as well as grain yield and thousand grain weight (Cabral et al. 2018; Groos et al. 2003; Ramya et al. 2010; Tura et al. 2020; Yu et al. 2018).

Telfer et al. (2021) identified three performance QTL between 3.5 and 12.9 cM on chromosome 7A in the SG population for grain yield per spike, spikelet fertility, and grain number per spike where the adapted allele was sourced from the Scout parent. This corresponds to QTL identified in the current study between 13.3 and 23.1 cM in the SM population on chromosome 7A; a grain yield performance QTL (*QYld.agt-SM.7A*) and TWT QTL responsive to grain fill temperature co-variates, with the Mace allele being the allele conferring adaption to stressed conditions. The parents conferring the favourable allele were different for the QTL identified in the two different populations and studies. This makes interpretation of the favourable allele combination difficult and will require additional investigation. This is a region that has previously shown potential for adaptation to heat stress for grain yield in delayed sowing studies (Bennett et al. 2012b; Hassan et al. 2018; Pinto et al. 2016), as well as chlorophyll content (Talukder et al. 2014a).

In the current study, a grain yield performance QTL (*QYld.agt-L2G.7A*) on chromosome 7A in the L2G population was identified flanking a TWT QTL (*QTwt.agt-SG.7A*) responsive to grain fill average maximum temperature in the SG population. For both QTL, Gladius was the source of the elite or stress-adapted allele. These two QTL were identified on either side of two performance QTL identified by Telfer et al. (2021) for spikelet number per spike identified in the SG and L2G populations where Scout and AUS17840 provided the elite allele, respectively, in controlled environment conditions. This region has been described by Bennett et al. (2012b) and Mason et al. (2013) for improved grain yield under heat stress conditions induced by delayed sowing, as well as thousand grain weight (Bennett et al. 2012b; Guan et al. 2018), grain number per spike (Guan et al. 2018), and stay green (Mason et al. 2013). This region was also found to be important for grain yield determination in other

adaptation studies (Tura et al. 2020) and thousand grain weight (Huang et al. 2004; Sun et al. 2008; Tsilo et al. 2010; Tura et al. 2020).

Telfer et al. (2021) identified two performance QTL on chromosome 6A (*QSfi.agt-SG.6A.2* and *QSfi.agt-L2G.6A*) that were flanked by three QTL identified in the current study (*QYld.agt-L2G.6A-1, QYld.agt-L2G.6A-2* and *QTwt.agt-RG.6A-2*). The populations involved within the two studies were not the same but did share Gladius as a common parent. The TWT response to stress is in repulsion to grain yield performance and responsive to temperature stress, while grain yield in the field appears to be linked positively to spikelet fertility in controlled environment conditions. This locus has been reported numerous times (Cui et al. 2014; Kuchel et al. 2007b; Mir et al. 2012; Pinto et al. 2016; Sun et al. 2008; Tura et al. 2020; Yu et al. 2018; Zhang et al. 2018) and is likely to be TaGW2 (Su et al. 2011) which confers a grain size advantage. Although TaGW2 was not mapped in the DH lines in this study, it was known to be segregating in some crosses, with Scout carrying the allele for large grain and Gladius the small grain allele. This suggests that the positive response in grain yield to heat stress seen in this study is linked to the TaGW2 allele for large grain.

Although some consistency between the field and in controlled environment condition studies can be demonstrated, there were more points of inconsistency. Likewise, links were found with other field studies conducted using delayed sowing, but these too were limited. This lack of commonality between the assay types suggests that the value of controlled environment studies is comparatively poor. It highlights the value of field-based genetic dissection of tolerance traits.

Heat stress adaptation in the context of breeding objectives

As proposed by Telfer et al. (2021) and further developed here, heat stress adaptation should be considered as the combination of overall performance as well as responsiveness to stress. A definition that similarly could be applied to other stress conditions.

There are two types of QTL identified in this study: performance QTL which were found to be significant across the breadth of the environments observed, and responsive QTL that varied in expression in response to the abiotic stress encountered across the environments tested. QTL that combines positive performance with positive response to stress would be most desirable to breeders. While this study identified examples of a positive performance allele for grain yield also conferring a positive response to screenings or TWT QTL, these were rare and support the conclusion that breeding for heat stress tolerance is complex. Perhaps more realistically, a breeder seeking heat stress tolerance could target performance QTL that has been shown not to be responsive to heat stress. The results of this study certainly support this as a more achievable target with three QTL for grain yield identified that meet this criterion. Similarly, QTL that only show responsiveness to heat stress, and are not linked to performance, may offer opportunities to breed varieties that are specifically adapted to heat prone environments. This study highlights the importance of considering both performance and responsiveness when investigating tolerance to abiotic stress tolerance. Either in isolation may lead to incomplete or even worse, misguided conclusions regarding genetic selection strategy.

Conclusions

This study demonstrates that adaptation to heat and drought can be assessed as the combination of performance and responsiveness to stress conditions. Furthermore, this two-dimensional framework for understanding and breeding for stress tolerance can be easily extended to other complex abiotic stresses. Loci for performance, with broad adaption across both stressed and unstressed environments in the absence of responsiveness, represent useful targets for breeders, with examples for grain yield identified in this study on chromosomes 2A and 7A. The responsiveness QTL identified offer opportunities to breed for specific adaption, including the grain yield locus found independent of performance on 5A that combines adaptation to heat stress during grain fill and drought stress. Finally, grain yield loci on 2B and 5B provide unique pathways to combine high performance with positive responsiveness to temperature and drought stress. These QTL, and others discussed in this study, present opportunities to further the development of diagnostic markers, firstly through validation in the populations used in this study and then wider validation in breeding populations.

Once validated these genetic regions represent opportunities for marker assisted selection by breeders. Additionally, the multi-environment framework used herein could be applied in the development of two-dimensional (performance and responsiveness) genomic prediction calibrations. This would enable the extension of current genomic selection principles, that are being increasingly used by breeding programs, to be extended into selection of adaptation to abiotic stress.

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Data availability The datasets generated during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Chapter 5. General Discussion

This study was established in response to a lack of applicable knowledge for breeding programs to select for improved adaptation to heat stress conditions, particularly those experienced in Mediterranean-type environments of southern Australia. Previous research had been targeted at traits conferring tolerance to heat stress conditions or using screening methods with relatively low relevance to real-world conditions and therefore low relevance for selection within breeding programs. Adaptation to heat stress conditions is complex with many, mostly quantitative, traits involved. This is further complicated by interactions with the timing of stress relative to plant developmental stage and additional GxE with other climatic and environmental factors. For breeders to routinely select for improved adaptation to heat stress conditions, they need strategies that can be employed within their breeding programs that provide representative and relevant data on adaptation to stress conditions and can be combined with their routine screening programs. Therefore, the key objectives of this study were to:

1. Investigate the role of heat stress in the adaptation of wheat to the southern Australian environment and identify the level of genetic diversity in adaptation to heat stress conditions currently available to breeders.
2. Investigate options for breeders to evaluate adaptation to heat stress conditions in relevant growing environments, using field-based and controlled environment assays, with consideration of overall adaptation and the ability to consider in conjunction with other breeding objectives.

3. Identify QTL that confer improved adaptation to heat stress conditions experienced in the Mediterranean-type environments of southern Australia and examine their relevance to breeders' selection objectives.

5.1. Overview of Study Outcomes

The key findings from the original research publications contained within this study are as follows:

- In environments prone to heat stress conditions, heat stress is a key driver of GxE interactions, of equal, or even greater contribution, than growing season rainfall.
- There is genetic diversity for adaptation to heat stress conditions experienced during both anthesis and grain filling already present within relevant commercial wheat varieties.
- Additional sources of genetic diversity are likely available from alternative and exotic sources that may offer additional value to breeders and the development of varieties adapted to heat stress conditions.
- Controlled environment assays, developed for high throughput and repeatable screening of germplasm for traits conferring adaptation to heat stress conditions, do show some relevance to field conditions, but may be of limited value and results should be used with caution.
- Adaptation to heat stress should be assessed as the combination of overall performance and responsiveness, as previous definitions of tolerance do not accurately reflect what is required for improved adaptation to heat stress conditions. Using this definition, a variety with high performance will be superior across a range of temperature conditions, and a variety with a positive response will perform more favourably in high-temperature stress conditions.

- Assessing adaptation to heat stress conditions using this performance and responsiveness framework is possible in the field using multiple representative field environments and temperature co-variates to determine variety performance and responsiveness. Such a system will therefore be compatible with best-practice breeding and variety evaluation trials.
- Various options exist within this adaptation framework for breeders and their selection objectives. The ideal combination would appear to be a high performance value and a positive response to heat stress. However, this combination may be relatively rare, and therefore a difficult selection target for breeders.
- Alternative selection targets for breeders include selection for performance in the absence of responsiveness as a lower-risk target and to achieve broad adaptation across a range of environments. Alternatively, responsiveness could be targeted to achieve specific adaptation to high temperature conditions.
- Grain yield QTL identified in multiple field conditions were identified that display elite performance and responsiveness to stressed environments (high temperature and drought), as well as QTL for performance in the absence of responsiveness and responsiveness in the absence of performance.

5.2. Improved Prospects for Breeders and the Selection of Improved Adaptation to Heat Stress Conditions

Previous definitions of tolerance to heat stress conditions used to identify improved adaptation to heat stress conditions have been problematic and potentially misleading. As demonstrated by Mohammadi, Armion et al. (2010), favouring lines with a low performance penalty under stress conditions inadvertently favours lines with low inherent performance ability. This is an unfavourable combination for breeders looking to improve overall performance as well as improving adaptation to stressed growing conditions. The ability to select for stable and high performance across a range of environments is crucial for breeders

selecting for adaptation to stressed environments. As a result, an updated definition of assessing adaptation to heat stress conditions was developed in this study (Telfer, Edwards et al. 2021) and is shown in Figure 3. The proposed framework which assesses overall adaptation as the combination of performance and responsiveness offers an improved definition for assessing adaptation to heat stress conditions over previous definitions, and a definition that is of greater relevance to breeders' selection objectives. Additionally, the proposed definition allows the identification of specific adaptation to stressed conditions, but also allows the performance value to be assessed to avoid the situation described by Mohammadi, Armion et al. (2010).

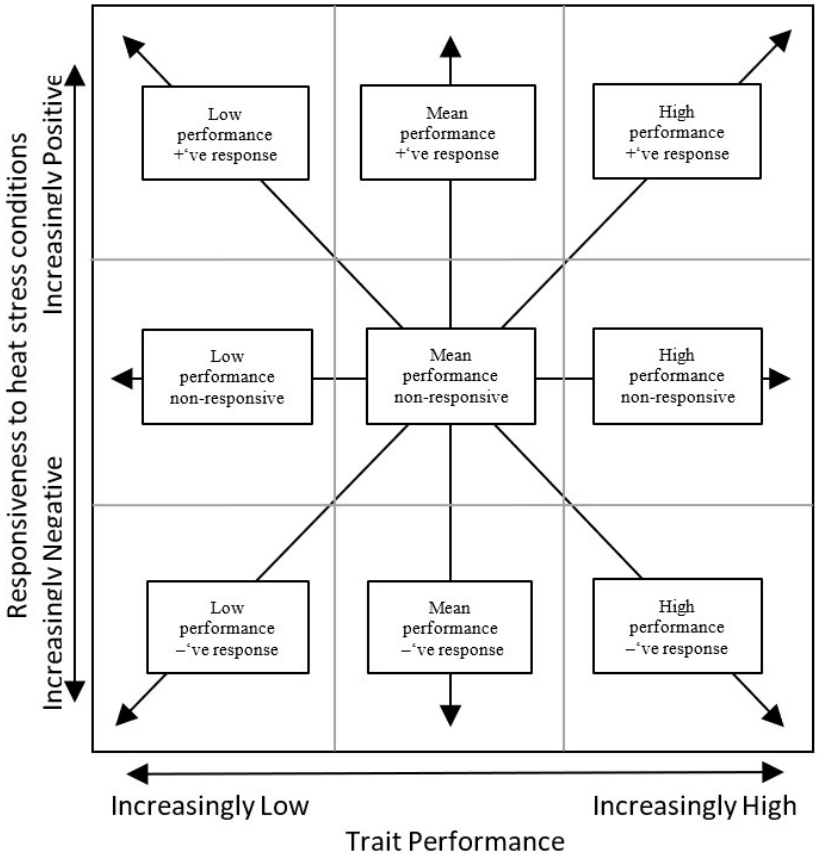

Figure 3. A framework to assess overall adaptation to heat stress conditions as the combination of performance and responsiveness (Telfer, Edwards et al. 2021).

The proposed framework to assess adaptation to heat stress conditions as the combination of performance and responsiveness was applied in this study in QTL mapping population studies conducted in both controlled environment conditions in Chapter 3 (Telfer, Edwards et al. 2021), and in field conditions across multiple representative conditions in Chapter 4 (Telfer, Edwards et al. 2022). There were QTL identified in field conditions for responsiveness and performance for a range of temperature co-variates determined for both the anthesis and grain fill periods as well as growing season rainfall. Comparisons can be drawn with the seminal work of Finlay and Wilkinson (1963). Performance, as defined in this study, is somewhat analogous to general adaptation as defined by Finlay and Wilkinson (1963), which can also be described as broad adaptation. The principles of Finlay and Wilkinson (1963) have long influenced breeders in Australia who generally evaluate their breeding material over a range of environments, looking to identify varieties with a certain level of broad adaptation, or stable and elite performance across a range of environments (Hollamby and Bayraktar 1996). This approach is likely to select for high performance in heat stress conditions if the target population of environments includes a sufficient range of heat stress conditions. This is demonstrated by a number of the varieties evaluated in Chapter 2 of this study (Telfer, Edwards et al. 2018), exhibiting high levels of adaptation to heat stress conditions. A number of the varieties evaluated originate from breeding programs situated in southern Australia that evaluate their breeding material in heat-stress-prone environments. This demonstrates that long held methodologies of selecting for broad adaptation have also been successful in selecting for improved adaptation to heat stress in environments such as Australia, and the value of continuing such methodologies. However, Chapter 4 (Telfer, Edwards et al. 2022) in this study demonstrated that using co-variates measured in each environment allows both performance and responsiveness to be quantified from the same dataset allowing for the targeted selection of improved adaptation to heat stress conditions and foreseeably improved rates of genetic gain.

Responsiveness, as defined by the heat stress adaptation framework in Figure 3, offers opportunities to select for specific adaptation to heat stress conditions. In variety evaluation trials responsiveness may be determined by analysing the performance data to determine the mean response across all varieties in the study to increasing stress, then determining the same for each variety to identify a positive or negative deviation from the mean. A positive deviation infers a positive response to increasing stress.

Historically, this may have been achieved by breeders observing and selecting varieties that do particularly well in stressed environments. Similar may also be achieved by using a heat stress nursery or identifying environments within those used for routine screening that has experienced temperature stress conditions and using relative variety performance as an indicator for specific adaptation to temperature stress conditions. An example of this is delayed sowing nurseries like those routinely used by the CIMMYT wheat breeding program to evaluate adaptation to heat stress conditions (Juliana, Singh et al. 2020). However, the relevance of screening in such an environment needs to be carefully considered as the representativeness to the target population of environments may be limited. Alternatively, a controlled environment assay could be used for targeted adaptation to heat stress conditions, as demonstrated by Telfer, Edwards et al. (2018). The results from controlled environment assays can have relevance to field conditions, however, caution is needed to ensure the representativeness of the assay to minimise confounding factors that could influence the results (stress caused by manual handling of plants, and CO_2 depletion from recirculated air were potential limitation of the assay used in the current study). In Chapter 4, this study was able to demonstrate that responsiveness could be identified and quantified in multi-environment variety evaluation trials that use best practice sowing times.

In the past, there have been ways that breeders can select for improved adaptation to heat stress conditions. However, as demonstrated in this study, the inclusion of temperature co-

variates within multi-environment trial analyses and considering adaptation as the combination of performance and responsiveness in a quantified manner, offer opportunities for breeders to value-add pre-existing screening programs to characterise and accelerate genetic gain for adaptation to heat stress conditions.

5.3. QTL Conferring Adaptation to Heat Stress Conditions and Inclusion in Breeding Germplasm

There have been numerous studies looking to identify QTL conferring an advantage for adaptation to heat stress conditions in the last couple of decades (Mohammadi, Zali et al. 2008, Pinto, Reynolds et al. 2010, Vijayalakshmi, Fritz et al. 2010, Bennett, Reynolds et al. 2012, Paliwal, Röder et al. 2012, Ali, Ibrahim et al. 2013, Mason, Hays et al. 2013, Shirdelmoghanloo 2014, Pinto, Lopes et al. 2016, Sharma, Singh et al. 2016, Bhusal, Sarial et al. 2017, Tahmasebi, Heidari et al. 2017, Guan, Lu et al. 2018, Hassan, Solouki et al. 2018, El Hassouni, Belkadi et al. 2019, Liu, Sukumaran et al. 2019, Tadesse, Suleiman et al. 2019). There appears, however, to be very little targeted selection for these QTL by breeding programs. Most of the genetic studies to date have identified genetic loci conferring an advantage in heat stress conditions induced within controlled environment assays, in the field using delayed sowing, or in the field using heat chambers or covers to aid in inducing high-temperature conditions. The reduced relevance of the assays compared to real-world conditions experienced in the field may be contributing to a reduced relevance of the loci identified or reduced knowledge of the loci in relevant growing conditions, therefore resulting in reduced uptake by breeders to use as a selection tool.

The relevance of the QTL identified in the current study, to local breeding programs, should be comparatively high due to the representativeness of the testing environments used. The environments used in the study were sampled from the wheat-producing areas of southern Australia, while achieving a range in temperature and water stress conditions, with experiments grown using regional best practice agronomy. This makes the dataset used for

QTL analysis of high relevance, and therefore the loci identified are also likely to have greater relevance to breeders selecting for adaptation to heat stress conditions in those environments.

Many of the QTL identified in this study occur in chromosome locations described in previous heat stress research using different methodologies. Examples include studies conducted by Bennett, Reynolds et al. (2012), Pinto, Reynolds et al. (2010), Pinto, Lopes et al. (2016), and Esten Mason, Hays et al. (2013) who conducted studies using delayed sowing methods to induce high-temperature stress. This suggests that some of the loci identified in delayed sowing conditions are relevant to conditions in southern Australia. However, several studies investigating GxE interactions and adaptation, without a specific target of heat stress or abiotic stress, also identified QTL in similar regions to those identified in this study, namely Tura, Edwards et al. (2020), Yu, Mao et al. (2018), and Tsilo, Hareland et al. (2010). This suggests that studies investigating general adaptation may achieve similar outcomes but will not be able to attribute the adaptation advantage to heat stress adaptation *per se*. This reaffirms that selection for broad adaptation in environments that include heat stress conditions does select for improved adaptation to heat stress. It also serves to demonstrate that the methods used in this study could be used as a validation tool for a wider range of environments, to demonstrate the relevance of loci identified using other methods and quantify their effect on adaptation to local heat stress conditions.

5.4. Opportunities for Further Research

Numerous QTL for performance and responsiveness were identified in this study. Although found to be relevant for the germplasm included, and for the environments sampled, these results should be evaluated further over a wider range of environments and seasons to better understand the range of responsiveness and the limits of adaptation. Evaluation at different latitudes, across a larger range of grain yield environments, including environments that rely on stored soil moisture, would allow for a greater understanding of the role and the limits of

the QTL identified. As mentioned in Chapter 4 (Telfer, Edwards et al. 2022), there are several QTL that offers opportunities for further evaluation to understand their role more deeply in adaptation and their underlying effects on crop physiology. Key QTL of interest identified included three grain yield QTL identified on chromosomes 2A and 7A for performance that were independent of responsiveness, therefore offering broad adaptation regardless of the level of stress experienced. A further two QTL were identified on chromosomes 2B and 5B that combined grain yield performance with positive responsiveness, reducing screenings and increasing test weight in heat and drought stress conditions. Finally, a QTL was identified for responsiveness on chromosome 5A, not associated with performance QTL, that combined a positive response in grain yield to increasing temperature and drought stress during grain fill. These QTL represent targets for development into diagnostic markers for use as selection tools by breeders. However, to improve the confidence in the value of these QTL, further validation across more environments and in breeding populations relevant to those environments would be required, followed by the development of diagnostic markers for selection.

The populations used are a good representation of varieties adapted to southern cereal-growing regions of Australia. Although the parents of the populations used may be largely outclassed as varieties by current commercial varieties, they represent genetic backgrounds or are direct parents of current elite commercial varieties grown in the targeted region. However, a wider selection of genetic diversity, both adapted and exotic, including from synthetic sources and from gene banks held throughout the world should be explored as there is likely further genetic diversity that can be identified that may add further value to wheat growing in the future. Additionally, these principles can be applied to other cereal-growing regions in Australia, and internationally, to identify QTL for improved adaptation that may have specific relevance to those geographic areas. Differences in latitude, rainfall, root and foliar diseases or pests, and many other factors, may change the role and relative importance of QTL in those environments.

The focus of this study was grain yield, yield-determining traits, and physical grain quality traits of commercial value. These remain the key traits of interest to breeders and growers as they represent key components of crop profitability. However, there is scope to apply the principles used in this study to investigate impacts on end-use quality, principally these are baking and noodle quality for wheat. This would help develop our understanding of the impact of variable and high-temperature conditions on upstream customers, which in turn may offer additional value to growers through a higher price achieved for higher quality and higher-value products.

A key limiting factor in evaluating adaptation to heat stress in relevant conditions across multiple environments is the resource-intensive nature of evaluating large populations in multiple environments. This remains a limitation, but the proposed framework (Figure 3) allows for breeding and variety evaluation trials to be value-added to specifically include adaptation to heat stress conditions. A second limitation of evaluating adaptation to heat stress conditions across multiple relevant environments is being able to effectively interpret or separate the impact of heat stress conditions on adaptation due to other confounding factors. These include factors such as variable maturity resulting in variable stress exposure amongst the genotypes included, and other co-occurring stresses such as nutritional deficiencies, disease, and other abiotic stresses such as drought stress. The framework presented in this study greatly diminishes this limitation of field studies and allows adaptation to heat stress conditions to be evaluated across multiple representative environments, maximising the value of the data to breeders.

5.5. Performance and Responsiveness as Breeder Selection Objectives

Considering adaptation to heat stress conditions as the combination of performance and responsiveness as proposed in this study (Telfer, Edwards et al. 2021, Telfer, Edwards et al. 2022), provides a range of opportunities for breeders and their selection objectives. As described in Chapter 4 (Telfer, Edwards et al. 2022), selecting for performance in the absence of responsiveness presents as a strategy for breeders to select for broad adaptation with elite

performance across a range of environmental conditions, both stressed and unstressed. Although this has been happening historically to an extent by selecting for stable performance across the breeders' target population of environments, targeted selection of performance as defined in Figure 3 presents an opportunity to do this more directly and without inadvertently selecting for adaptation to high-yielding and favourable environments to the detriment of stressed environments. This does not fit completely with previous definitions of 'tolerance' to stress conditions but presents a meaningful and tangible way of improving adaptation to stressed conditions, while simultaneously improving overall adaptation. Some previous definitions of tolerance, such as the susceptibility index, as defined by Fischer and Maurer (1978), aimed to select for a low penalty in trait expression under stress conditions. However, this may also inadvertently favour genotypes with a low-performance ability as discussed by Mohammadi, Armion et al. (2010). This is not what breeders are aiming to select for in new varieties, requiring a rethink of definitions used for tolerance and their impact on breeders' selection objectives. The definition of performance as defined and demonstrated in this study offers an alternative that allows for the selection of elite phenotype expression in both stressed and unstressed conditions, a much more favourable outcome for breeders.

The other component required to assess adaptation to stress conditions as defined in this study is responsiveness, which presents opportunities to breeders as a tool for targeting specific adaptation. Additional understanding of the extent and limits of the responsiveness may be important factors for consideration. In instances where performance and responsiveness co-occur it will be important to consider if positive performance attributes are genetically linked positively or negatively with responsiveness, with the latter diminishing the desired outcome in stressed conditions. This is a situation where it may be important to consider multiple phenotypic traits; different traits may exhibit different performance or responsiveness characteristics. For example, positive performance for grain yield may be linked with negative responsiveness to other economically important traits such as grain size

or test weight. This would create a situation that works to have a negative impact under increasingly stressful conditions. Alternatively, positive performance could be linked with positive responsiveness adding additional value for stress adaptation as demonstrated in this study (Telfer, Edwards et al. 2022), with QTL identified on chromosomes 2B and 5B that combined positive performance with positive responsiveness to increasingly stressful conditions. This would be a very favourable combination for breeders. In isolation, responsiveness may still offer value contributing to specific adaptation to stressed conditions but will be more limited in situations where an advantage may be evident.

5.6. Not Just a Framework for Heat Stress, but All Abiotic Stress Conditions

Heat stress was the primary focus of this study and was used to develop the framework for assessing overall adaptation as the combination of performance and responsiveness. However, there is a case for using this framework for assessing adaptation to other abiotic stresses such as drought stress, plant-available radiation, and frost. This was demonstrated in Chapter 4 (Telfer, Edwards et al. 2022) with the inclusion of growing season rainfall as a co-variate that allowed assessment of adaptation to variable rainfall conditions (drought stress) alongside that of heat stress. In doing so, numerous loci were identified that were responsive to both environmental stimuli in both positive and negative linkage. Further, this demonstrated the value of simultaneously investigating multiple stress conditions known to interact and impact on grain yield and the environment. In addition to abiotic stresses, it may be possible to apply this framework to other research areas, such as those investigating efficiency. For example, nutrient use efficiency to identify performance as well as responsiveness to nutrient applications.

This framework could be applied more generally to adaptation studies with a range of environmental co-variates measured to capture a range of factors that may be driving GxE interactions. There have been past examples, including that of Tura, Edwards et al. (2020),

who have investigated adaptation over a range of environments and identified QTL by environment interactions. The inclusion of the principles described in this study offers opportunities to further understand the underlying environmental drivers of QTL by environment interactions. Finally, the proposed framework will be of relevance to the field validation component of studies investigating heat stress and other abiotic stresses conducted in potentially non-representative conditions (including controlled environments and delayed sowing assays). Genotypes carrying candidate genes, or near-isogenic lines contrasting for genes of interest, can be evaluated over a range of representative environments, allowing performance and responsiveness to be assessed, leading to an improved assessment of the impact on adaptation and validating the relevance of candidate traits or genes for breeders.

5.7. Further Opportunities to Combine Assessment of Adaptation with Genomic Selection in Breeding Programs

Breeders are increasingly using GS as a routine part of their selection programs. Currently, GS methods are limited in their ability to incorporate GxE and it remains a current area of development to improve relevance to selection for plant breeding (Tolhurst, Mathews et al. 2019). The framework developed in this study to assess adaptation as the combination of overall performance and responsiveness using environmental or temperature co-variates presents an opportunity to be combined with current GS methods. This would allow breeders to deliberately target adaptation to heat stress conditions, as well as other abiotic stress or a combination of stress factors, within existing GS initiatives.

5.8. Concluding Remarks

Heat stress is a key abiotic stress limiting crop production in Mediterranean-type environments of southern Australia. However, adaptation to heat stress remains a complex selection target for breeders. The timing and magnitude of exposure to stress relative to plant development, as well as large variation in severity over seasons and environments, all lead to

large GxE. However, this study has provided a framework on how adaptation to heat stress conditions, and other abiotic stresses can be evaluated across multiple representative field trials that are relevant to the breeder's selection objectives. This includes the potential application in variety evaluation trials and breeding trials, where the use of climatic co-variates successfully allowed GxE to be attributed to temperature stress during both anthesis and grain filling as well as to growing season rainfall. This provides the opportunity for adaptation to be assessed as the combination of performance and responsiveness to better assess adaptation to heat and other abiotic stress conditions. In summary, this study presents a new framework for assessing adaptation to heat stress conditions, defined by concurrently considering performance and responsiveness, thereby greatly increasing relevance for researchers and breeders.

Appendix A. Chapter 2 Supplementary Material

All material is available online:

https://ars.els-cdn.com/content/image/1-s2.0-S0378429018314230-mmc1.docx

A.1. Supplementary Table 1

Genotypes used in the controlled environment and field experiments.

Genotype	Adaptation
AUS4683	Exotic
AUS4906	Exotic
AUS4926	Exotic
AXE	Adapted to southern Australia
CORRELL	Adapted to southern Australia
EGA GREGORY	Adapted to north-eastern Australian
EMU ROCK	Adapted to southern Australia
GLADIUS	Adapted to southern Australia
H45	Adapted to north-eastern Australian
HALBERD	Adapted to southern Australia
JANZ	Adapted to north-eastern Australian
KENNEDY	Adapted to north-eastern Australian
KUKRI	Adapted to southern Australia
LIVINGSTON	Adapted to north-eastern Australian
MACE	Adapted to southern Australia
RAC1629	Australian adapted breeders line
RAC1837	Australian adapted breeders line
RAC1859	Australian adapted breeders line
RAC875	Australian adapted breeders line
SCOUT	Adapted to southern Australia
SUNSTATE	Adapted to north-eastern Australian
SUNTOP	Adapted to north-eastern Australian
WYALKATCHEM	Adapted to southern Australia
YITPI	Adapted to southern Australia

Appendix B. Chapter 3 Supplementary Material

All material is available online:

https://static-content.springer.com/esm/art%3A10.1007%2Fs00122-021-03778-2/MediaObjects/122_2021_3778_MOESM1_ESM.xlsx

B.1. Supplementary Table 1

Consensus map and individual linkage maps for all populations affixed with RefSeq physical positions of QTL identified

B.2. Supplementary Table 2

Summary of experimental layouts for five heat stress experiments. Information includes the DH population, total number of lines, percentage of DH lines that had replicates (two main plots), total number of main plots, numbers of rows and columns of pots, sowing dates, and seed sources.

Experiment	Population	Lines Evaluated	% Rep DH lines	Main Plots	Rows	Cols	Sowing Dates	Seed Source
1	HK	126	100	276	46	12	1	-
2	RG	158	15	192	24	16	3	-
3	L2G	147	22	192	24	16	3	-
4	L1G	136	29	192	24	16	3	-
5	GSM	1087	11	1248	156	16	6	A, B

B.3. Supplementary Table 3

Pearson correlation coefficients of trait data used to describe and understand responsiveness to heat stress conditions used in the study. Data used to generate correlations uses data from all experiments described, including both temperature treatments.

	Thousand grain weight	Grain yield per spike	Spikelet number per spike	Grain number per spike	Spikelet Fertility
Thousand grain weight	1				
Grain yield per spike	0.40	1			
Spikelet number per spike	-0.02	0.52	1		
Grain number per spike	-0.09	0.85	0.62	1	
Spikelet Fertility	-0.09	0.81	0.40	0.95	1

Appendix C. Chapter 4 Supplementary Material

All material is available online:

https://static-content.springer.com/esm/art%3A10.1007%2Fs00122-021-04024-5/MediaObjects/122_2021_4024_MOESM1_ESM.xlsx

C.1. Supplementary Table 1

Consensus map and individual linkage maps for all populations affixed with RefSeq physical positions of QTL identified.

C.2. Supplementary Table 2

All QTL identified by trait measured, QTL type (responsive or performance; if responsive the climatic co-variate to which responsiveness was found), interval position (cM) (consensus map position), the effect of each QTL, P-Value, LOD, and physical position (RefSeq).

C.3. Supplementary Table 3

Genetic correlations between environments for each trait and population.

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