Towards the positional cloning of yield QTL on chromosome 1B for drought tolerance in wheat

By

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List of Abbreviations

Symbol	Definition
ACPFG	Australia Center for Plant Functional Genomics
ABA	Abscisic acid
ARC	Australia Research Council
BAC	Bacterial artificial chromosome
BLAST	Basic local alignment search tool
BLUPs	Best linear unbiased predictors
BPA	Bioplatform Australia
bp	Base pair
CIM	Composite interval mapping
cM	Centimorgan
CS	Chinese Spring
CT	Canopy temperature
°C	Degrees celsius
DAWN	Diversity among wheat geNome
DArT	Diversity arrays technology
DH	Doubled haploid
DNA	Deoxyribonucleic acid
F2:5	Fifth selfing generation derived from second filial generation
FOASTAT	Food and Agriculture organization statistical dataset
ha	Hectare
h^2	Broad sense heritability
HIF	Heterogeneous inbred families
HSP	Heatshock protein
GBS	Genotyping by sequencing
GRDC	Grain Research and Development Cooperation
IWGSC	International Wheat Genome Sequencing Consortium
ISBP	Insertion site-based polymorphism
KASP™	Kompetitive Allele Specific PCR
kg	Kilo gram
LOD	Logarithm of the odds
LSD	Least significant differences
MAS	Marker assisted selection

Mbp	Mega base pair
MPa	Mega Pascal
MET	Multi-environment trial
MIPS	Munich Information Centre for Protein Sequences
NDVI	Normalized difference vegetative index
NIL	Near isogenic line
PCR	Polymerase chain reaction
POTAGE	POPSEQ ordered *Triticum aestivum* gene expression
QTL	Quantitative trait locus
®	Registered trademark
RCBD	Randomized complete block design
RIL	Recombinant inbred line
RGR	Relative growth rate
ROS	Reactive oxygen species
SD	Standard deviation of mean
SE	Standard error
SA	South Australia
SIM	Single interval mapping
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeat
SOD	Superoxide dismutase
TPA	The Plant Accelerator
TGW	Thousand grain weight
TILLING	Targeting induced local lesions in genomes
™	Trademark
WGS	Whole genome shotgun sequencing
WGAIM	Whole genome average interval mapping
WUE	Water use efficiency

Abstract

Wheat feeds about 35% of the world's population and its productivity needs to be increased by 2.8% every year. Drought and heat have been reported to decrease grain yield up to 70 %. Fine mapping and dissecting the physiological effects of quantitative trait loci (QTL) for yield enable to find molecular markers and genes for breeding drought tolerant varieties. Here we focus on QTL with strong effects on yield and yield-related traits that were previously found on the chromosome 1B in three bread wheat (*Triticum aestivum* L.) mapping populations grown under abiotic stress. These QTL were first identified under low rain fed environments in three continents (Australia, Mexico and India) using double-haploid (DH) lines from Excalibur x Kukri (Edwards, 2012) and RAC875 x Kukri DH (Bennett et. al., 2012). The yield QTL on chromosome 1B of Excalibur x Kukri DH was highly expressed under severe drought stressed environments (yield < 500kg/ha) of South Australia and was not under the influence of phenology genes, and so was chosen for fine mapping. QTL for plant growth, relative leaf expansion and transpiration rate were also detected on chromosome 1B in a third mapping population derived from Drysdale x Gladius recombinant inbred lines (RIL), grown in glasshouse under well-watered and drought conditions, using a LemnaTec high throughput image phenotyping platform (Parent et al. (2015). The aims of this project were to: find whether or plant growth and transpiration QTL in Drysdale x Gladius coincide with a grain yield QTL, (2) fine map the yield QTL in Excalibur/Kukri near-isogenic lines (3) align the QTL from the three mapping populations onto chromosome 1B physical map and identify the candidate genes underlying yield under drought and heat stressed environments.

For aim 1, we selected Drysdale/Gladius RIL with recombination points in the region covering the QTL for growth and transpiration rate previously reported by Parent et al. (2015). We tested these RIL for two consecutive years (2014 and 2015) under severe drought and heat stresses in a rainout shelter (polytunnel). We found a yield QTL on chromosome 1B which was co-located with QTL for seeds/spikelet, seeds/spike, biomass, spike weight and plant height. All were expressed specifically under severe drought and high temperatures. Drysdale was the positive allele for all the QTL, except for plant height, and had a strong effect on number of seeds/spike under severe drought and heat stressed in Drysdale/Gladius. We also found that the yield QTL match a QTL for relative leaf area expansion rate and partially overlap with a QTL for transpiration rate. We hypothesized that Drysdale allele at the 1B loci contributes to biomass accumulation at early growth stage leading to a yield increase under dry and hot climate.

We aligned all the QTL for yield, yield components and physiological traits (growth, relative leaf expansion and transpiration rate) that were detected on the chromosome 1B in the three mapping populations onto the reference sequence RefSeq v1.0 and found co-locations among the QTL at the three regions. In the first region (7 Mbp), we found a co-location of yield QTL (*QYld.aww-1B.1*) from RAC875/Kukri and QTL for yield components and relative leaf expansion rate from Drysdale/Gladius. The second region spined 18.3 Mbp covered yield QTL in the three mapping populations, and also QTL for growth, leaf area expansion and transpiration rate in Drysdale/Gladius. Co-location of the QTL for yield and yield components with the QTL for physiological traits suggests a pleiotropic effect of the same gene and is indicative of potential function of the gene. The third region covered 17 Mbp and harboured a co-located yield and yield components QTL from the three mapping populations.

The co-located yield QTL from the three mapping populations in the third region covered a large interval that needed to be narrowed down to identify functional markers for molecular assisted breeding. We developed a high-resolution Excalibur/Kukri genetic map and fine mapped the yield QTL interval with SNP markers. Near-iso-genic lines (NIL) were developed from Excalibur/Kukri heterozygous inbred lines (RIL) (F2:5) that were recombinant in the yield QTL interval. The NIL were phenotyped under severe drought and high temperatures in a polytunnel using drip irrigation. We confirmed the yield and yield component QTL in Excalibur/Kukri NIL on the long arm of chromosome 1B. The Excalibur allele increased grain yield by 54.5%, biomass by 43%, fertile tillers by 32.8% and plant height by 14% compared to Kukri allele. The significant effect on grain yield resulted from significant number of productive tillers which was strongly correlated with seed number per plot. Based on the comparison of NIL haplotype, the yield QTL was narrowed down to 2.9 cM, corresponding to 2.2 Mbp on the chromosome 1B Chinese Spring reference sequence (IWGSC RefSeq v0.1). Using the Excalibur/Kukri NIL fine mapped yield QTL, we identified and annotated 42 genes on the 2.2 Mbp interval. Further expression analysis and characterization of the candidate gene/s will be necessary to identify the gene responsible for this grain yield QTL.

In this work, we found that chromosome 1B carries a locus of 17 Mbp that contributes to yield in Excalibur/Kukri population, a second locus that we narrowed down to 2.2 Mbp and 42 genes in Excalibur/Kukri. The data generated from this research adds to the currently available body of scientific knowledge related to the chromosome 1B genomic regions controlling yield under dry and hot environments. It will enable to complete the positional cloning of 1B QTL for yield.

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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Habtamu Tura

Signature..... ... Date....16/01/2018

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Thank you God for giving me the strength to keep going!

Dedication

To

*My mother **'Gimja Bekele'** and my father **'Seboka Tura'***

Chapter 1: General Introduction

Drought and heat stresses are the major abiotic stresses limiting wheat production on the globe. The problem is more pronounced in Australian environments where severe terminal drought coincides with heat waves during anthesis and grain filling period resulting in reduction of grain yield (Fleury et al. 2010; Pinto et al. 2010). Identification and cloning of QTL enable to introgress specific QTL by molecular assisted selection and to understand how to use them effectively by dissecting the physiology underlying the QTL to improve yield under dry and hot environments. Although positional cloning of yield QTL is challenging, particularly in a large and complex genome like the wheat one, recent advancement in phenotyping technologies and genomic resources make it now feasible.

This study targets QTL for yield, yield components and physiological traits on chromosome 1B in three Australian mapping populations (Excalibur/Kukri, RAC875/Kukri and Drysdale/Gladius) previously detected under different phenotyping platforms and drought treatments (Bennett et al. 2012a; Edwards 2012b; Parent et al. 2015). The three mapping populations were developed by the ACPFG using five modern Australian wheat cultivars. Excalibur and RAC875 were highly tolerant to drought while Kukri was susceptible to drought stress (Fleury et al. 2010). Drysdale was released by CSIRO for its high transpiration efficiency and high yield for New South Wales region (Condon et al. 2006). Gladius is derived from a cross between RAC875/Krichauff//Excalibur/Kukri and was selected by Australian Grain Technology in 2007 for its adaptation to southern region. Moreover, RAC875 and Gladius are highly tolerant to heat stress (Fleury et al. 2010). The overall objective of the study was to integrate yield QTL across the three mapping populations and fine map the 1B yield QTL to identify candidate genes controlling the yield QTL.

Chapter 2 will review the literature on the significance of drought and heat stress on wheat production, the knowledge on physiological and biochemical responses of wheat to drought, and tools and techniques to improve wheat yield in a dry and hot climate. Chapter 3 to Chapter 5 are research chapters. Chapter 3 will answer the question on whether the QTL for growth and relative leaf expansion rate co-locate and could explain the QTL for yield and yield components in Drysdale x Gladius RIL. It will describe QTL for yield and physiological traits in the Drysdale/Gladius RIL population, the QTL interaction with the environment, their co-location and their implications for future study. Chapter 4 will answer the question (2) on the construction of high resolution of Excalibur x Kukri DH genetic map and fine mapping of the

yield QTL using Excalibur/Kukri NIL. Chapter 5 will compare the physical positions of 14 QTL detected on chromosome 1B in the three mapping populations on the new wheat genome sequence assembly and analyse candidate genes underlying a yield QTL. The conclusion chapter will summarize the main findings of the study, its contribution to the body of knowledge on wheat tolerance to drought, practical implications for wheat improvement.

Chapter 2: Literature Review

2.1. Wheat crop characteristics

2.1.1. Production and economic importance of wheat

Wheat (*Triticum spp.*) is grown under a wide range of agro-climatic conditions and geographic regions of the world, ranking third among the cereal crops both in area coverage and total annual production (FAOSTAT 2013b). Wheat is an important crop for global food security, feeding about 35% of the world's population and contributing to about 20% of the calories and protein world-wide (Shiferaw et al. 2013). Due to its high yield potential, genetic diversity and high quality protein and processing properties (Shewry 2009; Worland et al. 1994), wheat is chosen as a strategic crop to feed the increasing global population, projected to reach 9.1 billion in 2050 (Alexandratos and Bruinsma 2012). Since the global demand for wheat is increasing (it is expected to reach a 1050 Mt by 2020), the global production will need to increase by 1.6 - 2.6% annually (Houshyar and Grundmann 2017). In other words, the global average grain yield must be increased from the current 2.7 t/ha to 3.8 t/ha (Houshyar and Grundmann 2017).

Average global wheat yield can exceed 10 t/ha under favourable environmental conditions such as sufficient water, nutrients and effective pest control (Shewry 2009). However, this potential is limited to 2.8 t/ha with prevailing abiotic and biotic stresses. Rust (stripe, leaf and stem) (Figlan et al. 2017), weeds (Shahbaz et al. 2017), viral/bacterial infections (Murray and Brennan 2009), insects and nematodes (Duveiller et al. 2007; Smiley and Nicol 2009) are among the biotic stresses which hamper wheat production. In addition, most of the wheat growing areas of the world experience abiotic stresses that adversely affect yield (Farooq et al. 2014; Semenov and Shewry 2011; Teixeira et al. 2013). Heat (Yang et al. 2017), drought (Páscoa et al. 2017), cold (Jiafeng et al. 2014), nutrient deficiency and toxicity (Ohki 1984), salinity and water logged soils (Khan and Khan 2017; Lutts et al. 2004) are considered the major abiotic stresses that induce wheat yield losses.

According to Rosegrant et al. (2001), the world market for wheat will grow significantly between 1997 and 2020 due to projected increases in world population. Current high demand for wheat makes it the third largest grain crop worldwide (Adjemian and Janzen 2014; Lagudah et al. 2001). Australia produces around 25 Mt of wheat every year, accounting for 56% of total

Australian grains production and is among the top ten wheat producing countries (FAOSTAT 2013a). Wheat production in Australia is operated by the Australian grains industry majorly in three geographical regions namely: the northern region (Queensland and New South Wales), the southern region (Victoria, Tasmania and South Australia), and the western region (Western Australia) (http://www.agriculture.gov.au/ag-farm-food/crops/wheat). The northern region has relatively high seasonal rainfall, stored soil moisture, production variability, and is the largest source of Australia's premium hard high-protein wheat than the other two regions. The southern and western regions have generally low soil fertility with many subsoil constraints and essentially depends on the winter rainfall. In the last past five years (2010 - 2015), the average total wheat grown area and production were 4.6 Mha and 8 Mt for northern region, 3.5 Mha and 7.9 Mt for southern and 4.9 Mha and 9 Mt for western region. About 70% of the Australian wheat production is exported, accounting for around 12% of the global wheat exports (ABARES, 2016) (http://www.agriculture.gov.au/abares). The main markets for Australian wheat are in the Asian and Middle Eastern regions, including Indonesia, Japan, South Korea, Malaysia, Vietnam and Sudan (http://www.agriculture.gov.au/ag-farm-food/crops/wheat). Thus, wheat is important for gross domestic product and represents a value of AUD 5 billion per year (Lagi et al. 2011).

2.1.2. Wheat biology

Crop plants of the genus *Triticum* are annual with winter and spring types. Australian bread and durum wheats are temperate climate cereals, of the spring type and planted in winter (late April to early July) (Lawes et al. 2016). The endosperm of wheat grain provides energy for the germinating seedling until the first leaf becomes photosynthetically functional (Simmons 1987). Temperature is a major climatic element which influences leaf appearance and extension (Kirby 1983). Stem development in wheat overlaps with the growth of other plant organs (Patrick 1972). Genotype (mainly controlled by the *Reduced height*, *Rht,* genes) and growing conditions determine the height of wheat plants which usually ranges between 30 and 150 cm (Austin and Jones 1975). Vegetative growth of winter wheat is on average 280-350 days, while spring wheat grow in 120-145 days. The shorter vegetative period for spring wheats is due to the absence of vernalisation requirement and warmer temperatures that promote tiller formation and growth, thus maturing the plant faster than winter wheats (Austin and Jones 1975). The beginning of the reproductive stage is marked by the appearance of double ridges on the vegetative apical meristem, the upper of which produces spikelet and its parts. The primary

tiller and other early established tillers complete their development and bear seeds more frequently than tillers that develop in later stages (Kirby 1983).

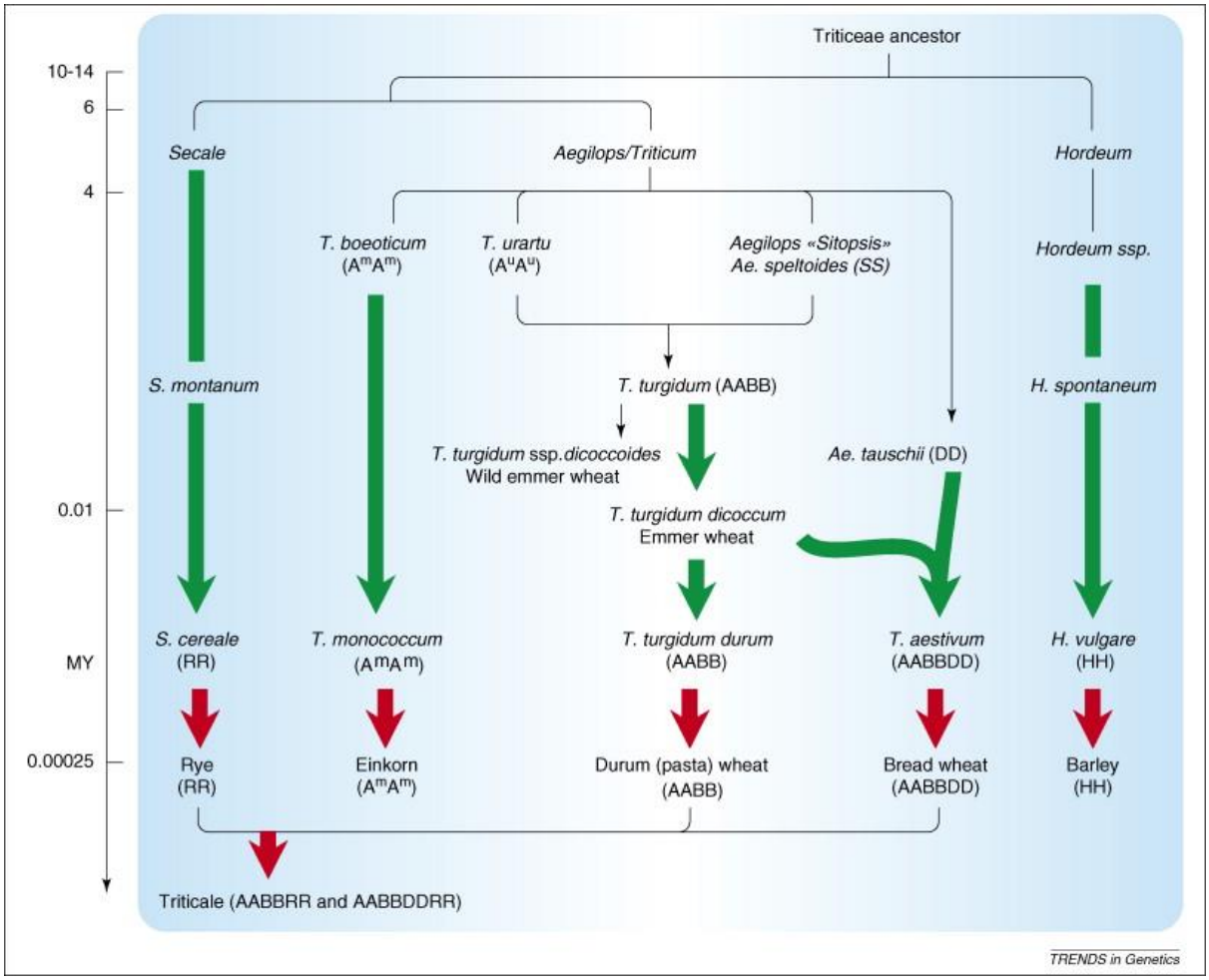

Figure 1. The evolution of the *Triticeae* species from a common ancestor adapted from (Feuillet et al. 2008)

2.1.3. Wheat genome

Bread wheat (*Triticum aestivum* L.) genome is hexaploid, comprising three genome progenitors (A, B and D). The first hybridization occurred between a diploid wheat progenitor closely related to *T. urartu* (genome AA) and the B genome progenitor which closely related to *Aegilops speltoides* (SS) and then eventually gave rise to durum wheat (2n=4x=28, AABB) (Feuillet et al. 2008) (Figure 1). The second hybridisation occurred between the tetraploid (AABB) and *Aegilops tauschii* (DD) (Kimber and Sears 1987). Kimber and Tsunewaki (1988) confirmed the hybridization occurred with the B genome cytoplasm to form the basic hexaploid, AABBDD (Fig 1). Bread wheat genome is large (~17 Gb) (Brenchley et al. 2012;

Consortium 2014) compared to that of human (3,000 Mb), rice (400 Mb) and *Arabidopsis thaliana* (130-140 Mb) (Martínez-Pérez et al. 1999). Due to the polyploid nature of its genome, gene redundancy is common, with at least a triplicate homoeoallelic set for most genes, which complicates genetic analysis (Lee et al. 2017). A locus named *Pairing homoeologous Ph1* prevents the homoeologous chromosomes from pairing, allowing wheat to behave like a diploid at meiosis (Griffiths et al. 2006). Hexaploid wheat contains 42 chromosomes in the 2N state and each of the three different genomes contributes seven chromosomes. The bread wheat genome is also characterized by its complexity, with repetitive sequences accounting for ~ 80% of the genome (Garbus et al. 2015), which complicates wheat genome sequencing even further.

Genetic variability in whet germplasm is an important tool for the breeders as it is the base of selection and progress in genetic gains of the trait of interest. Different authors studied the genetic variability within hexaploid and tetraploid wheat species (Bennett et al. 2012b; Blum et al. 2001; Mason et al. 2013; Moffatt et al. 1990; Onyemaobi et al. 2016a; Shirdelmoghanloo et al. 2016; Tiwari et al. 2013; Wu et al. 2014; Zivy 1987). Due to the limited number of initial hybridizations that gave origin to hexaploid wheat, genetic diversity in bread wheat is reduced when compared with its wild relatives.

2.1.4. Molecular markers resources in wheat

In order to track variation in progenies at the DNA level, breeders use molecular markers. Nowadays, Single Nucleotide Polymorphism (SNP) have become popular as basis for molecular markers because of their genome-wide abundance and amenability for high- to ultra-high-throughput detection platforms (Mammadov et al. 2012). In wheat, marker-assisted selection has been applied for simple traits (Gupta et al. 2010). High-plex genotyping platforms are being implemented to support improvement of polygenic traits.

Recently, a large number of SNP have been characterized in wheat, thanks to the availability of next generation sequencing technologies. A first Illumina iSelect 9K genotyping array of 9,000 gene-associated SNPs was developed for wheat genotyping (Cavanagh et al. 2013), followed by the development of a higher density iSelect 90K array, containing around 90,000 SNPs (Wang et al. 2014). In addition, a high-density Axiom array was created, containing 820,000 SNPs, from which a representative set of 35,000 SNP was selected to develop a "breeders array" (www.cerealsdb.uk.net). Automatic genotyping platforms such as

Kompetitive Allele Specific PCR (KASP™) marker assays have also been used for the selection of several agronomically important loci in wheat (Rasheed et al. 2016). KASP markers for traits of interest such as photoperiod sensitivity (*Ppd-D1*), plant height (*Rht*) and vernalisation requirement (*Vrn*) are available (www.cerealsdb.uk.net). With the reducing costs of sequencing, approaches such as genotyping by sequencing (GBS) (Polland et al. 2012) and population sequencing (POPSEQ) (Mascher et al. 2013, Chapmann et al. 2015) became possible in wheat. It is anchoring and ordering of markers/contig using by parental segregating mapping population. These resources and technologies provide researchers with significant capacity to undertake genetic analysis in wheat and provide opportunities to maximize genetic gains in wheat breeding.

2.1.5. Wheat genome sequence assembly

Wheat genome sequencing data are useful to study genetic variations among wheat germplasm, gene discovery and successful positional cloning to develop tolerance varieties for abiotic and biotic stresses. The International Wheat Genome Sequencing Consortium (IWGSC) (http://www.wheatgenome.org/) have used whole genome shotgun (WGS) sequencing and chromosome based approach to generate valuable genomic resources of wheat.

WGS using next-generation sequencing technologies is a relatively quick method of producing a draft sequence for a whole genome (Adams et al. 2000; Brenchley et al. 2012; Mardis 2008; Metzker 2010). Even though, the complex nature of the wheat genome limit the production of a high-quality *de novo* assembly of WGS sequences (Wicker et al. 2010), important information can be extracted and used as a resource for facilitating genes identification (Brenchley et al. 2012). A successful approach in wheat called Genome Zipper (Mayer et al. 2009) and then assembling those sequences following syntenic relationships with other grasses for gene models.

In addition to the WGS sequencing, the International Wheat Genome Sequencing Consortium aimed to provide a complete high quality wheat genome sequence (Feuillet and Eversole 2007), using a chromosome-based sequencing strategy. This approach required sorting and analysing individual chromosome arms using a flow cytometry (Doležel et al. 2007) to construct bacterial artificial chromosome (BAC) libraries (Šafář et al. 2010). The physical map was constructed from sequenced and aligned adjacent BAC clones and used to assemble a minimum tilling path

(MTP) through various fingerprinting technologies (Luo et al. 2003; Philippe et al. 2012). The MTP information was then used to sequence each of the individual chromosomes (Doležel et al. 2007; Doležel et al. 2012). The first reference sequence (IWGSC RefSeq v1.0, http://wheaturgi.versailles.inra.fr/Seq-Repository/) has been recently released. This is a vital resource for markers development and identification of genes in target loci. Diversity Among Wheat geNome (DAWN), an in-house University of Adelaide wheat genomics platform (Watson-Haigh *et al*., unpublished), is one of the bioinformatics tools used in this project to identify new SNP by using the IWGSC RefSeq v 1.0 and a large WGS dataset available for 16 wheat varieties founders of Australian breeding programs (Edwards et al. 2012).

2.2. Drought tolerance of wheat

2.2.1. Definitions of drought and impact

Drought is the inadequacy of available water in amount and distribution throughout the crop growth period and limit the genetic yield potential of the crop (Ji et al. 2010; Nevo and Chen 2010; Passioura 2007; Swindale and Bidinger 1981). Levitt (972) defined drought tolerance as the ability a plant to survive under water limited conditions by protecting the cellular functions from dehydration and desiccation mostly through physiological mechanisms such as osmotic adjustment, avoidance or tolerance to protein loss, adjustment in photo-respiration rates and other related cellular processes. Turner (1979) defined drought tolerance not only as the ability of a plant to survive, it is the capacity to produce acceptable amount of yield under water stress conditions. Turner emphasised that drought tolerant plants should not only survive under drought but also produce a harvestable yield under water limited conditions. Blum (1996) defined drought tolerance based on plant water relations and plant physiological functions under water stressed conditions, and various traits affecting plant performance under water stress such as plant developmental plasticity and plant phenology (Blum et al. 1999; Blum 1996).

Passioura (1997) pointed out that any definitions related to drought tolerance should focus on its useful meaning in agricultural productivity more than on plant survival mechanisms. Thus, for this study the definition by Turner (1979) has been adapted to: drought tolerance is the ability of a crop to produce economic yield with minimum loss in a water-deficit environment in comparison with a well-watered one. It is the cumulative effect of morphological,

physiological and biochemical tolerance mechanisms, and their interactions. It should also be measured/evaluated with the amount of final grain yield harvested per plant/plot rather than the survival rate of plant tissue.

The significance of drought effects on agricultural productivity varies with its intensity and frequency over locations, years, crop growth seasons, and its interaction with different environmental factors (Tardieu 2011a). Drought effect is more pronounced in Mediterranean type-environments which is characterized with mild wet-cool winter followed by dry summer such as in southern Australia (Specht 1973). In South Australia, drought stress is coupled with a sporadic heat and other abiotic stresses such as subsoil salinity, boron toxicity, nutrient deficiency, strong wind and irradiance and low air humidity (Fleury et al. 2010). The cyclic terminal drought, characterized by sporadic interruptions of rainfall during anthesis and grain filling, can cause total crop loss in Australia in extreme years (Fleury et al. 2010; Izanloo et al. 2008; Reynolds et al. 2007). More than 93% wheat production in Australia is based on dry land agriculture and frequently affected by terminal drought that significantly reduces wheat grain yield (Venkateswarlu et al. 2011). This results in about 3.5 million tonnes annual yield loss in Australia and limits the country's average yield to 1.7 t/ha, which is below the world average minimum yield (Gavran 2012; Ray et al. 2013).

Drought during reproductive and grain-filling phases of wheat (Fig 2) (terminal drought stress) causes reduction in yield (Farooq et al, 2014). This yield reduction is due to accelerated leaf senescence (Yang et al. 2003), oxidative damage to photo-assimilatory machinery (Farooq et al. 2009), reduced rates of carbon fixation and translocation of assimilate (Asada 2006), floret sterility due to pollen infertility (Cattivelli et al. 2008; Dorion et al. 1996), which in turn, reduces grain number and size by limiting its development (Ahmadi and Baker 2001; Nawaz et al. 2013). Reduction in grain size could also be associated with reduced sink capacity (Liang et al. 2001).

The growth stage of the wheat crop at which drought and/or heat occur will determine the yield components (seeds number and seeds weight) that will be affected by the stress. Drought and heat stress during reproductive stage (Zadoks' scale 37 to 69) (Zadoks et al. 1974) significantly affects both seed weight and seed number (Dolferus et al. 2013; Ji et al. 2010; Onyemaobi et al. 2016b; Powell et al. 2012), while the stress during the pre-anthesis stage mainly affect pollen

fertility which have a large influence on grain number and eventually resulting high yield losses (Farooq et al. 2011; Powell et al. 2012).

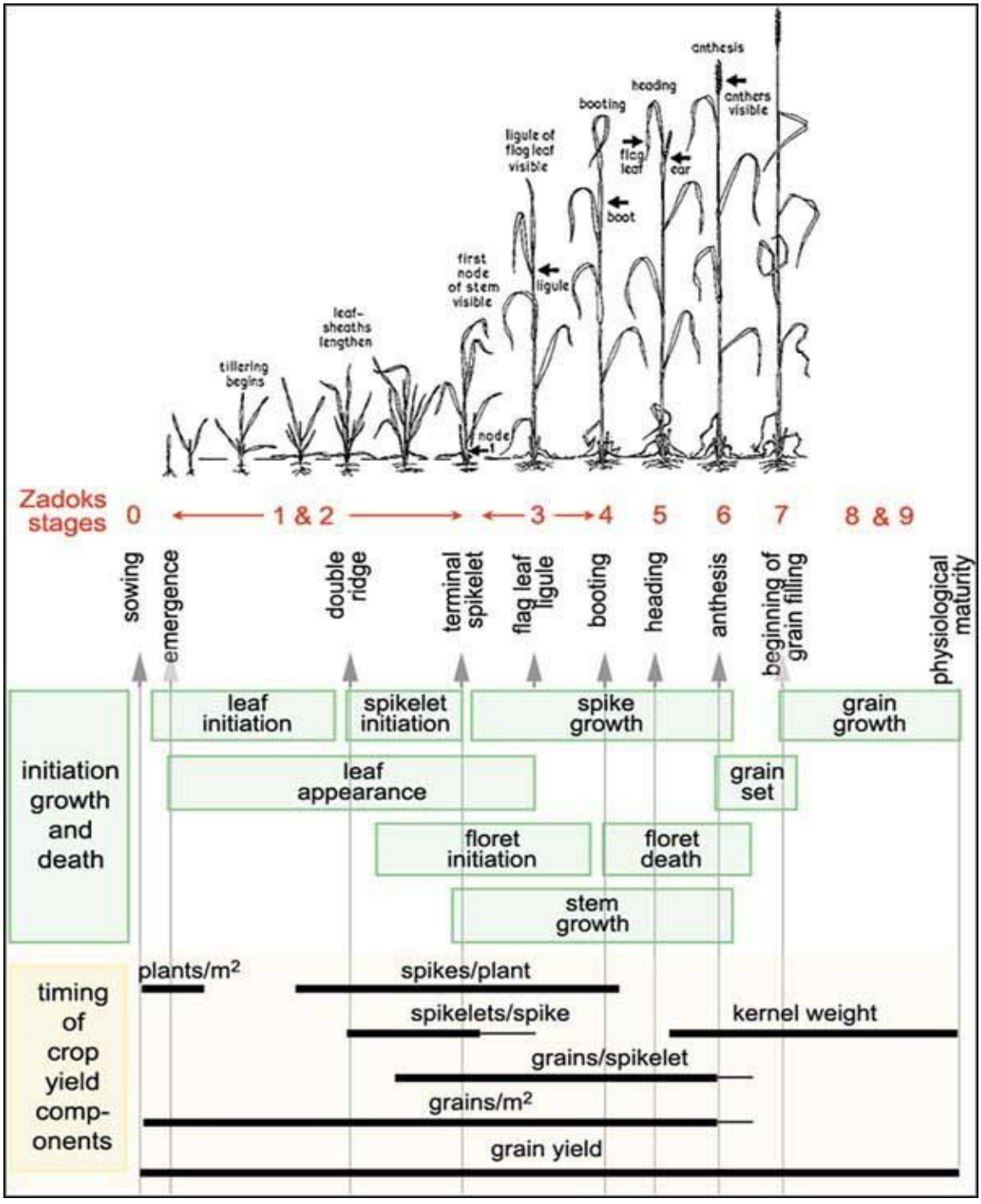

Figure 2. Zadoks stages of wheat and timing of yield components (Rawson and Macpherson 2000).

Drought and heat at the post-anthesis stage mostly affect grain weight (Ji et al. 2010; Pradhan and Prasad 2015; Prasad and Djanaguiraman 2014; Yang and Zhang 2006). Rise in global mean

temperatures due to global warming has a significant impact on crop production (Jones et al. 1999) due to damaging effect of heat on plant development and performance (Bita and Gerats 2013). Heat commonly occurs concomitantly with drought stress reducing overall crop production through negative effects on plant chlorophyll content, leaf photosynthesis, floret fertility, final grain number and weight (Perdomo et al. 2017; Pradhan et al. 2012). The degree of the damages may vary with duration of the stress and specific plant growth stages (Parent and Tardieu 2012). In Australian wheat growing regions, the booting stage coincides with rising temperatures in August-September (Wardlaw and Wrigley 1994) that are frequently above the optimum temperature (15ºC). Most of the wheat crops experience an average of 4 days of severe high temperatures above 35ºC and even on occasions temperatures that can reach 40 ºC. Tricker et al. (2018) has reviewed the effect of drought and heat on wheat growth and development, physiological mechanisms and genetic control of drought and/or heat tolerance as well as the potential physiological traits to overcome the combined effect of drought and heat stress. They concluded that under dry and hot climate it is all about water and heat exacerbates drought. Here below, we focused on the mechanisms of drought tolerance in plants.

2.2.2. Mechanisms of drought stress tolerance

As mentioned above, drought tolerance is a cumulative effect of morphological, physiological and biochemical tolerance mechanisms, and their interactions. Each of the mechanisms is well described below.

2.2.2.1. Drought escape

Plants can escape drought stress by speeding up phenological development, through early flowering and grain filling period. This allows plants to complete their life cycle prior to the onset of drought (Chaves et al. 2003; Turner 1979). There are ample genetic variation among wheat cultivars regarding the response to vernalisation and photoperiod which allows the crop to adapt to a wide range of agro-ecologies (Richards 2000). The three major sets of genes responsible for flowering time in wheat are: *vernalisation* (*Vrn*), *photoperiod* (*Ppd*) and *earliness per se (Eps).*They all have been identified and reported for their confounding effects on yield QTL expression across environments and genetic backgrounds (Pinto et al. 2010; Reynolds and Tuberosa 2008). The *Vrn* genes (Vrn_1 and Vrn_2) are located on chromosomes

group 5 and regulate the crop cycle duration and temperature required to induce flowering (Snape et al. 2001; Worland et al. 1994; YAN et al. 2004). The *Ppd* sensitivity genes (*Ppd-D$_1$*, *Ppd-B$_1$* & *Ppd-A$_1$*), located on the chromosomes group 2, control the timing of flowering and the response to day length (Beales et al. 2007; Worland et al. 1998). Earliness *per se (Eps)* is independent of environmental stimuli to induce flowering and were reported in many cultivars of wheat on chromosome 4A (Worland 1996).

Breeding for early maturing varieties has been a very successful strategy in areas prone to terminal drought stress and where deep-rooted access to water is limited by poor soil quality with physio-chemical properties such as those in Southern Australian (Araus et al. 2002; Parent et al. 2015).

2.2.2.2. Morphological mechanisms

Wheat semi-dwarf varieties carrying *Rht-B1b* (*Rht1*) and *Rht-D1b* (*Rht2*) alleles extensively used in the 1960's Green Revolution have relatively higher yield potential than taller varieties under different growth habits and environmental conditions (Richards 1992). In general, the semi-dwarf genes cause reduction in plant height improving lodging resistance and partitioning of assimilates to the developing grain (Evans 1993). Thus, they produce more grain numbers and overall yields by efficient partitioning of the biomass to grain (Taiz and Zeiger 2006). Reynolds et al. (2017) reported that high expression of biomass (source) and crossing to sources with good expression of sink traits like harvest index and thousand kernel weight lead to genetic gains for both yield and biomass.

In addition to the plant height, some wheat cultivars have modified root systems to increase hydraulic resistance so as to improve water uptake and use efficiency. For example, about 8% yield increment was reported in two Australian wheat cultivars (Cook and Kite) with reduced xylem diameter through backcrossing breeding program in dry environment of north-eastern Australia wheat belt (Richards and Passioura 1989). But, root growth to access ground water has been hindered by shallow top soil, soil structure and toxicity, and complete dryness in the case of Southern Australian regions (Nie et al. 2008).

There are also adaptations in leaf morphology during water deficit such as leaf rolling, folding and wilting (Reynolds et al. 2001), leaf thickness and erect leaf posture (Bogale et al. 2011;

Reynolds et al. 2000), leaf pubescence (Richards 1996a) and glume pubescence (Trethowan et al. 1998). These minimize leaf area exposure to radiation and temperature, which in turn helps to reduce water loss (Ludlow and Muchow 1990; Richards et al. 2002a). It was also reported that there is a possibility to increase wheat yield by 20% under Australian water limited conditions by increasing leaf area index and root length (Condon et al. 2002a; Passioura 2002). But, this will hold true for those areas with sufficient soil stored water and good soil structure for root development.

2.2.2.3. Physiological mechanisms

A number of physiological traits have been identified for drought tolerance that complement each other to maintain the final economic yield under stress conditions, while some may be interacting negatively. Thought, physiological traits have been proposed as selection tools for improving grain yield potential under dry and hot environments (Araus et al. 2002), its practicality in crop breeding is limited. Yield is defined as (plants/unit area) x (mean number of tillers with spikes/plant) x (mean number of grains/spike) x (mean grain weight) (Araus et al. 2003; Slafer 2003). Physiologists argue that one should target each of the subcomponents in the course of yield improvement. In the context of drought stress however, Passioura (1976) define and equate yield under water stress as water-limited YLD = WU x WUE x HI (Fig 4), with WU for water use, WUE for water use efficiency (biomass produced per unit of water used) and HI for harvest index.

Greater WUE is an obvious target for yield improvement in water limited areas. Transpiration efficiency (TE), or intrinsic WUE, is the ratio of instantaneous CO_2 assimilation to transpiration measured at the leaf level and is important in areas where sufficient residual soil moisture is available (Condon et al. 1993b). Since direct measurement of WUE in the field is a difficult task, its value can be determined indirectly by measuring carbon isotope-discrimination (CID). CID has a negative correlation with TE (Condon *et al.*, 1990). A low CID value is associated with lowering the CO_2 concentration in a leaf increasing the TE and then the WUE. It indicates a decrease of the $_{13}C$ discrimination during photosynthesis resulting from effective stomatal conductance (Araus et al. 2002; Araus et al. 2003). CID is a polygenic trait under strong additive genetic control with high value of narrow sense heritability (Rebetzke et al. 2008a; Rebetzke et al. 2006). It is also repeatable across environments and wheat mapping populations. Low Δ has been used as a proxy trait to select high biomass and grain yield in

wheat under targeting moisture stressed environment (Rebetzke et al. 2008a; Rebetzke et al. 2006).The variety Drysdale has been selected for its adaptation to southern New South Wales (NSW), Australia, based on its high WUE and grain yield (23% yield advantage over Diamondbird, the recommended variety in the region) using the CID as a proxy measurement (Richards 2006). This variety has a potential to exploit soil stored water during water stress period to maximize grain yield

Another way of improving WUE of wheat under water limited conditions is increasing leaf area index or crop growth rate. A rapid ground coverage at early stage reduces direct water evaporation from the soil (Passioura 2002). Several studies have been reported on the positive correlations among flag leaf area, WUE and photosynthetic rate, and their importance in wheat yield improvement under water stress environments (Hui et al. 2008; Rebetzke et al. 2002a; Shao et al. 2006; Wellmer et al. 2006). But, improvements in leaf level WUE may not always convert to higher crop yield (Condon et al. 2004a).

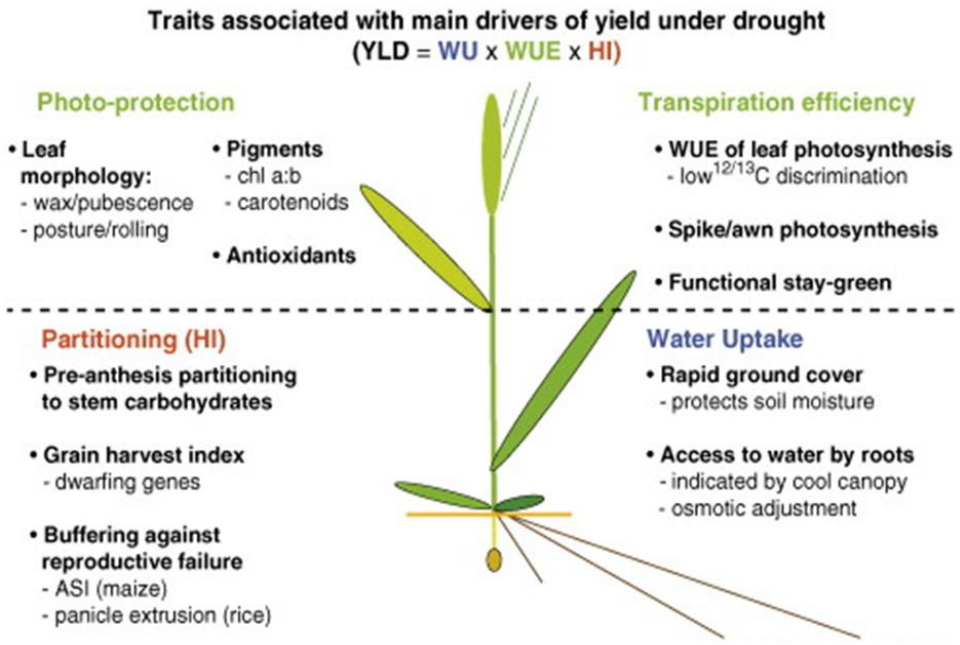

Figure 3. Conceptual model of the major traits defining yield under drought stressed environments as defined by Passioura (1996) which illustrates the interactions among water use (WU), water use efficiency (WUE) and harvest index (HI) and its impact on the final yield and yield components. Source: Reynolds and Tuberosa (2008).

Because, high value of WUE can be obtained from low values of photosynthesis and stomatal conductance, resulting in a low biomass accumulation, it might end in selection of genotypes with reduced grain yield (Blum 2005). Thus, it is important to give attention to the amount of biomass accumulated over time instead of only considering the value of WUE *per se* during selection for high WUE.

Another important morpho-physiological drought tolerance mechanism is the stomatal conductance. Araus et al. (2002) reported that advanced wheat cultivars with high stomatal conductance are correlated with cooler canopies, high photosynthesis rate, and maintain yield in water sufficient areas. In these cultivars, stomata are wide open increasing CO_2 entrance, assimilation and water flow from the soil to replace the transpired water (Reynolds et al. 2007; Saint Pierre et al. 2010a). However, stomatal conductance is markedly lowered to curb excess water loss in soil moisture deficit. Old varieties are characterized by lower stomatal conductance and low photosynthesis rate and yield regardless of soil moisture status (Siddique et al. 1990). An indirect measurement of stomatal conductance consists in measuring the canopy temperature depression (CTD) by using infrared thermometer (Fischer et al. 1998). Araus et al. (2002) pointed out the practical difficulties in measuring the CTD in field trials with low soil moisture, gusty winds and cloudy weather conditions of southern Australia. Shahinnia et al. (2016) suggested that stomatal traits could be an underlying mechanism increasing yield. They reported overlapping QTL for stomatal density and yield, kernel number per spike, and harvest index in RAC875/Kukri wheat population. Thus, stomatal traits might be useful to track specific yield QTL.

Normalized difference vegetative index (NDVI) is a measurement of canopy cover, greenness and biomass accumulation at a specific point of time (Araus et al. 2008). Drought tolerance plants maintain high NDVI values under moisture stress (Olivares-Villegas et al. 2007). Total photosynthesis can be increased by increasing total leaf area and daily duration of photosynthesis (Richards 2000). Increasing crop yield and biomass through extended duration of crop cycle has already been exploited by the breeders using *Vrn* and *Ppd* genes in marker assisted selection (Reynolds et al. 2005; Richards 2000). For example, an increase in wheat grain yield of 0.1 - 0.17 t/ha was reported for every extra day that flag leaf senescence was delayed (Pepler et al. 2005).

Understanding the above morpho-physiological drought tolerance mechanisms and their genetic basis is vital to improve yield and yield stability under drought conditions. It is also important to focus on those traits with high values of heritability and strong correlation with the final yield.

2.2.2.4. Biochemical mechanisms

Drought tolerance in crop plants is associated with various biochemical mechanisms, involving the phytohormone abscisic acid (ABA), which regulates stomatal conductance (Maldonado et al. 1997), and the synthesis of specific proteins (e.g. largely hydrophilic proteins, proteins that function to scavenge oxygen radicals, chaperone proteins, etc.) (Chandrasekar et al. 2000) (Figure 4).

When the cytoplasm starts to loose water, cells accumulate a range of solutes in order to compensate for the changes in osmotic pressure (Pnueli et al. 2002). These osmoprotectants include proline, glutamate, glycine-betaine, mannitol, sorbitol, fructans, polyols, trehalose, sucrose and oligosaccharides (Figure 4).

A number of proteins that accumulate during water loss are implicated in stabilizing and protecting cellular structures, enzymes, membranes and protein complexes during dehydration (Figure 4). These include some members of the late embryogenesis-abundant (O'Leary et al.) supergene family (Ingram and Bartels 1996), and small heat-shock proteins (sHSP) (Wehmeyer and Vierling 2000). sHSP and dehydrins (members of the *LEA*) may also protect cells during the course of dehydration and recovery when plants undergo a process of rehydration. HSPs family response to stress both in signalling and gene activation (Nollen and Morimoto 2002) and regulate over all cellular redox state (Arrigo 1998). They also interact with and regulate other stress response pathway such as the production of osmolytes (Diamant et al. 2001) and antioxidants (Panchuk et al. 2002).

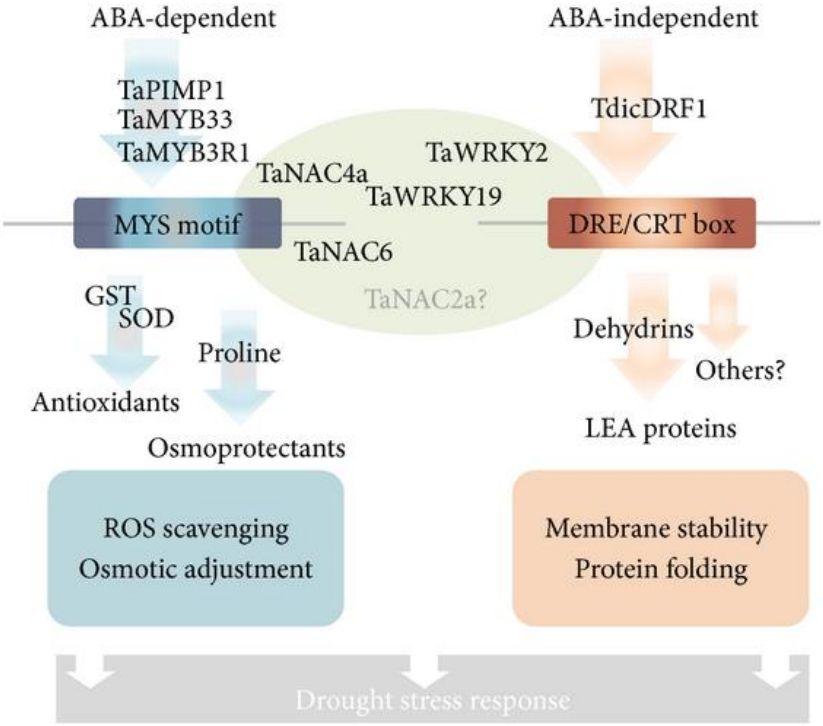

Figure 4. Summary of biochemical mechanisms including genes, proteins and osmoprotectants involved in wheat plant response to drought (Budak et al. 2013).

Various transcription factors and genes are expressed in response to drought in plants including wheat (Budak et al. 2013; Diab et al. 2008; Ghatak et al. 2017; Rahaie et al. 2010; Yoshida et al. 2014). The following genes that conferred drought tolerance in transgenic *Arabidopsis thaliana* have been identified in wheat: *myeloblastosis* genes *(MYB33* and *PIMPM*) encoding for *MY*B transcription factor involved in the ABA-dependent pathway (Qin et al. 2012); *dehydration-responsive element binding* protein (*DREB*) mediating the ABA-independent pathway (Agarwal et al. 2006; Morran et al. 2011) (Fig 5); *WRKY2* and *WRKY19* transcription factors (NIU et al. 2012a); *NAC (NAM/ATAM/CUC)* transcription factor (Tang et al. 2012), frequently reported in literature.

2.3. Breeding drought tolerant wheats

Yield is a quantitative trait under the control of several genes interacting with each other and the environment. Improvement of yield under water stressed environment needs a quantitative method to dissect the trait into heritable principal genetic components/units. Some of the

conventional and molecular/quantitative approaches of yield improvement under drought environment are discussed below.

2.3.1. Conventional breeding approaches and limitations

Many factors hamper the selection for drought tolerance directly in the field. Drought stress responses and overall plant performance under drought stress can be influenced by the sensitivities of specific developmental stages to drought, variation in phenology between genotypes, unpredictability of the timing and intensity of drought stress events in the field, and other uncontrollable environmental factors.

According to Baker (1969), the magnitude of the interaction between genotype and environment variance for yield is considerably larger than the genetic variance in wheat. This makes improvement of yield across different environments difficult. Conventional plant breeding using direct screening for grain yield through multi-location yield trials has been successful leading to high yielding semi-dwarf wheat varieties under optimal and moderately dry environments (Araus et al. 2002; Slafer et al. 2005; Tambussi et al. 2005). However, further yield increase is challenging mainly due to the slow process of yield selection and practical limitations of large plot screening under drought (Quarrie et al. 1999; Richards 2006).

To overcome these problems, Quarrie et al. (1999) pointed out the importance of selecting for other traits that contribute to higher yields and thought to be strongly associated with drought tolerance but less influenced by environmental conditions. Ideally these traits are of high heritability, genetically associated to grain yield under drought, easily measured, identified during early growth stage and have a proven effect in drought tolerance and yield stability (Richards 2006). The presence of sufficient genetic variability and lack of yield penalties under favourable conditions are also desirable features. Limited success has been reported on the use of physiological traits as selection criteria in breeding program. Successful examples are the Australian varieties Drysdale (with 10% yield advantage over standard cultivars) and Rees which have been bred using the carbon isotope discrimination technologies as indirect selection techniques for water use efficiency/transpiration efficiency (Condon et al. 2004a; Passioura 2002; Rebetzke et al. 2002a; Richards et al. 2002a).

2.3.2. Molecular breeding approach using QTL mapping

A quantitative trait locus (QTL) is a specific region on a chromosome that underlies quantitative trait such as yield or yield under drought. QTL are detected by genotype-phenotype association analysis using linkage mapping (Maccaferri et al. 2008; Quarrie et al. 2006b; Salvi and Tuberosa 2005c). A large number of QTL for yield and yield components under drought and/or heat stress have been reported in wheat (Bennett et al. 2012b; Maccaferri et al. 2008; Maphosa et al. 2014; Mir et al. 2012a; Pinto et al. 2010; Quarrie et al. 2005a).

Three mapping populations, 233 Excalibur/Kukri DH lines, 322 RAC875/Kukri DH lines and 242 Drysdale/Gladius RIL, have been developed at the ACPFG, University of Adelaide, by crossing five well characterized Australian wheat cultivars contrasting in drought tolerance (Izanloo et al. 2008) to map and dissect yield QTL under water stressed conditions. QTL for yield and yield components were detected in multi-location trials in Australia and Mexico by Edwards (2012a), Bennett et al. (2012a) and Maphosa et al (2014). QTL for growth and transpiration rates have also been detected in the Drysdale/Gladius population under control and drought conditions using the imaging Lemnatec platform of The Plant Accelerator (TPA) (Parent et al. 2015). In addition, several QTL for yield and yield related traits have been detected elsewhere under various moisture regimes in different mapping populations (Table 1).

Integrating marker-assisted selection methods with conventional breeding can increase the overall genetic gains and breeding efficiency. Some successful stories have been reported in other crops. For example, rice yield under drought conditions could be improved by selecting QTL for root traits, osmotic adjustment, leaf drying and leaf rolling that were coincident with yield QTL using linked markers (Babu et al. 2003; Lanceras et al. 2004). In recent years, on yield QTL pyramiding enabled to develop drought tolerant rice cultivars with a higher yield advantage using molecular marker assisted backcrossing (MABC) (Ahmed et al. 2013; Kumar et al. 2013; Shamsudin et al. 2016; Swamy and Kumar 2013). In maize, introgression of favourable alleles at five target regions using MABC techniques increased yield by 50% under water-limited environments (Ribaut and Ragot 2007). There are also some reports on the coincidence of wheat yield QTL with other traits, for example QTL for flag leaf senescence in winter wheat, but no significant yield gain has been recorded under drought (Tuberosa and Salvi 2006; Verma et al. 2004). More recently, there was a promising report on pyramiding

yield QTL in wheat using marker-assisted recurrent selection under dry environments (Alvarez et al. ; Gahlaut et al. 2017).

The contribution of MABC in developing drought tolerant varieties is hampered by various factors. The QTL interval are often very large which can lead to negative linkage drag with other traits. Cleaning of the introgressed region might became a lengthy and expensive process. As commented above on G x E, QTL for yield under drought are usually unstable across experiments (years and sites) making reproducibility of the results difficult. Lastly, it is difficult to know the epistasis effect and, as a result, backcrossing might fail to create tolerant varieties (Barker et al. 2005; Fleury et al. 2010; Francia et al. 2005; Mir et al. 2012b). Some of these issues would be solved by discovering the gene underlying these QTL by positional cloning.

Table 1 Summary of QTL identified for different traits on chromosome 1B in wheat under drought and/or heat or non-stressed conditions

NO.	Traits	Population	Environment	Reference	Remark
1	Spike number/plant, signle seed weight, seeds/spike, spike length	Drysdale/Gladius RIL	semi controlled field trial (polytunnel), Australia	Parent et al (2017)	Partially co-located with QTL for plant height
2	Growth rate, transpiration rate, leave expansion rate	Drysdale/Gladius RIL	Drought stress in glasshouse, controlled, Australia	Parent et al (2015)	Partially co-located with yield & yield components QTL
3	Grain yield, plant height, days to heading	Rio Blanco (PI 531244)/IDO444 (PI 578278) RIL	Drought stress, rain fed, USA	Zhang et al (2014)	
4	Grain yield, Kernel per spike	RAC875/Kukri DH	rain fed field trial, terminal drought and heat, Australia	Bennett et al (2012a)	Co-located with yield and yield component QTL
5	Spikes per plant, thousand kernel weight, spikelet per spike, fertile spikelet per spike, sterile spikelet per spike, spike length, plant height	Hanxuan 10/Lumai 14 DH	Drought stress and well-watered, control, China	Wu et al (2012)	
6	Thousand kernel weight	HTRI 11712/HTRI 105 $F_{2:3}$ families	Terminal drought, under irrigation and controlled , Germany	Nezhad et al (2012)	
7	Grain yield, TKW, test weight, fertile seeds per head, sterility, osmotic potential	Excalibur/Kukri DH	rain fed field trial, terminal drought and heat, Australia	Edwards, (2012)	Colocated with yield and yield component QTL
8	Grain weight, grain weight spike-1, grain number spike-1, spike m-2, spike weight, spike harvest index, and harvest index	Oste-Gata/Massara-1 RIL	Terminal drought, under irrigation, controlled, Iran	Golabadi et al (2011)	
9	Grain yield, number of grains per ear and chlorophyll a fluorescence	Chinese Spring/SQ1 DH	Water stressed, rainout shelter, controlled, Europe	Czyczyło-Mysza et al (2011)	
10	Agronomic, phonological and physiological traits	Seri M82/Babax RIL	Water and heat stressed , under irrigation, controlled, Mexico	Pinto et al (2010)	
11	Grain number, spike number, grain weight, days to anthesis, harvest index, plant height	Seri M82/Babax F_7 sister line	Water stress, rain fed, controlled , Australia	McIntyre et al (2010)	
12	Grain yield, osmotic potential, chlorophyll content, harvest index, dry matter, days to heading, days to maturity	Durum wheat (Langdon)/Wild emmer wheat (G18-16) RIL	Drought stress, under irrigation, Polytunnel, Israel	Peleg et al (2009)	
13	Grain yield , plant height, days to heading	Crown/Lvevo RIL	Rain-fed and irrigation ,controlled, Europe	Maccaferri et al (2008)	
14	Yield, anthesis and height	Seri M 82/Babax RIL	Water stress, rain fed and irrigation, controlled, Australia	Mathews et al (2008)	
15	Carbon iso-top discrimination, plant height, water soluble carbohydrate	Cranbrook/Halberd,Sunco/Tasman, and CD87/Katepwa DH	Rain fed and irrigation, controlled, Australia	Rebetzke et al (2008b) Rebetzke et al (2008a)	
16	Stem water soluble carbohydrate, thousand kernel weight, grain filling efficiency, remobilization efficiency	Hanxuan10/Lumai14 DH	Drought stress and well-watered,controlled, China	Yang et al (2007)	

2.3.3. Positional cloning of drought tolerance QTL: The way to candidate genes

Positional cloning is a stepwise genomic approach (Figure 5) to narrow down a QTL region to the shortest possible genetic interval by adding new markers and phenotyping recombinant lines. Recombinant inbred lines (RIL) are homozygous lines derived from individuals of an F_2 population by single seed descent method (Figure 6). Large number of RIL populations (more than 1000) are needed for fine mapping. Near isogenic lines (NIL) also enable positional cloning. NIL are defined as pairs of lines that differ for a chromosome segment carrying a locus of interest but are otherwise very similar in their genetic background. The NIL populations can be generated by i) marker-assisted backcross introgression (transferring a QTL allele from donor parent to selected RIL through backcrossing) ii) selecting and selfing RIL heterozygous at the QTL region and iii) advanced backcross QTL analysis (ABQA) (transferring chromosome segment from wild relatives to elite lines coupled with phenotypic selections) (Salvi and Tuberosa 2005a). The second approach using heterozygous RIL (Figure 6) is much easier and quicker (if a RIL population is already available) than the more traditional method of backcrossing (Allard 1960). Thus, NIL are a powerful tool in searching the genes underlying the target QTL (Boopathi 2013).

Since about 10,000 RIL are available at ACPFG for the three mapping populations, only the second method will be used for NIL generation in this study (Figure 6). In QTL-NIL population, the target QTL is considered as a single Mendelian factor strongly linked to the closest marker (Alonso-Blanco and Koornneef 2000).

So far, positional cloning has been successful for QTL for traits under the major gene effect such as disease resistance, quality, dwarfing and maturity traits in wheat (Gupta et al. 2010; Salvi and Tuberosa 2005a). More recently, boron tolerant genes in wheat have been identified (Pallotta et al. 2014). Currently this approach is more successful in cloning genes underlying qualitative traits such as disease, phenology and quality traits in barley and other fully sequenced species (Wang et al. 2012b).

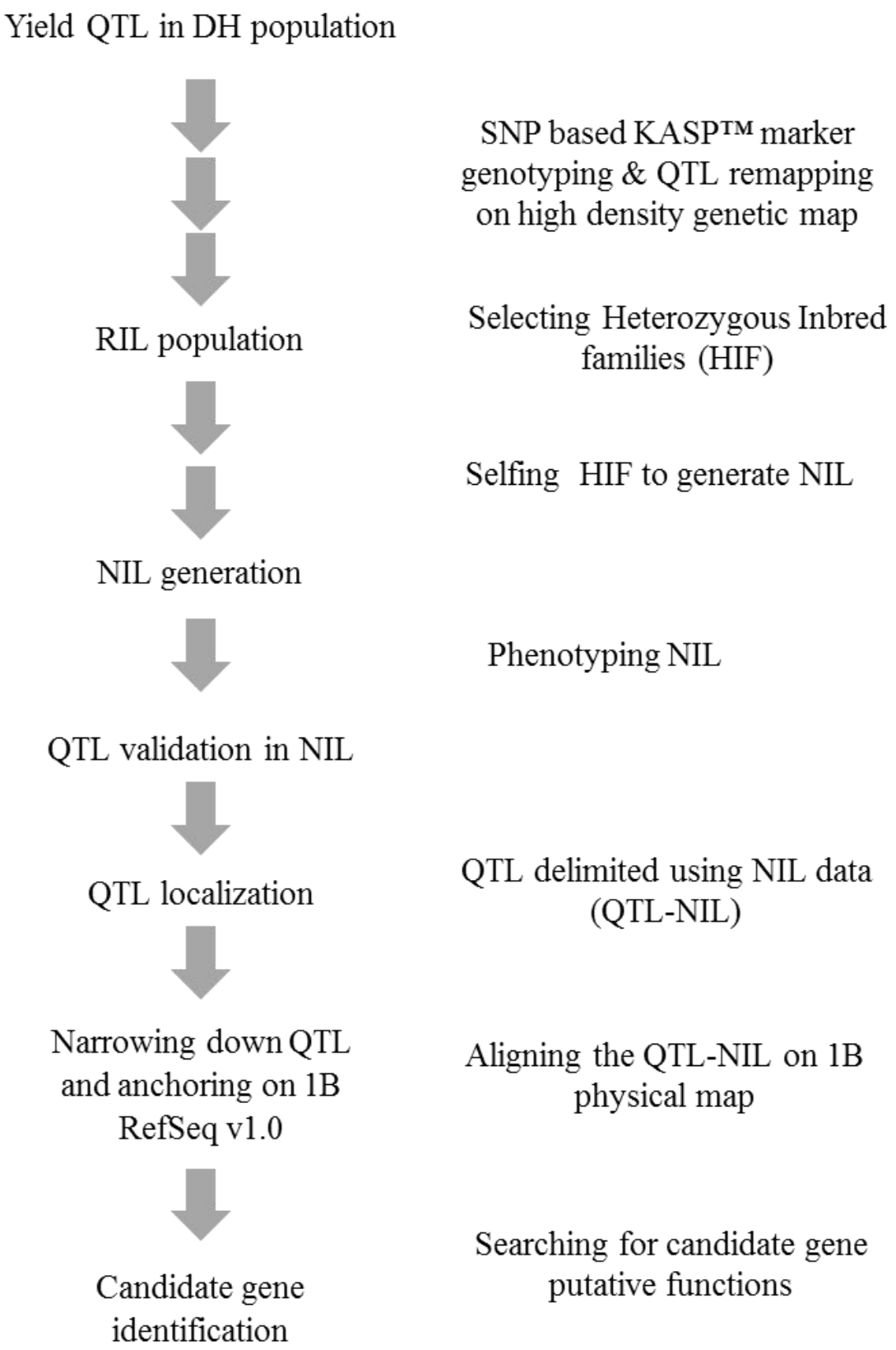

Figure 5. Major steps in positional cloning to be followed modified from Salvi and Tuberosa (2005b)

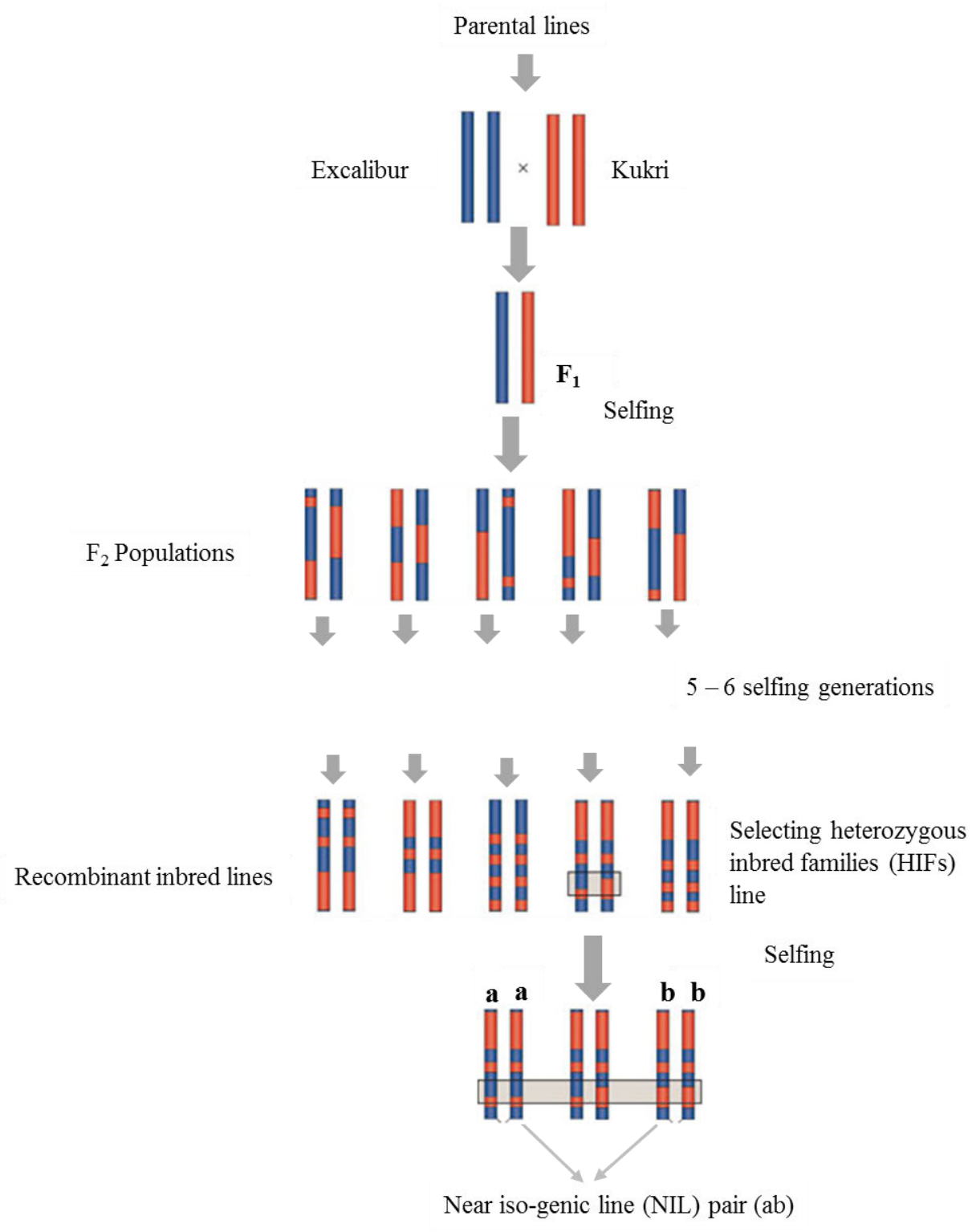

Figure 6. Development of RIL from parental lines and NIL from heterozygous RIL.

2.4. Aims of the research

QTL for yield and yield components were previously detected on the chromosome 1B under rain-fed conditions in Australia and Mexico in Excalibur/Kukri (Edwards et al., 2012) and RAC875/Kukri populations (Bennett et al. 2012b). QTL for plant growth and transpiration rate were also found on chromosome 1B in Drysdale/Gladius population in glasshouse conditions (using an imaging platform of The Plant Accelerator). These loci coincided with QTL for spike number/plant in semi-controlled conditions (Parent et al. (2015), but not in a field study on the same population by Maphosa et al. (2014). Preliminary comparison of the QTL across the three mapping populations showed some common markers in the QTL intervals. Based on this, we raised the following questions:

1. Did the genetic variation explained by QTL for growth and transpiration rate at early growth stage in wheat translate to final grain yield under terminal drought and heat stress?
2. What was the environmental conditions that triggered the expression of the QTL for yield and yield components in Drysdale/Gladius?
3. Are the QTL across the three mapping populations collocated?
4. Could we identify candidate genes underlying the 1B yield QTL?

To address these questions, this PhD work had the following objectives:

1. Find out if chromosome 1B carries QTL for yield or yield components in Drysdale/Gladius population in trials under well-watered and stressed conditions
2. Validate and fine map the 1B yield QTL in Excalibur/Kukri DH using NIL
3. Anchor the 1B QTL for yield, yield components and physiological traits on chromosome 1B Chinese Spring reference sequence
4. Identify candidate genes underlying yield QTL

2.5. References

Adams MD, Celniker SE, Holt RA, Evans CA, Gocayne JD, Amanatides PG, Scherer SE, Li PW, Hoskins RA, Galle RF (2000) The genome sequence of *Drosophila melanogaster*. Science 287:2185-2195

Adjemian M, Janzen JP (2014) USDA Economic Research Service-Wheat Prices Driven By Supply and Demand, Not Speculators

Agarwal PK, Agarwal P, Reddy M, Sopory SK (2006) Role of DREB transcription factors in abiotic and biotic stress tolerance in plants. Plant Cell Reports 25:1263-1274

Ahmadi A, Baker D (2001) The effect of water stress on grain filling processes in wheat. The Journal of Agricultural Science 136:257-269

Ahmed HU, Henry A, Mauleon R, Dixit S, Vikram P, Tilatto R, Verulkar SB, Perraju P, Mandal NP, Variar M (2013) Genetic, physiological, and gene expression analyses reveal that multiple QTL enhance yield of rice mega-variety IR64 under drought. PLoS One 8:e62795

Alexandratos N, Bruinsma J (2012) World agriculture towards 2030/2050: the 2012 revision. ESA Working paper Rome, FAO

Allard W (1960) Principles of Plant Breeding (John Willey and Sons. Inc. London)

Alonso-Blanco C, Koornneef M (2000) Naturally occurring variation in Arabidopsis: an underexploited resource for plant genetics. Trends in plant0 science 5:22-29

Alvarez S, Marsh EL, Schroeder SG, Schachtman DP (2008) Metabolomic and proteomic changes in the xylem sap of maize under drought. Plant, Cell & Environment 31:325-340

Araus J, Slafer G, Reynolds M, Royo C (2002) Plant breeding and drought in C3 cereals: what should we breed for? Annals of botany 89:925-940

Araus J, Villegas D, Aparicio N, Del Moral L, El Hani S, Rharrabti Y, Ferrio J, Royo C (2003) Environmental factors determining carbon isotope discrimination and yield in durum wheat under Mediterranean conditions. Crop Science 43:170-180

Araus JL, Slafer GA, Royo C, Serret MD (2008) Breeding for yield potential and stress adaptation in cereals. Critical Reviews in Plant Science 27:377-412

Arrigo A (1998) Small stress proteins: chaperones that act as regulators of intracellular redox state and programmed cell death. Biological Chemistry 379:19-26

Asada K (2006) Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol 141:391-396

Austin R, Jones H (1975) The physiology of wheat. Part III. Plant Breeding Institute Annual Report 1974:20-71

Babu RC, Nguyen BD, Chamarerk V, Shanmugasundaram P, Chezhian P, Jeyaprakash P, Ganesh S, Palchamy A, Sadasivam S, Sarkarung S (2003) Genetic analysis of drought resistance in rice by molecular markers. Crop Science 43:1457-1469

Baker R (1969) Genotype-environment interactions in yield of wheat. Canadian Journal of Plant Science 49:743-751

Barker T, Campos H, Cooper M, Dolan D, Edmeades G, Habben J, Schussler J, Wright D, Zinselmeier C (2005) Improving drought tolerance in maize. Plant Breed Rev 25:173-253

Beales J, Turner A, Griffiths S, Snape JW, Laurie DA (2007) A pseudo-response regulator is misexpressed in the photoperiod insensitive Ppd-D1a mutant of wheat (Triticum aestivum L.). Theoretical and Applied Genetics 115:721-733

Bennett D, Izanloo A, Reynolds M, Kuchel H, Langridge P, Schnurbusch T (2012a) Genetic dissection of grain yield and physical grain quality in bread wheat (Triticum aestivum L.) under water-limited environments. Theoretical and Applied Genetics 125:255-271

Bennett D, Reynolds M, Mullan D, Izanloo A, Kuchel H, Langridge P, Schnurbusch T (2012b) Detection of two major grain yield QTL in bread wheat (Triticum aestivum L.) under heat, drought and high yield potential environments. Theoretical and Applied Genetics 125:1473-1485

Blum A (2005) Drought resistance, water-use efficiency, and yield potential—are they compatible, dissonant, or mutually exclusive? Crop and Pasture Science 56:1159-1168

Blum A, Klueva N, Nguyen H (2001) Wheat cellular thermotolerance is related to yield under heat stress. Euphytica 117:117-123

Blum A, Zhang J, Nguyen H (1999) Consistent differences among wheat cultivars in osmotic adjustment and their relationship to plant production. Field Crops Research 64:287-291

Blum U (1996) Allelopathic interactions involving phenolic acids. Journal of Nematology 28:259

Bogale A, Tesfaye K, Geleto T (2011) Morphological and physiological attributes associated to drought tolerance of Ethiopian durum wheat genotypes under water deficit condition. Journal of Biodiversity and Environmental Sciences 1:22-36

Bohnert HJ, Sheveleva E (1998) Plant stress adaptations—making metabolism move. Curr Opin Plant Biol 1:267-274

Boopathi NM (2013) Mapping Population Development. Genetic Mapping and Marker Assisted Selection. Springer, pp 23-37

Brenchley R, Spannagl M, Pfeifer M, Barker GL, D'Amore R, Allen AM, McKenzie N, Kramer M, Kerhornou A, Bolser D (2012) Analysis of the bread wheat genome using whole-genome shotgun sequencing. Nature 491:705-710

Budak H, Kantar M, Yucebilgili Kurtoglu K (2013) Drought tolerance in modern and wild wheat. The Scientific World Journal 2013

Cattivelli L, Rizza F, Badeck F-W, Mazzucotelli E, Mastrangelo AM, Francia E, Marè C, Tondelli A, Stanca AM (2008) Drought tolerance improvement in crop plants: an integrated view from breeding to genomics. Field Crops Res 105:1-14

Cavanagh CR, Chao S, Wang S, Huang BE, Stephen S, Kiani S, Forrest K, Saintenac C, Brown-Guedira GL, Akhunova A (2013) Genome-wide comparative diversity uncovers multiple targets of selection for

improvement in hexaploid wheat landraces and cultivars. Proceedings of the national academy of sciences 110:8057-8062

Chandrasekar V, K Sairam R, Srivastava G (2000) Physiological and biochemical responses of hexaploid and tetraploid wheat to drought stress. Journal of Agronomy and Crop Science 185:219-227

Chaves MM, Maroco JP, Pereira JS (2003) Understanding plant responses to drought—from genes to the whole plant. Functional plant biology 30:239-264

Condon A, Richards R, Farquhar G (1993) Relationships between carbon isotope discrimination, water use efficiency and transpiration efficiency for dryland wheat. Crop and Pasture Science 44:1693-1711

Condon A, Richards R, Rebetzke G, Farquhar G (2002) Improving intrinsic water-use efficiency and crop yield. Crop Science 42:122-131

Condon A, Richards R, Rebetzke G, Farquhar G (2004) Breeding for high water-use efficiency. Journal of Experimental Botany 55:2447-2460

Consortium IWGS (2014) A chromosome-based draft sequence of the hexaploid bread wheat (Triticum aestivum) genome. Science 345:1251788

Diab AA, Kantety R, Ozturk N, Benscher D, Nachit M, Sorrells M (2008) Drought-inducible genes and differentially expressed sequence tags associated with components of drought tolerance in durum wheat

Diamant S, Eliahu N, Rosenthal D, Goloubinoff P (2001) Chemical chaperones regulate molecular chaperones in vitro and in cells under combined salt and heat stresses. Journal of Biological Chemistry 276:39586-39591

Doležel J, Kubaláková M, Paux E, Bartoš J, Feuillet C (2007) Chromosome-based genomics in the cereals. Chromosome Research 15:51-66

Doležel J, Vrána J, Šafář J, Bartoš J, Kubaláková M, Šimková H (2012) Chromosomes in the flow to simplify genome analysis. Functional & integrative genomics 12:397-416

Dolferus R, Powell N, Xuemei J, Ravash R, Edlington J, Oliver S, Van Dongen J, Shiran B (2013) The physiology of reproductive-stage abiotic stress tolerance in cereals. Molecular Stress Physiology of Plants. Springer, pp 193-216

Dorion S, Lalonde S, Saini HS (1996) Induction of male sterility in wheat by meiotic-stage water deficit is preceded by a decline in invertase activity and changes in carbohydrate metabolism in anthers. Plant Physiol 111:137-145

Duveiller E, Singh RP, Nicol JM (2007) The challenges of maintaining wheat productivity: pests, diseases, and potential epidemics. Euphytica 157:417-430

Edwards J (2012) A genetic analysis of drought related traits in hexaploid wheat. PhD thesis. University of Adelaide, Adelaide, Australia

Evans L (1993) Crop evolution, adaptation, and yield. Cambridge Univ. Press, Cambridge, UK. Crop evolution, adaptation, and yield Cambridge Univ Press, Cambridge, UK:-

FAOSTAT D (2013a) Food and agriculture organization of the United Nations. Statistical database.

FAOSTAT F (2013b) Food and agriculture organization of the United Nations. Statistical database.

Farooq M, Bramley H, Palta JA, Siddique KHM (2011) Heat stress in wheat during reproductive and grain-filling phases. Critical Reviews in Plant Sciences 30:491-507

Farooq M, Hussain M, Siddique KH (2014) Drought stress in wheat during flowering and grain-filling periods. Critical Review in Plant Sciences 33:331-349

Farooq M, Wahid A, Kobayashi N, Fujita D, Basra S (2009) Plant drought stress: effects, mechanisms and management. Agronomy for sustainable development 29:185-212

Feuillet C, Eversole K (2007) Physical mapping of the wheat genome: a coordinated effort to lay the foundation for genome sequencing and develop tools for breeders. Israel Journal of Plant Sciences 55:307-313

Feuillet C, Langridge P, Waugh R (2008) Cereal breeding takes a walk on the wild side. Trends in Genetics 24:24-32

Figlan S, Baloyi T, Hlongoane T, Terefe T, Shimelis H, Tsilo T (2017) Adult plant resistance of selected Kenyan wheat cultivars to leaf rust and stem rust diseases. Cereal Research Communications 45:68-82

Fischer R, Maurer R (1978) Drought resistance in spring wheat cultivars. I. Grain yield responses. Australia Journal of Agricultural Research 29:897-912

Fischer R, Rees D, Sayre K, Lu Z-M, Condon A, Saavedra AL (1998) Wheat yield progress associated with higher stomatal conductance and photosynthetic rate, and cooler canopies. Crop Science 38:1467-1475

Fleury D, Jefferies S, Kuchel H, Langridge P (2010) Genetic and genomic tools to improve drought tolerance in wheat. Journal of Experimental Botany 61:3211-3222

Francia E, Tacconi G, Crosatti C, Barabaschi D, Bulgarelli D, Dall'Aglio E, Valè G (2005) Marker assisted selection in crop plants. Plant Cell, Tissue and Organ Culture 82:317-342

Gahlaut V, Jaiswal V, Tyagi BS, Singh G, Sareen S, Balyan HS, Gupta PK (2017) QTL mapping for nine drought-responsive agronomic traits in bread wheat under irrigated and rain-fed environments. PloS one 12:e0182857

Garbus I, Romero JR, Valarik M, Vanžurová H, Karafiátová M, Cáccamo M, Doležel J, Tranquilli G, Helguera M, Echenique V (2015) Characterization of repetitive DNA landscape in wheat homeologous group 4 chromosomes. BMC Genomics 16:375

Gavran M (2012) Australian plantation statistics 2012 update. ABARES

Ghatak A, Chaturvedi P, Weckwerth W (2017) Cereal crop proteomics: Systemic analysis of crop drought stress responses towards marker-assisted selection breeding. Frontiers in Plant Science 8

Gupta P, Langridge P, Mir R (2010) Marker-assisted wheat breeding: present status and future possibilities. Molecular Breeding 26:145-161

Hoekstra FA, Golovina EA, Buitink J (2001) Mechanisms of plant desiccation tolerance. Trends Plant Sci 6:431-438

Houshyar E, Grundmann P (2017) Environmental impacts of energy use in wheat tillage systems: A comparative life cycle assessment (LCA) study in Iran. Energy 122:11-24

Hui Z, ZhengBin Z, HongBo S, Ping X, Foulkes M (2008) Genetic correlation and path analysis of transpiration efficiency for wheat flag leaves. Environmental and experimental botany 64:128-134

Ingram J, Bartels D (1996) The molecular basis of dehydration tolerance in plants. Annual Review of Plant Biology 47:377-403

Izanloo A, Condon AG, Langridge P, Tester M, Schnurbusch T (2008) Different mechanisms of adaptation to cyclic water stress in two South Australian bread wheat cultivars. Journal of Experimental Botany 59:3327-3346

Ji X, Shiran B, Wan J, Lewis DC, Jenkins CL, Condon AG, Richards RA, Dolferus R (2010) Importance of pre- anthesis anther sink strength for maintenance of grain number during reproductive stage water stress in wheat. Plant, Cell & Environment 33:926-942

Jiafeng J, Xin H, Ling L, Jiangang L, Hanliang S, Qilai X, Renhong Y, Yuanhua D (2014) Effect of cold plasma treatment on seed germination and growth of wheat. Plasma Science and Technology 16:54

Khan MM, Khan N (2017) Effect of Water Logging and Salinity Stress on Physiological and Biochemical Changes in Tolerant and Susceptible Varieties of *Triticum aestivum* L. Intrnational Journal Current Microbiology Applied Science 6:975-981

Kimber Gv, Sears E (1987) Evolution in the genus Triticum and the origin of cultivated wheat. Wheat and wheat improvement 2:154-164

Kirby SH (1983) Rheology of the lithosphere. Reviews of Geophysics 21:1458-1487

Kumar A, Singh ON, Mandal NP, Dwivedi JL, Variar M, Kikuchi S, Satoh K, Mauleon R, Dixit S, Leung H (2013) Genetic, physiological, and gene expression analyses reveal that multiple QTL enhance yield of rice mega-variety IR64 under drought

Lagi M, Bar-Yam Y, Bertrand KZ, Bar-Yam Y (2011) The food crises: A quantitative model of food prices including speculators and ethanol conversion

Lagudah ES, Dubcovsky J, Powell W (2001) Wheat genomics. Plant Physiology and Biochemistry 39:335-344

Lanceras JC, Pantuwan G, Jongdee B, Toojinda T (2004) Quantitative trait loci associated with drought tolerance at reproductive stage in rice. Plant Physiology 135:384-399

Lawes R, Huth N, Hochman Z (2016) Commercially available wheat cultivars are broadly adapted to location and time of sowing in Australia's grain zone. European Journal of Agronomy 77:38-46

Lee T, Hwang S, Kim CY, Shim H, Kim H, Ronald PC, Marcotte EM, Lee I (2017) WheatNet: A genome-scale functional network for hexaploid bread wheat, Triticum aestivum. Molecular Plant

Levitt J (972) Responses of plants to encironmental stresses. cademic Press, New York, N.Y

Liang J, Zhang J, Cao X (2001) Grain sink strength may be related to the poor grain filling of indica-japonica rice (Oryza sativa) hybrids. Physiology Plant 112:470-477

Loudet O, Chaillou S, Camilleri C, Bouchez D, Daniel-Vedele F (2002) Bay-0× Shahdara recombinant inbred line population: a powerful tool for the genetic dissection of complex traits in Arabidopsis. TAG Theoretical and Applied Genetics 104:1173-1184

Ludlow M, Muchow R (1990) A critical evaluation of traits for improving crop yields in water-limited environments. Advances in agronomy 43:107-153

Luo M-C, Thomas C, You FM, Hsiao J, Ouyang S, Buell CR, Malandro M, McGuire PE, Anderson OD, Dvorak J (2003) High-throughput fingerprinting of bacterial artificial chromosomes using the snapshot labeling kit and sizing of restriction fragments by capillary electrophoresis. Genomics 82:378-389

Lutts S, Almansouri M, Kinet J-M (2004) Salinity and water stress have contrasting effects on the relationship between growth and cell viability during and after stress exposure in durum wheat callus. Plant Science 167:9-18

Maccaferri M, Sanguineti MC, Corneti S, Ortega JLA, Salem MB, Bort J, DeAmbrogio E, del Moral LFG, Demontis A, El-Ahmed A (2008) Quantitative trait loci for grain yield and adaptation of durum wheat (Triticum durum Desf.) across a wide range of water availability. Genetics 178:489-511

Maldonado CA, Zuñiga GE, Corcuera LJ, Alberdi M (1997) Effect of water stress on frost resistance of oat leaves. Environment Expiremental Botany 38:99-107

Mammadov J, Aggarwal R, Buyyarapu R, Kumpatla S (2012) SNP markers and their impact on plant breeding. International Journal of Plant Genomics 2012

Maphosa L, Langridge P, Taylor H, Parent B, Emebiri LC, Kuchel H, Reynolds MP, Chalmers KJ, Okada A, Edwards J (2014) Genetic control of grain yield and grain physical characteristics in a bread wheat population grown under a range of environmental conditions. Theoretical and Applied Genetics 127:1607-1624

Mardis ER (2008) Next-generation DNA sequencing methods. Annual Review Genomics Hum Genet 9:387-402

Martínez-Pérez E, Shaw P, Reader S, Aragón-Alcaide L, Miller T, Moore G (1999) Homologous chromosome pairing in wheat. Journal of Cell Science 112:1761-1769

Mason RE, Hays DB, Mondal S, Ibrahim AM, Basnet BR (2013) QTL for yield, yield components and canopy temperature depression in wheat under late sown field conditions. Euphytica 194:243-259

Metzker ML (2010) Sequencing technologies—the next generation. Nature Reviews Genetics 11:31-46

Mir R, Kumar N, Jaiswal V, Girdharwal N, Prasad M, Balyan H, Gupta P (2012a) Genetic dissection of grain weight in bread wheat through quantitative trait locus interval and association mapping. Molecular Breeding 29:963-972

Mir RR, Zaman-Allah M, Sreenivasulu N, Trethowan R, Varshney RK (2012b) Integrated genomics, physiology and breeding approaches for improving drought tolerance in crops. Theoretical and Applied Genetics 125:625-645

Moffatt J, Sears R, Paulsen G (1990) Wheat high temperature tolerance during reproductive growth. I. Evaluation by chlorophyll fluorescence. Crop Science 30:881-885

Murray GM, Brennan JP (2009) Estimating disease losses to the Australian wheat industry. Australasian Plant Pathology 38:558-570

Nawaz F, Ashraf MY, Ahmad R, Waraich EA (2013) Selenium (Se) seed priming induced growth and biochemical changes in wheat under water deficit conditions. Biological Trace Elements Research 151:284-293

Nevo E, Chen G (2010) Drought and salt tolerances in wild relatives for wheat and barley improvement. Plant, Cell & Environment 33:670-685

Nie Z, Miller S, Moore G, Hackney B, Boschma S, Reed K, Mitchell M, Albertsen T, Clark S, Craig A (2008) Field evaluation of perennial grasses and herbs in southern Australia. 2. Persistence, root characteristics and summer activity. Australian Journal of Experimental Agriculture 48:424-435

NIU CF, Wei W, ZHOU QY, TIAN AG, HAO YJ, ZHANG WK, Ma B, Lin Q, ZHANG ZB, ZHANG JS (2012) Wheat WRKY genes TaWRKY2 and TaWRKY19 regulate abiotic stress tolerance in transgenic Arabidopsis plants. Plant,Cell & Environment 35:1156-1170

Nollen EA, Morimoto RI (2002) Chaperoning signaling pathways: molecular chaperones as stress-sensingheat shock'proteins. Journal of Cell Science 115:2809-2816

Ohki K (1984) Manganese deficiency and toxicity effects on growth, development, and nutrient composition in wheat. Agronomy Journal 76:213-218

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

Onyemaobi I, Liu H, Siddique KH, Yan G (2016a) Both male and female malfunction contributes to yield reduction under water stress during meiosis in bread wheat. Frontiers in Plant Science 7

Onyemaobi I, Liu H, Siddique KH, Yan G (2016b) Both male and female malfunction contributes to yield reduction under water stress during meiosis in bread wheat. Frontiers in Plant Science 7:2071

Pallotta M, Schnurbusch T, Hayes J, Hay A, Baumann U, Paull J, Langridge P, Sutton T (2014) Molecular basis of adaptation to high soil boron in wheat landraces and elite cultivars. Nature 514:88-91

Panchuk II, Volkov RA, Schöffl F (2002) Heat stress-and heat shock transcription factor-dependent expression and activity of ascorbate peroxidase in Arabidopsis. Plant Physiology 129:838-853

Parent B, Shahinnia F, Maphosa L, Berger B, Rabie H, Chalmers K, Kovalchuk A, Langridge P, Fleury D (2015) Combining field performance with controlled environment plant imaging to identify the genetic control of growth and transpiration underlying yield response to water-deficit stress in wheat. Journal of Experimental Botany 66:5481-5492

Páscoa P, Gouveia C, Russo A, Trigo R (2017) The role of drought on wheat yield interannual variability in the Iberian Peninsula from 1929 to 2012. International Journal of Biometeorology 61:439-451

Passioura J (1997) Drought and drought tolerance. Drought tolerance in higher plants: genetical, physiological and molecular biological analysis.Balhassen, E. (Ed.), Springer, pp 1-5

Passioura J (2002) Soil conditions and plant growth. Plant, cell & environment 25:311-318

Passioura J (2007) The drought environment: physical, biological and agricultural perspectives. Journal of Experimental Botany 58:113-117

Patrick J (1972) Distribution of assimilate during stem elongation in wheat. Australian Journal of Biological Sciences 25:455-468

Pepler S, Gooding M, Ford K, Ellis R (2005) A temporal limit to the association between flag leaf life extension by fungicides and wheat yields. European Journal of Agronomy 22:363-373

Philippe R, Choulet F, Paux E, van Oeveren J, Tang J, Wittenberg AH, Janssen A, van Eijk MJ, Stormo K, Alberti A (2012) Whole Genome Profiling provides a robust framework for physical mapping and sequencing in the highly complex and repetitive wheat genome. BMC genomics 13:47

Pinto RS, Reynolds MP, Mathews KL, McIntyre CL, Olivares-Villegas J-J, Chapman SC (2010) Heat and drought adaptive QTL in a wheat population designed to minimize confounding agronomic effects. Theoretical and Applied Genetics 121:1001-1021

Pnueli L, Hallak- Herr E, Rozenberg M, Cohen M, Goloubinoff P, Kaplan A, Mittler R (2002) Molecular and biochemical mechanisms associated with dormancy and drought tolerance in the desert legume Retama raetam. The Plant Journal 31:319-330

Powell N, Ji X, Ravash R, Edlington J, Dolferus R (2012) Yield stability for cereals in a changing climate. Functional Plant Biology 39:539-552

Pradhan GP, Prasad PVV (2015) Evaluation of wheat chromosome translocation lines for high temperature stress tolerance at grain filling stage. PloS one 10:e0116620

Prasad PVV, Djanaguiraman M (2014) Response of floret fertility and individual grain weight of wheat to high temperature stress: sensitive stages and thresholds for temperature and duration. Functional Plant Biology 41:1261-1269

Qin Y, Wang M, Tian Y, He W, Han L, Xia G (2012) Over-expression of TaMYB33 encoding a novel wheat MYB transcription factor increases salt and drought tolerance in Arabidopsis. Molecular biology reports 39:7183-7192

Quarrie S, Quarrie SP, Radosevic R, Rancic D, Kaminska A, Barnes J, Leverington M, Ceoloni C, Dodig D (2006) Dissecting a wheat QTL for yield present in a range of environments: from the QTL to candidate genes. Journal of Experimental Botany 57:2627-2637

Quarrie S, Steed A, Calestani C, Semikhodskii A, Lebreton C, Chinoy C, Steele N, Pljevljakusić D, Waterman E, Weyen J (2005) A high-density genetic map of hexaploid wheat (Triticum aestivum L.) from the cross Chinese Spring× SQ1 and its use to compare QTLs for grain yield across a range of environments. Theoretical and Applied Genetics 110:865-880

Quarrie SA, Stojanović J, Pekić S (1999) Improving drought resistance in small-grained cereals: A case study, progress and prospects. Plant Growth Regulation 29:1-21

Rahaie M, Xue G-P, Naghavi MR, Alizadeh H, Schenk PM (2010) A MYB gene from wheat (Triticum aestivum L.) is up-regulated during salt and drought stresses and differentially regulated between salt-tolerant and sensitive genotypes. Plant Cell Reports 29:835-844

Rasheed A, Wen W, Gao F, Zhai S, Jin H, Liu J, Guo Q, Zhang Y, Dreisigacker S, Xia X (2016) Development and validation of KASP assays for genes underpinning key economic traits in bread wheat. Theoretical and Applied Genetics 129:1843-1860

Ray DK, Mueller ND, West PC, Foley JA (2013) Yield trends are insufficient to double global crop production by 2050. PLoS One 8:e66428

Rebetzke G, Condon A, Farquhar G, Appels R, Richards R (2008) Quantitative trait loci for carbon isotope discrimination are repeatable across environments and wheat mapping populations. Theoretical and Applied Genetics 118:123-137

Rebetzke G, Condon A, Richards R, Farquhar G (2002) Selection for reduced carbon isotope discrimination increases aerial biomass and grain yield of rainfed bread wheat. Crop Science 42:739-745

Rebetzke G, Richards R, Condon A, Farquhar G (2006) Inheritance of carbon isotope discrimination in bread wheat (Triticum aestivum L.). Euphytica 150:97-106

Reynolds JF, Bugmann H, Pitelka LF (2001) How much physiology is needed in forest gap models for simulating long-term vegetation response to global change? Challenges, limitations, and potentials. Climatic Change 51:541-557

Reynolds M, Dreccer F, Trethowan R (2007) Drought-adaptive traits derived from wheat wild relatives and landraces. Journal of Experimental Botany 58:177-186

Reynolds M, MUJEEB- KAZI A, Sawkins M (2005) Prospects for utilising plant- adaptive mechanisms to improve wheat and other crops in drought- and salinity- prone environments. Annals of Applied Biology 146:239-259

Reynolds M, Skovmand B, Trethowan R, Pfeiffer W (2000) Evaluating a conceptual model for drought tolerance. Molecular approaches for genetic improvement of cereals for stable production in water-limited environments, Ribaut J-M and D. Poland, (eds):49-53

Reynolds M, Tuberosa R (2008) Translational research impacting on crop productivity in drought-prone environments. Current Opinion in Plant Biology 11:171-179

Reynolds MP, Pask AJ, Hoppitt WJ, Sonder K, Sukumaran S, Molero G, Saint Pierre C, Payne T, Singh RP, Braun HJ (2017) Strategic crossing of biomass and harvest index—source and sink—achieves genetic gains in wheat. Euphytica 213:257

Ribaut J-M, Ragot M (2007) Marker-assisted selection to improve drought adaptation in maize: the backcross approach, perspectives, limitations, and alternatives. Journal of Experimental Botany 58:351-360

Richards G (1996) Cultural tourism in Europe. Greg Richards

Richards JC (1992) Longman dictionary of language teaching and applied linguistics. Longman

Richards R (2000) Selectable traits to increase crop photosynthesis and yield of grain crops. Journal of Experimental Botany 51:447-458

Richards R, Passioura J (1989) A breeding program to reduce the diameter of the major xylem vessel in the seminal roots of wheat and its effect on grain yield in rain-fed environments. Crop and Pasture Science 40:943-950

Richards R, Rebetzke G, Condon A, Van Herwaarden A (2002) Breeding opportunities for increasing the efficiency of water use and crop yield in temperate cereals. Crop Science 42:111-121

Richards RA (2006) Physiological traits used in the breeding of new cultivars for water-scarce environments. Agricultural Water Management 80:197-211

Rosegrant MW, Paisner MS, Meijer S, Witcover J (2001) 2020 Global food outlook: Trends, alternatives, and choices. International Food Policy Research Institute

Šafář J, Šimková H, Kubaláková M, Číhalíková J, Suchánková P, Bartoš J, Doležel J (2010) Development of chromosome-specific BAC resources for genomics of bread wheat. Cytogenetic and Genome Research 129:211-223

Saint Pierre C, Crossa J, Manes Y, Reynolds MP (2010) Gene action of canopy temperature in bread wheat under diverse environments. Theoretical and Applied Genetics 120:1107-1117

Salvi S, Tuberosa R (2005a) How many genes underlie a primary QTL? Trends in Plant Science 10:297-304

Salvi S, Tuberosa R (2005b) How many genes underlie a primary QTL? Trends in Plant Science 6:297-304

Salvi S, Tuberosa R (2005c) To clone or not to clone plant QTLs: present and future challenges. Trends in Plant Science 10:297-304

Sarkar NK, Kundnani P, Grover A (2013) Functional analysis of Hsp70 superfamily proteins of rice (Oryza sativa). Cell Stress and Chaperones 18:427-437

Semenov MA, Shewry PR (2011) Modelling predicts that heat stress, not drought, will increase vulnerability of wheat in Europe. Scientific Reports 1

Shahbaz M, Muhammad S, Ahmad F, Anwar M, Sajjad M, Riaz M, Nabi G, Waqar MQ (2017) Efficacy of different herbicides for controlling narrow leave weeds in wheat crop at different locations under adaptive research zone Sheikhupura. Pure and Applied Biology.

Shahinnia F, Le Roy J, Laborde B, Sznajder B, Kalambettu P, Mahjourimajd S, Tilbrook J, Fleury D (2016) Genetic association of stomatal traits and yield in wheat grown in low rainfall environments. BMC Plant Biology 16:150

Shamsudin NAA, Swamy BPM, Ratnam W, Cruz MTS, Raman A, Kumar A (2016) Marker assisted pyramiding of drought yield QTLs into a popular Malaysian rice cultivar, MR219. BMC genetics 17:30

Shao M, Tang X, Zhang Y, Li W (2006) City clusters in China: air and surface water pollution. Frontiers in Ecology and the Environment 4:353-361

Shewry P (2009) Wheat. Journal of Experimental Botany 60:1537-1553

Shiferaw B, Smale M, Braun H-J, Duveiller E, Reynolds M, Muricho G (2013) Crops that feed the world 10. Past successes and future challenges to the role played by wheat in global food security. Food Security 5:291-317

Shirdelmoghanloo H, Taylor JD, Lohraseb I, Rabie H, Brien C, Timmins A, Martin P, Mather DE, Emebiri L, Collins NC (2016) A QTL on the short arm of wheat (Triticum aestivum L.) chromosome 3B affects the stability of grain weight in plants exposed to a brief heat shock early in grain filling. BMC Plant Biology 16:1

Siddique K, Tennant D, Perry M, Belford R (1990) Water use and water use efficiency of old and modern wheat cultivars in a Mediterranean-type environment. Crop and Pasture Science 41:431-447

Simmons SR (1987) Growth, development, and physiology. Wheat and Wheat Improvement doi:10.2134/agronmonogr13.2ed.c3,13:77-113

Singh K, Chugh V, Sahi GK, Chhuneja P (2012) Wheat: Mechanisms and genetic means for improving heat tolerance. Improving Crop Resistance to Abiotic Stress, Volume 1 & Volume 2:657-694

Slafer G, Araus J, Royo C, Moral L (2005) Promising eco- physiological traits for genetic improvement of cereal yields in Mediterranean environments. Annual Applied Biology 146:61-70

Smiley RW, Nicol JM (2009) Nematodes which challenge global wheat production. Wheat Science and Trade BF Carver, ed Wiley-Blackwell, Ames, IA 10:171-187

Snape J, Butterworth K, Whitechurch E, Worland A (2001) Waiting for fine times: genetics of flowering time in wheat. Euphytica 119:185-190

Sonah H, Bastien M, Iquira E, Tardivel A, Légaré G, Boyle B, Normandeau É, Laroche J, Larose S, Jean M (2013) An improved genotyping by sequencing (GBS) approach offering increased versatility and efficiency of SNP discovery and genotyping. PloS One 8:e54603

Specht RL (1973) Structure and functional response of ecosystems in the Mediterranean climate of Australia. Mediterranean Type Ecosystems. F. di Castri et al. (eds.), Mediterranean Type Ecosystems Springer, pp 113-120

Swamy BPM, Kumar A (2013) Genomics-based precision breeding approaches to improve drought tolerance in rice. Biotechnology advances 31:1308-1318

Swindale L, Bidinger F (1981) The human consequences of drought and crop research priorities for their alleviation. The physiology and biochemistry of drought resistance in plants New York, Academic Press USA:1-13

Taiz L, Zeiger E (2006) Stress physiology. Plant Physiology. 4th Edition, Sinauer Associates Inc. Publishers, Massachusetts.

Tambussi EA, Nogués S, Araus JL (2005) Ear of durum wheat under water stress: water relations and photosynthetic metabolism. Planta 221:446-458

Tang Y, Liu M, Gao S, Zhang Z, Zhao X, Zhao C, Zhang F, Chen X (2012) Molecular characterization of novel TaNAC genes in wheat and overexpression of TaNAC2a confers drought tolerance in tobacco. Physiologia Plantarum 144:210-224

Tardieu F (2011) Any trait or trait-related allele can confer drought tolerance: just design the right drought scenario. Journal of Experimental Botany:63(1):25-31

Teixeira EI, Fischer G, van Velthuizen H, Walter C, Ewert F (2013) Global hot-spots of heat stress on agricultural crops due to climate change. Agricultural and Forest Meteorology 170:206-215

Tiwari C, Wallwork H, Kumar U, Dhari R, Arun B, Mishra V, Reynolds M, Joshi AK (2013) Molecular mapping of high temperature tolerance in bread wheat adapted to the Eastern Gangetic Plain region of India. Field Crops Research 154:201-210

Trethowan R, Reynolds M, Skovmand B, van Ginkel M (1998) "The effect of glume pubescence on floret temperature in wheat". 1998 Agronomy Abstracts, American Society of Agronomy, Baltimore, MD, USA),p 161

Tuberosa R, Salvi S (2006) Genomics-based approaches to improve drought tolerance of crops. Trends in Plant Science 11:405-412

Turner NC (1979) Drought resistance and adaptation to water deficits in crop plants. Stress Physiology in Crop Plants Eds H Mussel and RC Staples Wiley, New York:344-372

Venkateswarlu B, Shanker AK, Shanker C, Maheswari M (2011) Crop stress and its management: perspectives and strategies. Springer Science & Business Media

Verma V, Foulkes M, Worland A, Sylvester-Bradley R, Caligari P, Snape J (2004) Mapping quantitative trait loci for flag leaf senescence as a yield determinant in winter wheat under optimal and drought-stressed environments. Euphytica 135:255-263

Wang L-N, Liu Y-F, Zhang Y-M, Fang R-X, Liu Q-L (2012) The expression level of Rosa Terminal Flower 1 (RTFL1) is related with recurrent flowering in roses. Molecular biology reports 39:3737-3746

Wang S, Wong D, Forrest K, Allen A, Chao S, Huang BE, Maccaferri M, Salvi S, Milner SG, Cattivelli L (2014) Characterization of polyploid wheat genomic diversity using a high- density 90 000 single nucleotide polymorphism array. Plant Biotechnology Journal 12:787-796

Wehmeyer N, Vierling E (2000) The expression of small heat shock proteins in seeds responds to discrete developmental signals and suggests a general protective role in desiccation tolerance. Plant PhysiolOGY 122:1099-1108

Wellmer F, Alves-Ferreira M, Dubois A, Riechmann JL, Meyerowitz EM (2006) Genome-wide analysis of gene expression during early Arabidopsis flower development. PLoS Genetics 2:e117

Wicker T, Buchmann JP, Keller B (2010) Patching gaps in plant genomes results in gene movement and erosion of colinearity. Genome Research 20:1229-1237

Worland A, Appendino M, Sayers E (1994) The distribution, in European winter wheats, of genes that influence ecoclimatic adaptability whilst determining photoperiodic insensitivity and plant height. Euphytica 80:219-228

Worland A, Korzun V, Röder M, Ganal M, Law C (1998) Genetic analysis of the dwarfing gene Rht8 in wheat. Part II. The distribution and adaptive significance of allelic variants at the Rht8 locus of wheat as revealed by microsatellite screening. Theoretical and Applied Genetics 96:1110-1120

Worland AJ (1996) The influence of flowering time genes on environmental adaptability in European wheats. Euphytica 89:49-57

Wu X, Cheng R, Xue S, Kong Z, Wan H, Li G, Huang Y, Jia H, Jia J, Zhang L (2014) Precise mapping of a quantitative trait locus interval for spike length and grain weight in bread wheat (*Triticum aestivum* L.). Molecular Breeding 33:129-138

Yan H, Kang M, De Reffye P, Dingkuhn M (2004) A dynamic, architectural plant model simulating resource- dependent growth. Annals of Botany 93:591-602

Yang J, Zhang J (2006) Grain filling of cereals under soil drying. New Phytologist 169:223-236

Yang J, Zhang J, Wang Z, Zhu Q, Liu L (2003) Involvement of abscisic acid and cytokinins in the senescence and remobilization of carbon reserves in wheat subjected to water stress during grain filling. Plant, Cell and Environment 26:1621-1631

Yang K, Lim CJ, Hong JK, Park CY, Cheong YH, Chung WS, Lee KO, Lee SY, Cho MJ, Lim CO (2006) Identification of cell wall genes modified by a permissive high temperature in Chinese cabbage. Plant Science 171:175-182

Yang X, Tian Z, Sun L, Chen B, Tubiello FN, Xu Y (2017) The impacts of increased heat stress events on wheat yield under climate change in China. Climatic Change:140 (3) 605-620

Yoshida T, Mogami J, Yamaguchi-Shinozaki K (2014) ABA-dependent and ABA-independent signaling in response to osmotic stress in plants. Current opinion in plant biology 21:133-139

Yost HJ, Lindquist S (1986) RNA splicing is interrupted by heat shock and is rescued by heat shock protein synthesis. Cell 45:185-193

Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. Weed Research 14:415-421

Zang Q-W, Wang C-X, Li X-Y, Guo Z-A, Jing R-L, Zhao J, Chang X-P (2010) Isolation and characterization of a gene encoding a polyethylene glycol-induced cysteine protease in common wheat. Journal of Biosciences 35:379-388

Zivy M (1987) Genetic variability for heat shock proteins in common wheat. Theoretical and Applied Genetics 74:209-213

Chapter 3: Co-location of yield and yield component QTL with QTL for relative leaf expansion rate on chromosome 1B in the Drysdale/Gladius spring wheat population grown under severe drought and heat stress

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Chapter 3: Co-location of yield and yield components with QTL for relative leaf expansion rate on chromosome 1B in the Drysdale/Gladius spring wheat population grown under severe drought and heat stress

3.1. Abstract

Grain yield is the outcome of physiological and developmental processes interacting with the environment during the whole plant growth period. It is important to dissect yield into its biological and environmental attributes so as to improve yield in dry and hot environments. This study was conducted to test if there were genetic loci controlling yield and yield components on chromosome 1B in the Drysdale/Gladius population under terminal drought and heat stress conditions. We used recombinant inbred lines that showed a recombination event within the 1B QTL for growth, relative leaf expansion and transpiration rate previously identified by Parent et al. (2015) in this population. QTL for grain yield, seeds/spikelet, early vigour, biomass, flag leaf area, vegetative canopy temperature and plant height were discovered on chromosome 1B, explaining 2.8% to 51% of the genetic variation. The Drysdale provided the desirable allele for all traits. These QTL were expressed only under severe drought and hot conditions. Lines carrying the Drysdale allele had an average of five more seeds/spike than lines carrying the Gladius allele. The increase might be due to the improvement in floral fertility resulting from biomass production that supports grain number and yield. We also confirmed the colocation of these QTL with a QTL for rate of leaf area expansion measured in a previous study using an imaging platform. The Drysdale allele increased plant growth and biomass which could result in the yield increase. Relative leaf expansion rate measured with an imaging platform may provide a proxy trait for further fine mapping of this yield QTL. The markers linked to these QTL might also be used to select potential drought tolerant lines at early growth stage under controlled conditions to develop high yielding cultivars for dry and hot environments.

3.2. Introduction

Wheat feeds about 30% of the world's population and is a strategic crop for global food security (FAOSTAT 2015; Shiferaw et al. 2013). Wheat contributes about 20% of the daily dietary calories and protein intake world-wide (Shiferaw et al. 2013). Although breeding has increased genetic gain and yield in wheat under a wide range of environments, there is a huge gap between wheat demand and production that has to be filled (Lopes et al. 2012; Rosegrant and Agcaoili 2010). Wheat productivity needs to be increased by more than 2.8% every year to feed the increasing world population, projected to reach 9.1 billion at the end of 2050 (Alexandratos and Bruinsma 2012). Current yield increase is nearly 1% per annum but is difficult to improve due to its complex quantitative nature and its strong interaction with the environment (Fleury et al. 2010). Drought and heat can decrease wheat yield by up to 47 % (Blum et al. 2001) or 66 % (Lopes and Reynolds 2011) under normal production conditions or result in total crop loss in extreme cases.

The significance of drought effects on agricultural productivity varies with its intensity and frequency over locations, years, crop growth seasons, and its interaction with different environmental factors (Tardieu 2011a). Cyclic drought, characterized by sporadic interruptions of rainfall during anthesis and grain filling can cause total crop loss in Australia in extreme years (Izanloo et al. 2008; Reynolds et al. 2007). The drought effect is particularly pronounced in Mediterranean type-environments such as in southern Australia, where it is coupled with a cyclic heat and other abiotic stresses such as subsoil salinity, boron toxicity, nutrient deficiency, strong wind and irradiance and low humidity (Fleury et al. 2010). This results in about 3.5 million tons annual yield loss in Australia and limits the country's average yield to 1.7 t/ha, which is below the world average (Gavran 2012; Ray et al. 2013).

Despite these challenges, exploitation of the existing genetic variability in wheat germplasm remains the basis for improvement of quantitative traits, such as yield in dry and hot environments. Wheat varieties respond differently to different drought and heat stress scenarios. Manipulation of diversity to improve drought and heat tolerance can be achieved through identification of genomic regions that are responsible for these variations (Bonneau et al. 2013a; Maphosa et al. 2015; Maphosa et al. 2014; Tahmasebi et al. 2016). Thus, a better understanding of the genetic loci controlling yield under drought and heat is important for breeding.

Previous studies have detected yield QTL on chromosome 1B (*QYld.aww.1B*) in Excalibur/Kukri DH lines (Edwards, 2012) (detail in chapter 4) and in RAC875/Kukri DH population (Bennett *et al.*, 2012) (detail in chapter 5 under section 5.3.2) under rain-fed multi-location trials in South Australia. A co-located QTL for growth rate (*QGRO.atw.1B*), relative leaf expansion rate ($QRER_{AVE}$*,atw.1B*) and transpiration rate (*QTR.atw.1B*) were identified by Parent et al. (2015) in recombinant inbred lines of Drysdale/Gladius using the high through-put phenotyping platform of The Plant Accelerator under well-watered conditions. A QTL was also found for tillering number (*QSnp.atp11.1B*) in Drysdale/Gladius under semi-controlled conditions at a range of soil moisture and watering regimes by Parent et al (2017). However, Maphosa et al, 2014 did not find a yield QTL on 1B in Drysdale/Gladius RIL. Drysdale and Gladius have different pedigree. Drysdale is selected for its high transpiration efficiency (Condon et al. 2006) while Gladius is adapted to drought and heat stressed environments (Fleury et al., 2010). Comparison of yield QTL positions on chromosome 1B in Excalibur/Kukri and RAC875/Kukri DH with QTL for growth, leaf expansion and transpiration rate in Drysdale/Gladius RIL has shown that four markers (adw13, adw3, adw22 and adw36) were shared between these QTL. Since Gladius is derived from Excalibur, RAC875 and Kukri crosses, we expected to detect common QTL between the three mapping populations. This suggests that there would be a yield QTL on the 1B chromosome of Drysdale/Gladius that might be explained by genetic variation of growth and transpiration rate. Therefore, this study was conducted to verify this hypothesis and test whether there were genetic loci controlling yield and yield components on chromosome 1B in the Drysdale/Gladius population.

3.3. Materials and methods

3.3.1. Plant material

A recombinant inbred line population of 5,000 lines was derived from a cross between Drysdale (Hartog/3Quarrion) and Gladius (RAC875/Krichauff//Excalibur/Kukri/3/RAC875/Krichauff/ 4/RAC875//Excalibur/Kukri). Both parental lines are well adapted to low and medium moisture environments but have different drought tolerance mechanisms (Fleury et al. 2010). Gladius is an erect and waxy leaf variety released by Australian Grain Technologies in 2007 for its high yield under severe drought condition of southern Australia. Drysdale was developed by Commonwealth Scientific and Industrial Research Organization (CSIRO) and released in

2001. It is a high yielding variety that has been selected for its high water use efficiency adapted to the New South Wales region, where sufficient underground soil moisture is available during the terminal drought period (Condon et al. 2006).

In 2014, we selected 96 RIL that showed recombination points in the region of the QTL for growth, relative leaf expansion and transpiration rate previously identified using a high throughput Lemnatec image phenotyping platform (The Plant Accelerator, TPA, Urrbrae, South Australia) by Parent et al. (2015). The lines were selected from a screen of 1,000 Drysdale/Gladius RIL based on their recombination in the interval delineated by four KASP™ markers (adw13, adw3, adw22 and adw36) for the QTL for plant growth, relative leaf expansion and transpiration rate. The 96 lines were also selected as they were fixed for the photoperiod genes *Ppd-B1* and *Ppd-D1*.

In 2015, another set of 2,000 Drysdale/Gladius RIL were genotyped using four SNP markers (adw1061145, adw1005607, adw22, and adw36) from the region of *QSsp.adw-1B* found for seeds/spikelet detected in 2014 trial. One hundred and ninety one recombinant lines were selected for the 2015 trial and were not genotyped for phenology genes. Forty-five RIL were in common between the two sets found by Parent et al. (2015). The aim of the 2015 trial was to validate the QTL for seeds/spikelet (*QSsp.adw-1B)* detected in 2014 and to find yield QTL in Drysdale/Gladius RIL.

3.3.2. Field trials

Two trials were conducted in the 2014 and 2015 cropping seasons under semi-controlled field conditions using a polyurethane rain-out shelter (called polytunnel) and a birdcage at Urrbrae, University of Adelaide, South Australia (35° S 139° E). The polytunnel and birdcage were equipped with drip irrigation facilities and gypsum blocks (KG100 model, Adelaide, Australia) was used to measure soil moisture and soil temperature. Six gypsum blocks were installed at 10 cm and 40 cm depth at three different positions within a 7 m interval, both in the polytunnel and birdcage. The sensor recorded the soil moisture status every minute. Average values over 24 hrs periods were used to describe the climatic scenarios of the trials. Well-watered and severe drought conditions were reached when water-soil-tension read -0.1 (at field water capacity) and -0.6 MPa, respectively as reported in (Parent et al. 2015). Average daily air temperature and humidity in the polytunnel were calculated from three mobile loggers placed

at three different points in the polytunnel. Data from the on-site automatic weather station (MWS model, Hunter Industries, Australia) were used to record air temperature and humidity for the birdcage trial in 2014.

All trials were fertilized with nitrogen, phosphorous and potassium at planting as per recommendation (80 - 40 - 80, N P_2O_5 K_2O_5 kg/ha) for wheat in South Australia. Two thirds of the nitrogen fertilizer were applied during planting and the remaining third was applied at tillering stage. Bayfidan® 250 EC with active ingredient of Triadimenol 250 g/l at 0.4 ml rate Bayer Crop Sciences, Australia) was applied to control fungal disease infestations during vegetative growth stage.

In 2014, 96 Drysdale/Gladius RIL were grown under two treatments: drought in the polytunnel and well-watered condition in the birdcage. Planting was carried out on August 7, 2014, later than the farmers' planting time for wheat (April/May) in South Australia to expose the plants to high temperatures during flowering and grain filling. The RIL were arranged in a randomized complete block design (RCBD) with two replications in the polytunnel and partial replication (where 34% of the lines replicated twice) in the birdcage. The parental lines were replicated four times in both treatments. A total of 16 plants were grown in two 80 cm rows, with 10 cm between rows and between plants in the same row. Two rows of filler plants were planted at first and last plots for each replication to avoid border effects. Both treatments were maintained in well-watered conditions with soil water tension > -0.1 MPa during the vegetative stage. Drought treatment was induced by withholding irrigation when 50% of the population reached early booting stage (Zadoks' growth scale 40) (Zadoks et al. 1974) to ensure terminal drought stress. The control experiment in the birdcage was kept well-watered with soil-moisture tension > -0.1 MPa until physiological maturity as in (Parent et al. 2015).

In 2015, 191 RIL were sown in the polytunnel on July 4. The lines were randomly allocated to the experimental plots and blocks in a RCBD in two replications. The two parental lines (Drysdale and Gladius) were used as checks and replicated 8 times. Land preparation, planting, plot size, plant density per plot, irrigation facilities and fertilizer application were similar to the 2014 trial. The trial was all under drought treatment: irrigation was stopped at early booting stage.

3.3.3. Plant phenotyping

Days to heading (DTH), anthesis (DTA) and maturity (DTM) were scored as the number of days between sowing and the day when 50% of the plants in the plot reached heading, anthesis and physiological maturity, respectively. Grain filling period (GFP) was calculated as the time between anthesis and maturity. Plant height (PH) was the distance (cm) from the ground to the tip of the spike excluding the awns as measured at maturity.

Fertile tillers/plot (FT) were the number of fertile spikes per plot counted at maturity. Sterile tillers/plot (ST) were the number of tillers without fertile spikes. Number of spikelets/spike (SpS), spike length (SL) and number of seeds/spike (SS) were averaged from five spikes sampled randomly at harvest. Spike length (cm) was measured from the first rachis to the tip of the last floret, excluding the awn. Seeds were bulked from five spikes, counted with a seed counter (Pfueffer GmBH, Germany) and averaged to calculate the number of seeds/spike. The average seeds/spike was divided by the average number of spikelet/spike to calculate the number of seeds/spikelet (SSp). Grain yield (Yld, g/plant) was measured by weighing grain harvested from the whole plot (two rows of 16 plants) and divided by the total number plants per plot. Thousand grains weight (TGW) was measured per plot in grams by weighing five hundred seeds randomly sampled and multiplied by two to get TGW in grams. Biomass/plant (BM) was the weight (g) of the above ground plant after harvest and oven dry. Harvest index (HI, %) was the ratio between grain yield and biomass multiplied by 100.

Early vigour was visually scored from 1 to 5 where 1 refer to poor seedling establishment (weak and thin seedling) and 5 refers to the best seedling establishment (leafy and vigorous seedlings) (Regan et al. 1992). Normalized difference vegetative index (NDVI) was measured per plot twice a week during vegetative growth using a GreenSeeker® Handheld Crop Sensor (Trimble Navigation limited, USA) (Rouse et al. 1974). Flag leaf area (FLA, cm^2) was the average flag leaf area measured on three randomly selected plants per plot at booting stage using a portable area meter (LI-300A Portable Area meter, LI-COR Inc., USA). Canopy temperature (CT, C) was measured using a portable infrared thermometer (Sixth Sense LT300 IRT) between 11:00 am and 2:00 pm on windless and clear days. The thermometer was fixed to a stick to keep it at a constant height and angle above the canopy (Olivares-Villegas et al. 2007). CT was measured three times and averaged within a five day interval during the vegetative (CTv, from stem

elongation to booting) and grain-filling stages (CTg, from heading to anthesis) (Pask et al. 2012).

3.3.4. Statistical analysis of 2014 - 2015 trials

Statistical analysis of phenotypic traits was done using Genstat® edition 16 (International 2013). A generalized linear model with randomization was used for both years' polytunnel trials, while a spatial model was used for the birdcage trial. Genotypes were set as a fixed effect in both models. Analysis of variances (ANOVA) and Pearson linear (two sided) correlation analysis were applied in order to compute the statistical significance of the variance and correlation coefficients for the data from different experiments and different parameters. Broad sense heritability (h^2) calculated using the variance components predicted from a restricted maximum likelihood of the mixed model. The best linear unbiased predictions were estimated to determine the genetic effect of each line and trait mean for QTL analysis. The genotype was fitted as the random variable in the model and broad sense heritability calculated following the formula developed by Cullis et al. (2006):

$$h^2 = 1 - \text{PEV}/2\boldsymbol{\sigma}^2_G$$

where PEV and $\boldsymbol{\sigma}^2_G$ are phenotypic error variance and genotypic variance, respectively.

3.3.5. Sequence analysis and identification of new SNP

We used a total of 9 SNP markers that delineated the QTL for seeds/spikelet (*QSsp.adw-1B*) (Supp. Table 1): 6 'BS' markers were designed from the Breeders' 35k Axiom® array sequences (BS00021710, BS00022093, BS00076394, BS00006033, BS00022851, BS00042340); 2 markers were converted from genotype-by-sequencing (GBS) (adwkasp24 and adwkasp29); and 1 marker came from the Illumina 90k iSelect SNP (adw226394). The markers sequences were anchored on the Chinese Spring whole genome assembly (IWGSC WGA v0.4) (https://wheat-urgi.versailles.inra.fr/; Alaux et al. 2016) by BLASTN using an in-house portal (Crop Bioinformatics Group, University of Adelaide, CroBiAd). Scaffolds exhibiting a percentage of identity > 98% and e-values between 8e-22 to 2e-51 with marker sequences were selected. These scaffolds were then analysed with Diversity Among Wheat geNome (DAWN), an in-house wheat genomics platform enabling identification of sequence variants (CroBiAd) (Watson-Haigh et al., unpublished). DAWN runs on the Integrative Genomics Viewer (IGV) platform and combines the whole genome assembly of Chinese

Spring (IWGSC WGA v0.4), the whole genome sequences of 16 cultivars (also called BPA dataset, from BioPlatforms Australia) (Edwards et al. 2012), a RNA-Seq dataset (International Wheat Genome Sequencing 2014) and the Munich Information Centre for Protein Sequences (MIPS HCS) gene models (http://pgsb.helmholtz-muenchen.de/plant/wheat/index.jsp). The BPA dataset includes Drysdale and Gladius parental lines, with a 10X coverage and 100 bp paired-end Illumina HiSeq sequence reads (https://researchdata.ands.org.au/bpa-wheat-cultivars/2614).

DAWN aligned the BPA reads onto the IWGSC WGA v0.4 assembly to identify SNP between Drysdale and Gladius in selected scaffolds. DAWN setting is highly specific and identified SNP are homeolog specific. Only the SNP found within a sequence stretch of 200 bp, containing ≥ 50% GC to increase the chance that the SNP to be selected from generic region and a depth of read coverage >10, were selected. Using the nucleotide positions of the SNP in the scaffolds and the Fetch-Seq tool in DAWN, 100 bp sequence segments containing the SNP were retrieved and used to design KASP™ markers (Semagn *et al.*, 2014) with the Kraken software (Supplementary table 3.2). KASP markers were also homeolog specific. This is verified by segregation of the mapping population.

3.3.6. Genotyping and genetic map construction

The Drysdale/Gladius chromosome 1B genetic map was constructed using genotypic data of a total of 371 RIL by combining subsets used in the three experiments described above. Genomic DNA was extracted from 8 weeks old leaves collected from one plant per line with the 96-well format DNA extraction protocol for freeze-dried seedling leaves (Dietrich et al. 2002). Single nucleotide polymorphism (SNP) were genotyped using the KBioscience Competitive Allele-Specific Polymerase chain reaction assay (He et al. 2014; Semagn et al. 2014). All KASP™ markers were designed using the Kraken software and assayed using a SNP Line (LGC Genomics, Teddington, UK). For each KASP™ SNP, two allele specific forward primers (A1 and A2) and one common reverse primer (C1) were designed by importing SNP sequences into KrakenTM software (Version 13.4.18.11833) (LGC Genomics, Middlesex, United Kingdom). All the markers were assessed on parents and all the lines of the mapping population using the LGC genomics KASP™ assay. The KASP™ protocol used is available online from LGC genomics, Middlesex, United Kingdom (www.lgcgroup.com/our-science/genomics-solutions/genotyping/kaspgenotyping-chemistry). After genotyping, the SNP calls were

converted to A or B in accordance to the genotypes of parents (Drysdale and Gladius, respectively) and "-" for missing data.

A total of 93 SNP based KASP™ markers were designed from various sources described below and mapped on 371 Drysdale/Gladius RIL. Sixteen markers prefixed "adwkasp" were converted from the Illumina 90k iSelect SNP (Wang et al. 2014). Five KASP™ markers were converted from genotype-by-sequencing markers (GBS) that were mapped on Excalibur/Kukri DH genetic map (Chapter 4) and polymorphic to Drysdale/Gladius; those are prefixed as "adw" and followed with a seven digit number. Thirty one 'BS' markers were designed from the Breeders' 35k Axiom® array sequences available at Cereals DB Website (http://www.cerealsdb.uk.net/cerealgenomics/CerealsDB) (Wilkinson et al. 2012). At last 41 KASP™ were designed from SNP identified in DAWN and prefixed "adw". KASP assays are highly specific in wheat: markers matching the segregation ratio expected in the RIL were mapped as homeolog specific (the mapping step demonstrating they are 1B specific); markers that don't have correct segregation pattern were considered non-homeolog specific and removed from the map construction.

RIL were also genotyped for *Ppd-D1* and *Ppd-B1* phenology genes as described in (Maphosa et al. 2014). Ppd-D1_F, Ppd-D1_R1 and Ppd-D1_R2 primers were used from (Beales et al. 2007) to amplify the *Ppd-D1* using the PCR setting from Eagles et al. (2009). Díaz et al. (2012) protocol was used for *Ppd-B1*. Two early flowering genes *TARGET OF EAT3* (*TaTOE1-B1*) and *Triticum aestivum FLOWERING LOCUS T3-B1* (*TaFT3-B1)* recently reported on chromosome 1BS and 1BL, respectively, were also genotyped (Zikhali et al. 2017). Markers were assigned to a linkage group at a threshold value for logarithm of the odds (LOD) > 3 and then ordered within the linkage group with the RECORD algorithm of ICiMapping version 4 (Meng et al. 2015). Marker segregation distortion was tested by a chi-square (χ^2) test ($p < 0.05$). Markers with genetic distance ≥ 50 cM were removed from the linkage group and double crossovers were manually curated. The final genetic distance between markers was calculated by the rippling function with sum of adjacent recombination frequencies (SARF) criterion with 5 windows size (Meng et al. 2015).

3.3.7. QTL analysis

We used three datasets for QTL mapping:2014 polytunnel trial (96 RIL) under well-watered and severe drought treatment, and 2015 polytunnel trial (191 RIL) under severe drought, and the TPA data (135 RIL) under well-watered conditions from Parent et al. (2015). Traits showing significant variations among the RIL were subjected to a step wise QTL analysis using QTL Cartographer version 2.5 (Wang et al. 2012d). Single marker analysis was first performed to identify markers significantly associated with a trait, followed by simple interval mapping to know the tentative location/s of the QTL on the chromosome. QTL position was then determined by composite interval mapping using the standard model (Model 6) with forward/backward regression method at a significant threshold of $p < 0.05$ (Wang et al. 2012d). The model was adjusted at a walk speed of 1 cM intervals and a maximum of 5 markers as co-factors per model. The threshold to accept the presence of QTL was defined by 1,000 permutations test (Churchill and Doerge 1994). The phenotypic variation explained by a QTL (R^2) and the additive effect were generated by the composite interval mapping model.

3.4. Results

3.4.1. Environmental conditions

Irrigation was stopped at early booting stage so that plants experienced a severe drought during anthesis and the grain filling period in both years similar to the conditions experienced by crops in the southern Australian wheat belt. The target drought and temperature regimes for this experiment were defined based on the climate data from Bureau of Meteorology climatic data collected at different research cites of Southern Australia over the period 1981-2010 (http://www.bom.gov.au/climate/averages/tables/ca_sa_names.shtml) with a long term average rainfall ranged from 218-300 mm and average maximum temperature ranged from 22 -25 ^{o}C in southern Australia. In 2014, plants in the birdcage experienced well-watered and mild heat conditions with daily average temperatures > 25°C only for 5 days between heading and maturity and a maximum of two and three consecutive days at heading and grain filling, respectively (Suppl. Fig. 1). The polytunnel trial was under severe drought during anthesis and grain filling with the depletion of both the top soil (10 cm) and underground soil moisture (40 cm) with soil water tensions < -0.6 MPa (Suppl. Fig. 1). Plants were under heat stress with daily average temperatures higher than 25°C for more than twelve days between heading and

maturity, including 5 consecutive days at critical stages of heading and anthesis (Suppl. Fig. 1). A maximum temperature of 45°C that extended for more than 6 hours was recorded for some days during anthesis and grain filling in the 2014 polytunnel trial (raw data not shown). Overall the polytunnel trial experienced warmer temperatures than the birdcage, especially during flowering and grain filling in the 2014 trial.

In 2015, irrigation was withheld during stem elongation; earlier than in 2014. The experiment was under severe drought stress from booting to maturity, coupled with an average daily temperature > 25°C (Supp. Fig 2). Average temperatures were extreme (> 35°C) during grain filling. Overall the trial was under warmer temperatures in 2015 than 2014. Polytunnel trials in both years were under severe drought stress during the critical reproductive stage and combined with high temperature at grain filling period in both years. Thus, we successfully created experimental conditions which were similar to the southern Australian conditions with terminal drought and heat stress.

3.4.2. Phenotypic variation and correlations

Although Drysdale and Gladius did not show significant differences for most traits, analysis of variance revealed highly significant variations among the Drysdale/Gladius RIL for all traits except for grain filling duration, biomass/plant and harvest index under well-watered condition in the 2014 trial and grain filling period in 2015 (Table 1). There was continuous and transgressive segregation among the RIL for all traits in both years (Supp. Fig. 3, 4 and 5).

A wide range of broad sense heritability values was observed across traits in both years, from 15% for HI to 91% for spikelet/spike, indicating variable proportions of genetic to environment variance among traits (Table 1). Spikelet/spike and seeds/spikelet were the most heritable yield components under drought with h^2 percentages of 91% and 75% in 2015 and 83% and 71% in 2014 polytunnel trials, respectively. This showed that there is a significant amount of genetic variance in the control of the traits that contribute to yield in a dry environment. The lowest heritability of 15% and 20% were recorded for grain filling duration in 2015 and 2014 well-watered, respectively (Table 1).

High heritability values ranging from 70 to 93% were observed for days to heading and anthesis, indicating strong genetic control of maturity traits in the Drysdale/Gladius mapping

population. The population segregated for *Ppd-D1* and *Ppd-B1* but did not segregate for *TaTOE1-B1* and *TaFT3-B1*. As differences in phenology can affect greatly yield components under drought (Fleury et al. 2010), the 2015 trial mapping population was separated into two groups based on their similarity in days to anthesis to remove the prevailing effect of flowering time from the QTL analysis (Fig. 1). Group 1 comprised 148 lines flowering within a ten days window between 87 and 96 DAS. Group 2 included a long tail of 43 lines flowering late (flowering between 98 and 117 DAS). We focused the QTL analysis on group 1 that flowered in a relatively short period of time.

Most of the yield and yield component traits showed similar and consistent correlation trends under well-watered and drought treatments in both years with higher coefficients of correlation recorded in the 2015 trial (Tables 2 and 3). Almost all traits showed significant positive correlations with grain yield and each other across the treatment in both years.

Significant positive correlations ranging from low to high values (r^2 = 0.30 to 0.80) were observed between yield, seeds/spike, biomass, TGW and HI under well-watered and drought conditions in both years. Biomass showed the highest correlation with yield (r^2 = 0.80 and 0.79 under well-watered and drought, respectively) in 2014 followed by seeds/spike under well-watered in 2014 (r^2 = 0.67) and under drought in 2015 (r^2 = 0.53) (Tables 2 and 3). Early vigour, NDVI and vegetative stage canopy temperature showed significant positive correlations with grain yield and seeds number (Table 3).

Strong and negative correlations were observed between yield components and canopy temperature measured at grain filling period (CT_g). Grain filling period was significantly and positively correlated with yield and yield components across trials and years. In the 2014 trial, yield and all yield components showed a significant negative correlation with maturity traits (HD, AD and MD) under drought conditions, while a significant positive correlation was observed under well-watered condition. There was no significant correlation between grain yield and maturity traits in 2015 in the group 2, of lines chosen to have a similar maturity.

3.4.3. QTL for yield and yield components on chromosome 1B

Analysis of the 2014 dataset showed no QTL on chromosome 1B for traits measured under well-watered conditions. Four QTL were detected under drought for four different traits but at

a different location of chromosome 1B (Table 4). *QSsp.adw-1B* for seeds/spikelet was flanked by the markers adw580 (71.9 cM) and adw582 (100.8 cM) and explained 16.2% of the phenotypic variance (R^2) with Drysdale contributing the positive allele and an additive effect of 5 seeds/spike (Table 4). QTL for early vigour (*QVig.adw-1B*, 11-12.4 cM) and flag leaf area (*QLfa.adw-1B*, 51.8-58.1 cM) explained 11.3% and 11.4% of the phenotypic variance, respectively. QTL for plant height (*QPH.adw-1B.2*, 131-164 cM) was also detected under the drought treatment in 2014 which explained the largest phenotypic variance of 51%. Drysdale contributed the positive allele for flag leaf area, while the Gladius allele increased early vigour and plant height.

As no QTL were found under well-watered conditions in 2014, only one treatment (drought) was applied in 2015. Preliminary analysis of the entire 191 Drysdale/Gladius RIL population used in the 2015 trial showed co-located QTL at two hotspot regions of chromosome 1B in Drysdale/Gladius RIL map (Table 4). In the first region, a QTL for grain yield (*QYld.adw-1B.1*) spanned from adw653 (61.2 cM) to adw10 (92.1 cM) and overlapped with QTL for plant height (*QPh.adw-1B.1*), canopy temperature (*QCt$_v$.adw-1B*), and biomass (*QBm.adw-1B*). In the second region, QTL for yield (*QYld.adw-1B.2*), seeds/spikelet (*QSsp.adw-1B*), seeds/spike (*QSS.adw-1B*), days to heading (*QDth.adw-1B*), days to anthesis (*QDta.adw-1B*), days to maturity (*QDtm.adw-1B*) and plant height (*QPh.adw-1B.2*) were co-located and flanked by BS00032039 (164.2 cM) and adw522 (192.5 cM). These QTL explained 2.8% to 51% of the phenotypic variance. Drysdale contributed the positive alleles for yield and yield components while Gladius increased the number of days to maturity and plant height under the 2015 drought trial.

The 2015 trial was reanalysed excluding lines from groups 1 and 3 and using 148 lines with similar anthesis date (Fig. 1). We found partially co-located QTL for yield (*QYld.adw-1B.1*), seed/spikelet (*QSsp.adw-1B*), biomass (*QBM.adw-1B*) and plant height (*QPh.adw-1B.1*) in the first QTL hotspot region, spanning from adw653 (61.2 cM) to adw582 (100.8 cM) (Table 4). Only the yield QTL (*QYld.adw-1B.2*), spanning from adw526 (198.2 cM) to adw37 (211.8 cM), was observed in the second hotspot region. The Drysdale allele increased the traits for all these QTL.

We re-analysed the QTL for leaf area expansion, growth rate, transpiration rate and other physiological traits on 135 Drysdale/Gladius RIL using the data generated by Parent et al.

(2015) (Table 4) and our genetic map of chromosome 1B constructed from 241 Drysdale/Gladius RIL. A QTL for average relative leaf expansion rate (*$QRER_{AVE}$.adw*-1B) was detected in the interval spanned from adw8 (86.6 cM) to adw582 (100.8 cM) on the new map. The QTL was co-located with yield QTL (*QYLd.adw-1B.1*) and seeds/spikelet (Q*Ssp.adw-1B*). Moreover, QTL for growth rate, transpiration rate and transpiration rate per unit of leaf area were also detected as reported by Parent et al. (2015) but did not co-locate with yield and yield components QTL from 2014 and 2015 trials (Table 4). Drysdale provided the positive allele for all re-analysed QTL as reported by Parent et al. (2015) except the QTL for transpiration rate per unit of leaf area at 104.4 cM.

3.5. Discussion

Unlike previous studies of Drysdale/Gladius population (Maphosa et al. 2014; Parent et al. 2015), we found QTL for yield and yield components on chromosome 1B. A cluster of partially co-located QTL for grain yield (*QYld.adw-1B.1*), seeds/spikelet (*QSSp.adw-1B*) and biomass (*QBm.adw-1B*) were found in the region spanned from adw653 (61.9 cM) to adw582 (100.8 cM) (Tables 4 and 5, Fig. 2). A second yield QTL peak (*QYld.adw-1B.2*) was also detected at markers adw526 (102.8 cM) and adw608 (111 cM) of the chromosome 1B. Drysdale/Gladius yield QTL (*QYld.adw-1B.2*) partially overlapped with previously reported yield QTL (*QYld.aww-1B.2*) in the RAC875/Kukri double haploid population (Chapter 5, under section 5.3.2) (Bennett *et al.*, 2012) and yield QTL (*QYld.aww-1B.2)* detected in the Excalibur/Kukri double haploid population (Edwards 2012c) under multi-location trials of South Australia (detail in Chapter 4). As we couldn't find sequences of previously reported QTL for yield and yield components on chromosome 1B in the literature (Table I, chapter 2, section 2.3.2), we couldn't relate our findings to previous work.

Yield in wheat is usually affected by phenology. We found significant negative correlation between grain yield and maturity traits under drought showing a yield penalty for late maturing lines for the drought environment (Araus et al. 2008). Absence of significant correlation between yield and maturity traits in 2015 in the sub-population (148 RIL), indicated that the maturity effect was effectively removed from the yield and yield components QTL by excluding early and late maturing lines from the sub-population. The QTL for yield and yield components on chromosome 1B in Drysdale/Gladius in region I were found on a RIL subset

with similar date of anthesis (Fig. 1) indicating that these QTL are not due to differences in phenology.

Yield QTL are often subject to a strong G x E interactions, and other QTL have been reported to be specific to the environmental conditions (Bennett et al. 2012b; Bonneau et al. 2013a; Millet et al. 2016; Quarrie et al. 2006a). The yield and yield component QTL of region I were only detected under severe drought and heat stress and no QTL was detected under well-watered conditions (Table 4). Thus, these yield QTL in chromosome 1B are expressed only under severe drought and heat conditions across the three mapping populations.

Drysdale contributed the positive allele for all the QTL found on region I. RIL carrying the Drysdale allele had longer spikes, more fertile spikelets and heavier seeds though the parental performance *per se* was inconsistent across the years and treatments for these traits in this study (Table 4). *QSSp.adw-1B* for seeds/spikelet contributed an increment of 5 seeds/spike. This indicates that fertile spikes is the main driver of yield differences in Drysdale/Gladius under drought and heat stress. Grain number/spike has previously been reported as the strongest determinant of grain yield increases (Fischer 2007; Miralles and Slafer 2007) and the yield component predominantly used to improve grain yield potential in wheat breeding (Peltonen-Sainio et al. 2007). However, this can only be fully realised if the increases in grain number are complemented with maintained grain weight (Calderini et al. 2013; Griffiths et al. 2015). In our study, although the QTL for TGW was below the LOD threshold, there were significant differences among Drysdale/Gladius RIL for TGW, indicating compensation between the grains number and grain weight under severe drought and heat stress in Drysdale/Gladius RIL, which was also supported with a negative correlation observed between grains weight and grains number.

We found that the QTL for yield (*QYld.adw-1B.1*) and yield components (*QSsp.adw-1B* and *QBm.adw-1B)* were co-located with QTL for relative leaf expansion rate (*QRER.adw-1B*) in QTL hotspot region I (Tables 4 and 5). The Drysdale allele increases leaf area expansion that contributed to biomass and eventually grain yield. The coincidence of QTL suggests the contribution of leaf area growth to yield performance under drought and heat stress. We also detected a QTL for plant height (*QPh.adw-1B.1,* 74 -77 cM) co-located with yield QTL in region I, suggesting that overall plant growth was affected. The co-location of yield and yield

component QTL observed in this study is supported by the significant positive correlation observed between yield and yield components (Tables 2 and 3).

Positive selection for a high rate of leaf area expansion at the early growth stage may help the plant to accumulate biomass in the winter season and maintain grain yield during moisture deficit later in the season. Assimilates such as water soluble carbohydrates can be stored in stem, flag leaf and spikes during the vegetative phase of growth when water is available and can be remobilized during grain filling under terminal drought stress (Blum 1997; Quarrie et al. 2006c). Early vigour is therefore regarded as a positive trait for a dry climate as it can increase water and light use efficiency resulting in high yield potential (Sloane 1999).

Drysdale is known for its water use efficiency (WUE) (Richards 2006) and was released as high yielding cultivar for North South Wales where stored soil moisture is available. In the southern Australia, the soil is shallow, thus wheat yield depends heavily on within-winter-season rain fall. The Drysdale allele at the region I QTL may contribute to an effective use of available winter moisture at the vegetative stage to accumulate assimilates that can be later translocated to maintain spike fertility during terminal drought and heat stress. This is supported by the co-location of QTL for seeds/spikelet and biomass in region I. The Drysdale allele increases early growth phase biomass that eventually contributed to final grain yield. Further analysis on QTL for WUE in Drysdale/Gladius RIL is required to test the hypothesis and complement our results.

We found a second QTL hotspot region that spanned from 118.2 to 164.2 cM for growth and transpiration rate measured in pots using a high-throughput platform (Parent et al 2015) (Tables 4 and 5). This region did not co-located with any of the QTL for yield or yield components detected in the 2014 and 2015 trials. The RIL segregating for the QTL showed differences between alleles for high growth and transpiration rate in both well-watered and mild drought stress conditions so the QTL was reported as a stable QTL (Parent et al. 2015) which was confirmed by a second study in a polytunnel under a range of soil moisture and temperatures in the polytunnel (Parent et al 2017). Parent et al. (2017) reported that these QTL controlled spike number/plant, single seed weight, seeds number/spike and spike length. However, due to the strong trade-off between yield components, no yield effect was observed. Here, we validated these QTL and showed they were stable across water conditions but there was no significant yield effect at this locus.

Finally, we found a QTL for plant height (*QPh.adw-1B.2*, 164.2 - 192.5 cM) which controls 51% of the phenotyping variation but did not co-located with any yield and yield components QTL. Though no height locus has been reported on 1B so far, there might be other pleiotropic loci controlling plant height and influencing the performance of lines under drought environments in Drysdale/Gladius which may need to be further investigated.

3.6. Conclusion

A yield QTL on Drysdale/Gladius chromosome 1B was expressed only under severe drought and high temperature conditions and predominantly contributed to seeds/spikelet that from high floret fertility. The yield QTL was co-located with biomass and relative leaf expansion rate indicating that the Drysdale allele increases early growth and assimilate accumulation during the favourable winter season. These assimilates can then be remobilized to maintain seeds/spike and then eventually grain yield under stress. To our knowledge, this study is the first report of QTL for grain yield and yield components under severe drought and heat stress conditions in the field that match a QTL for leaf expansion rate under controlled conditions measured using a high throughput imaging platform. Thus, the result of this study will help us to further fine map the yield QTL in Drysdale/Gladius using rate of leaf expansion as a proxy trait. This result also helps understanding the mechanisms underlying this yield QTL under drought and hot environments. The markers linked to these QTL could also be used by breeders to select potential drought tolerant lines at early growing stages under controlled conditions to develop high yielding cultivars for dry and hot environments.

3.7. References

Alexandratos N, Bruinsma J (2012) World agriculture towards 2030/2050: the 2012 revision. ESA Working paper Rome, FAO

Araus JL, Slafer GA, Royo C, Serret MD (2008) Breeding for yield potential and stress adaptation in cereals. Critical Reviews in Plant Science 27:377-412

Beales J, Turner A, Griffiths S, Snape JW, Laurie DA (2007) A pseudo-response regulator is misexpressed in the photoperiod insensitive Ppd-D1a mutant of wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics 115:721-733

Bennett D, Reynolds M, Mullan D, Izanloo A, Kuchel H, Langridge P, Schnurbusch T (2012) Detection of two major grain yield QTL in bread wheat (*Triticum aestivum* L.) under heat, drought and high yield potential environments. Theoretical and Applied Genetics 125:1473-1485

Blum A (1997) Improving wheat grain filling under stress by stem reserve mobilisation. Wheat: Prospects for Global Improvement. Springer, pp 135-141

Blum A, Klueva N, Nguyen H (2001) Wheat cellular thermotolerance is related to yield under heat stress. Euphytica 117:117-123

Bonneau J, Taylor J, Parent B, Bennett D, Reynolds M, Feuillet C, Langridge P, Mather D (2013) Multi-environment analysis and improved mapping of a yield-related QTL on chromosome 3B of wheat. Theoretical and Applied Genetics 126:747-761

Calderini D, Quintero A, Hasan A, Wingen L, Griffiths S, Girousse C, Mouzeyar S, Le Gouis J (2013) Improving grain filling and potential grain size. International Workshop of the Wheat Yield Consortium, 3 Proceedings; CENEB, CIMMYT Cd Obregon, Sonora (Mexico); 5-7 Mar 2013. CIMMYT, p 39

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963-971

Condon AG, Farquar G, Rebetzke GJ, Richards RA (2006) The application of carbon isotope discrimination in cereal improvement for water-limited environments. Drought adaptation in cereals:171-219

Díaz A, Zikhali M, Turner AS, Isaac P, Laurie DA (2012) Copy number variation affecting the Photoperiod-B1 and Vernalization-A1 genes is associated with altered flowering time in wheat (Triticum aestivum). PLoS One 7:e33234

Dietrich CR, Cui F, Packila ML, Li J, Ashlock DA, Nikolau BJ, Schnable PS (2002) Maize Mu transposons are targeted to the 5′ untranslated region of the gl8 gene and sequences flanking Mu target-site duplications exhibit nonrandom nucleotide composition throughout the genome. Genetics 160:697-716

Eagles HA, Cane K, Vallance N (2009) The flow of alleles of important photoperiod and vernalisation genes through Australian wheat. Crop and Pasture Science 60:646-657

Edwards D, Wilcox S, Barrero RA, Fleury D, Cavanagh CR, Forrest KL, Hayden MJ, Moolhuijzen P, Keeble-Gagnere G, Bellgard MI, Lorenc MT, Shang CA, Baumann U, Taylor JM, Morell MK, Langridge P, Appels R, Fitzgerald A (2012) Bread matters: a national initiative to profile the genetic diversity of Australian wheat. Plant Biotechnology Journal 10:703-708

Edwards J (2012) A genetic analysis of drought related traits in hexaploid wheat. The University of Adelaide, School of Agriculture. Food and Wine (PhD thesis)

FAOSTAT F (2015) Food and Agriculture Organization of the United Nations Statistics Division. Rome: FAO

Fischer R (2007) Understanding the physiological basis of yield potential in wheat. The Journal of Agricultural Science 145:99

Fleury D, Jefferies S, Kuchel H, Langridge P (2010) Genetic and genomic tools to improve drought tolerance in wheat. Journal of Experimental Botany 61:3211-3222

Gavran M (2012) Australian plantation statistics 2012 update. ABARES

Griffiths S, Wingen L, Pietragalla J, Garcia G, Hasan A, Miralles D, Calderini DF, Ankleshwaria JB, Waite ML, Simmonds J (2015) Genetic dissection of grain size and grain number trade-offs in CIMMYT wheat germplasm. PloS One 10:e0118847

He C, Holme J, Anthony J (2014) SNP genotyping: the KASP assay. Crop Breeding: Methods and Protocols:75-86

International VSN (2013) GenStat for Windows, 16th Edn. VSN International Hemel Hempstead, UK

International Wheat Genome Sequencing C (2014) A chromosome-based draft sequence of the hexaploid bread wheat (T*riticum aestivum*) Genome. Science 345:1251788

Izanloo A, Condon AG, Langridge P, Tester M, Schnurbusch T (2008) Different mechanisms of adaptation to cyclic water stress in two South Australian bread wheat cultivars. Journal of Experimental Botany 59:3327-3346

Lopes M, Reynolds M, Manes Y, Singh R, Crossa J, Braun H (2012) Genetic yield gains and changes in associated traits of CIMMYT spring bread wheat in a "historic" set representing 30 years of breeding. Crop Science 52:1123-1131

Lopes MS, Reynolds MP (2011) Drought adaptive traits and wide adaptation in elite lines derived from resynthesized hexaploid wheat. Crop Science 51:1617-1626

Maphosa L, Langridge P, Taylor H, Emebiri LC, Mather DE (2015) Genetic control of grain protein, dough rheology traits and loaf traits in a bread wheat population grown in three environments. Journal of Cereal Science 64:147-152

Maphosa L, Langridge P, Taylor H, Parent B, Emebiri LC, Kuchel H, Reynolds MP, Chalmers KJ, Okada A, Edwards J (2014) Genetic control of grain yield and grain physical characteristics in a bread wheat population grown under a range of environmental conditions. Theoretical and Applied Genetics 127:1607-1624

Meng L, Li H, Zhang L, Wang J (2015) QTL IciMapping: Integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. The Crop Journal 3:269-283

Millet EJ, Welcker C, Kruijer W, Negro S, Coupel-Ledru A, Nicolas SD, Laborde J, Bauland C, Praud S, Ranc N (2016) Genome-wide analysis of yield in Europe: allelic effects vary with drought and heat scenarios. Plant Physiology 172:749-764

Miralles DJ, Slafer GA (2007) Sink limitations to yield in wheat: how could it be reduced? Journal of Agricultural Science:145úm 2, p 139-149

Parent B, Bonneau J, Maphosa L, Kovalchuk A, Langridge P, Fleury D (2017) Quantifying wheat sensitivities to environmental constraints to dissect G x E in the field. Plant Physiology:pp-00372

Parent B, Shahinnia F, Maphosa L, Berger B, Rabie H, Chalmers K, Kovalchuk A, Langridge P, Fleury D (2015) Combining field performance with controlled environment plant imaging to identify the genetic control of growth and transpiration underlying yield response to water-deficit stress in wheat. Journal of Experimental Botany 66:5481-5492

Peltonen-Sainio P, Kangas A, Salo Y, Jauhiainen L (2007) Grain number dominates grain weight in temperate cereal yield determination: Evidence based on 30 years of multi-location trials. Field Crops Research 100:179-188

Quarrie S, Pekic Quarrie S, Radosevic R, Rancic D, Kaminska A, Barnes J, Leverington M, Ceoloni C, Dodig D (2006a) Dissecting a wheat QTL for yield present in a range of environments: from the QTL to candidate genes. Journal of Experimental Botany 57:2627-2637

Quarrie SA, Pekic Quarrie S, Radosevic R, Rancic D, Kaminska A, Barnes JD, Leverington M, Ceoloni C, Dodig D (2006b) Dissecting a wheat QTL for yield present in a range of environments: from the QTL to candidate genes. Journal of Experimental Botany 57:2627-2637

Ray DK, Mueller ND, West PC, Foley JA (2013) Yield trends are insufficient to double global crop production by 2050. PLoS One 8:e66428

Regan KL, Siddique KHM, Turner NC, Whan BR (1992) Potential for increasing early vigour and total biomass in spring wheat. II. Characteristics associated with early vigour. Aust J Agric Res 43:541-553

Reynolds M, Dreccer F, Trethowan R (2007) Drought-adaptive traits derived from wheat wild relatives and landraces. Journal of Experimental Botany 58:177-186

Richards RA (2006) Physiological traits used in the breeding of new cultivars for water-scarce environments. Agricultural Water Management 80:197-211

Rosegrant M, Agcaoili M (2010) Global food demand, supply, and price prospects to 2010. International Food Policy Research Institute, Washington, DC USA

Semagn K, Babu R, Hearne S, Olsen M (2014) Single nucleotide polymorphism genotyping using Kompetitive Allele Specific PCR (KASP): overview of the technology and its application in crop improvement. Molecular Breeding 33:1-14

Shiferaw B, Smale M, Braun H-J, Duveiller E, Reynolds M, Muricho G (2013) Crops that feed the world 10. Past successes and future challenges to the role played by wheat in global food security. Food Security 5:291-317

Sloane D (1999) Early vigour: its role in enhancing the productivity of wheat grown in South Australia

Tahmasebi S, Heidari B, Pakniyat H, McIntyre CL (2016) Mapping QTLs associated with agronomic and physiological traits under terminal drought and heat stress conditions in wheat (Triticum aestivum L.). Genome 60:26-45

Tardieu F (2011) Any trait or trait-related allele can confer drought tolerance: just design the right drought scenario. Journal of Experimental Botany:err269

Wang S, Basten C, Zeng Z (2012) Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC, 2010.

Wang S, Wong D, Forrest K, Allen A, Chao S, Huang BE, Maccaferri M, Salvi S, Milner SG, Cattivelli L (2014) Characterization of polyploid wheat genomic diversity using a high- density 90 000 single nucleotide polymorphism array. Plant Biotechnology Journal 12:787-796

Wilkinson PA, Winfield MO, Barker GL, Allen AM, Burridge A, Coghill JA, Edwards KJ (2012) CerealsDB 2.0: an integrated resource for plant breeders and scientists. BMC bioinformatics 13:219

Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. Weed Research 14:415-421

Zikhali M, Wingen LU, Leverington- Waite M, Specel S, Griffiths S (2017) The identification of new candidate genes Triticum aestivum FLOWERING LOCUS T3- B1 (TaFT3- B1) and TARGET OF EAT1 (TaTOE1- B1) controlling the short day photoperiod response in bread wheat. Plant, Cell & Environment

Tables and Figures

Table 1. Descriptive statistics and ANOVA results for yield, yield components and maturity traits of Drysdale/Gladius RIL population under drought and well-watered conditions in 2014 and 2015 trials.

Years	**2014 trial**										**2015 trial**				
Treatment	**Well-watered**					**Drought**					**Drought**				
Genotype + parentals	**Drysdale**	**Gladius**	**96 RILs**			**Drysdale**	**Gladius**	**96 RILs**			**Drysdale**	**Gladius**	**148 RILs**		
Traits	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**h^2 (%)**	**F-test**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**h^2 (%)**	**F-test**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**h^2 (%)**	**F-test**
Morphological and physiological traits															
Early vigor (scale)	3 5 ± 1 0	3 0 ± 0 0	3 1 ± 1 3	63	***	3 5 ± 1 3	3 5 ±0 6	3 4 ± 1 1	34	*	3 5 ± 0 6	2 9 ± 0 4	3 3 ± 1	77	***
Leaf area (cm^2)	26 5 ± 3 6	20 3 ± 0 6	21 2 ± 5 9	55	**	32 2 ± 5 9	26 9 ± 6 7	24 5 ± 6 5	62	**	—	—	—	—	—
NDVI	—	—	—	—	—	—	—	—	—	—	0 6 ± 0 1	0 0 6 ± 0 0	0 6 ± 0 1	49	***
Canopy temperature at vegetative stage	—	—	—	—	—	—	—	—	—	—	16 1 ± 1 6	16 3 ±1 8	15 7± 1 9	23	***
Canopy temperature at grain filling stage	—	—	—	—	—	—	—	—	—	—	18 2 ± 3 6	17 7 ± 4 4	15 7± 1 9	30	***
Plant height (cm)	76 0 ± 5 4	83 3 ± 1 5	78 1 ± 7 8	80	**	75 3 ± 8 7	68 ± 5 3	69 8 ± 6 7	51	***	75 7 ± 3 7	64 1 ± 3 8	68 4 ± 7 3	70	***
Maturity traits															
Days to heading	75 8 ± 2 8	73 0 ± 0 0	75 6 ± 5 5	87	***	70 3 ±2 1	72 5 ± 1 0	73 5 ± 5 1	93	***	87 6 ± 2 4	88 9 ± 2 4	88 1 ± 3 4	76	**
Days to anthesis	79 0 ± 1 4	76 8 ± 0 5	78 9 ± 5 4	90	***	74 0 ± 1 6	75 8 ± 1 5	76 7 ± 4 9	90	***	90 9 ± 2 0	92 5 ±2 1	91 5 ± 2 4	70	**
Days to maturity	119 5 ± 4 2	113 8 ± 1 5	103 9 ± 1 81	68	***	102 0 ± 1 4	105 0 ± 1 2	103 9 ± 4 6	78	**	118 9 ± 4 9	120 9 ± 5 1	118 4 ± 4 6	36	**
Grain filling period	37 0 ± 1 2	40 5 ± 3	37 6 ± 3 9	20	ns	28 0 ±1 8	29 3 ±1 3	27 2 ± 3 3	35	*	28 0 ± 3 4	28 4 ± 3 2	26 9 ± 3 1	15	ns
Yield components															
Fertile tillers/plant	4 2 ± 0 3	3 6 ± 0 5	3 5 ± 1 1	56	***	2 2 ± 0 5	2 2 ± 0 9	2 3 ± 0 8	25	ns	2 1 ± 0 5	2 2 ± 0 5	2 2 ± 0 6	61	***
Sterile tillers/plant	1 0 ± 1 0	1 0 ± 0 5	1 2 ± 0 7	45	**	1 8 ± 0 3	1 2 ± 0 4	1 7 ± 0 8	67	**	1 0 ± 0 3	0 9 ± 0 3	0 9 ± 0 5	35	**
Spike length (cm)	8 8 ± 0 1	8 1 ± 0 3	8 4 ± 0 9	73	**	8 6 ± 0 4	7 8 ± 0 4	8 1 ± 0 8	72	**	10 2 ± 0 6	8 2 ± 0 3	9 1 ± 0 8	86	***
Spikelet/spike	17 7 ± 1 1	17 8 ± 1 1	18 0 ± 1 9	79	***	18 1 ± 0 3	17 2 ± 0 8	17 8 ± 0 4	83	**	20 4 ± 0 7	16 4 ± 0 3	18 4 ± 1 8	91	***
Seeds/spikelet	2 8 ± 0 3	2 8 ± 0 1	2 6 ± 0 4	66	***	2 7 ± 0 3	2 4 ± 0 3	2 4 ± 0 4	71	**	2 7 ± 0 2	2 6 ± 0 2	2 4 ± 0 4	75	***
Seeds/spike	49 9 ± 2 9	49 5 ± 2 4	47 5 ± 8 9	69	***	45 8 ± 5 3	43 9 ± 3 7	43 1 ± 7 5	55	**	53 6 ± 5 1	44 4 ± 3 2	43 5 ± 7 5	69	***
Grain yield/plant (g)	1 7 ± 0 1	1 6 ± 0 1	1 7 ± 0 3	71	**	1 6 ± 0 2	1 5 ± 0 8	1 4 ± 0 2	38	ns	2 5 ± 1 1	2 1 ± 0 7	1 95 ± 0 9	47	***
Biomass/plant (g)	3 6 ± 1 0	3 5 ± 1 0	3 4 ± 0 7	32	ns	3 3 ± 0 3	3 1 ± 0 4	3 1 ± 0 5	53	**	6 5 ± 1 5	4 8 ± 1 8	5 5 + 1 9	56	***
Harvest index(%)	49 5 ±14 0	50 1 ± 13 8	48 9 ± 5 6	35	ns	46 5 ± 3 5	41 4 ± 6 9	45 1 ± 5 1	48	*	41 7 ± 18 0	38 7 ± 8 7	34 6 ± 6 7	23	**
Thousand grain weight (g)	46 5 ±2 7	48 3 ± 3 5	46 6 ± 5 2	56	**	40 7 ± 5 0	38 7 ± 4 0	38 6 ± 5 2	63	**	27 8 ± 5 7	27 0 ± 6 3	25 7 ± 5 8	50	**

— : trait not measured

SD = standard deviation, h^2 = heritability, **P <0.001, *P <0.01 and P < 0.05 and ns.

Table 2. Correlations between yield, yield components and maturity traits of Drysdale /Gladius RIL population under drought (below diagonal) and well-watered (above diagonal) conditions in the 2014 trial.

	Vig	**LA**	**FT**	**DTH**	**DTA**	**DTM**	**GFP**	**PH**	**SL**	**SpS**	**SSp**	**SS**	**YLD**	**BM**	**HI**	**TGW**
Vig	-	0.18*	0.45***	-0.05	-0.05	0.01	0.09	0.46***	0.50***	0.35***	0.38***	0.47***	0.32***	0.19*	0.19*	0.26**
LA	0.22***	-	0.14	-0.42***	-0.42***	-0.12	0.40***	-0.07	0.21*	-0.06	0.29***	0.18*	0.11	-0.02	0.18*	0.17
FT	0.37***	0.32***	-	0.32***	0.33***	0.47***	0.25**	0.51***	0.54***	0.50***	0.40***	0.57***	0.33***	0.27**	0.1	0.15
DTH	-0.16*	-0.59***	-0.05	-	0.98***	0.76***	-0.20*	0.43***	0.35***	0.58***	-0.14	0.22*	0.25**	0.48***	-0.34***	-0.16
DTA	-0.18**	-0.57***	-0.05	0.96***	-	0.76***	-0.22**	0.45***	0.37***	0.57***	-0.13	0.22*	0.26**	0.49***	-0.34***	-0.14
DTM	-0.07	-0.43***	0.03	0.75***	0.76***	-	0.46***	0.41***	0.45***	0.58***	0.12	0.42***	0.43***	0.52***	-0.13	0.01
GFP	0.17**	0.25***	0.12	-0.38***	-0.43***	0.26***	-	-0.01	0.16	0.09	0.37***	0.33***	0.30***	0.11	0.27***	0.19*
PH	0.45***	0.16*	0.27***	-0.22**	-0.17*	-0.12	0.08	-	0.64***	0.56***	0.15	0.42***	0.37***	0.38***	-0.01	0.29***
SL	0.32***	0.24***	0.41***	0.14	0.15*	0.17*	0.01	0.45***	-	0.79***	0.27***	0.64***	0.48***	0.42***	0.06	0.22**
SpS	0.16*	-0.22**	0.19**	0.61***	0.59***	0.54***	-0.14	0.21**	0.61***	-	0.11	0.63***	0.41***	0.46***	-0.08	-0.02
SSp	0.33***	0.36***	0.26***	-0.52***	-0.53***	-0.33***	0.33***	0.27***	0.12	-0.35***	-	0.83***	0.57***	0.34***	0.35***	0.02
SS	0.42***	0.23***	0.39***	-0.14*	-0.17*	0.003	0.25***	0.40***	0.53***	0.30***	0.78***	-	0.67***	0.53***	0.21**	-0.02
YLD	0.22**	0.01**	0.20**	-0.24***	-0.25*	-0.06	0.30**	0.35***	0.22**	0.07	0.33***	0.29***	-	0.80***	0.27***	0.30***
BM	0.14*	0.18	0.17*	0.09	0.06	0.22	0.22**	0.28***	0.30***	0.29***	0.12	0.31***	0.79***	-	-0.34***	0.1
HI	0.19**	0.30***	0.14*	-0.55***	-0.54***	-0.4	0.24***	0.23***	-0.02	-0.25***	0.42***	0.26***	0.65***	0.05	-	0.30***
TGW	0.14***	0.19**	-0.01	-0.32***	-0.29***	-0.21**	0.14*	0.15*	-0.04	-0.19**	-0.15***	0.38***	0.28***	0.18**	0.26***	-

Vig = Early vigour, LA = flag leaf area, FT = fertile tillers, DTH = days to heading, DTA = days to anthesis, DTM = days to maturity, GFP = grain filling duration, PH = plant height, SL = spike length, SpS = Spikelet/Spike, SSp = Seeds/Spikelet, SS = seed/spike, YLD = grain yield, BM = biomass, HI = harvest index and TGW = thousand grain weight, and Values are Pearson correlation coefficients, with significance levels indicated by ***, **, * $p < 0.001$, $p < 0.01$, $p < 0.05$, respectively.

Table 3. Correlations among yield and yield components for 148 Drysdale/Gladius RIL with similar anthesis date in the 2015 trial under drought.

	Vig	**NDVI**	**CTV**	**CTg**	**FT**	**ST**	**DTH**	**DTA**	**DTM**	**GFP**	**PH**	**SL**	**SpS**	**SSp**	**SS**	**YLD**	**BM**	**HI**	**TGW**
Vig	-																		
NDVI	0.66***	-																	
CTV	0.17**	0.25***	-																
CTg	0.22***	0.41***	0.17**	-															
FT	0.45***	0.33	0.03	-0.04	-														
ST	0.04	0.22***	-0.03	0.11	-0.38***	-													
DTH	-0.06	0.12*	0.25***	0.12	0.12*	0.20***	-												
DTA	0.01	0.18***	0.21***	0.09*	0.14**	0.15**	0.97***	-											
DTM	-0.02	0.08	0.20***	-0.07	0.21***	0.07	0.92***	0.93***	-										
GFP	-0.03	-0.1	0.07	-0.34***	0.27***	-0.07	0.54***	0.52***	0.79***	-									
PH	0.37***	0.26***	0.24***	-0.09	0.48***	-0.3	0.26***	0.35***	0.41***	0.39***	-								
SL	0.36***	0.59***	0.31***	0.25***	0.29***	0.16**	0.51***	0.53***	0.50***	0.29***	0.44***	-							
SpS	0.19***	0.40***	0.18***	0.21***	0.21***	0.25***	0.66***	0.61***	0.57***	0.34***	0.25***	0.75***	-						
SSp	0.41***	0.29***	0.38***	0.01	0.30***	-0.23***	-0.03	0.07	0.1	0.13**	0.58***	0.25***	-0.08	-					
SS	0.46***	0.40***	0.37***	0.05	0.36***	-0.13*	0.12*	0.17**	0.19***	0.19***	0.59***	0.52***	0.35***	0.87***	-				
YLD	0.38	0.16**	0.18**	-0.30***	0.72***	-0.38***	0.06	0.09	0.26***	0.49***	0.61***	0.24***	0.13*	0.49***	0.53***	-			
BM	0.09	0.1	0.12*	-0.11	0.20***	0.02	0.12*	0.13*	0.17**	0.21***	0.17**	0.15**	0.09	0.13*	0.12*	0.26***	-		
HI	0.14**	-0.06	0.26***	-0.37***	0.35***	-0.29***	0.15**	0.20***	0.37***	0.55***	0.55***	0.05	0	0.55***	0.46***	0.72***	0.28***	-	
TGW	0.06	-0.07	0.16**	-0.42***	0.25***	-0.12*	0.27***	0.28***	0.48***	0.68***	0.43***	0.17**	0.14*	0.21***	0.20***	0.67***	0.21***	0.75***	-

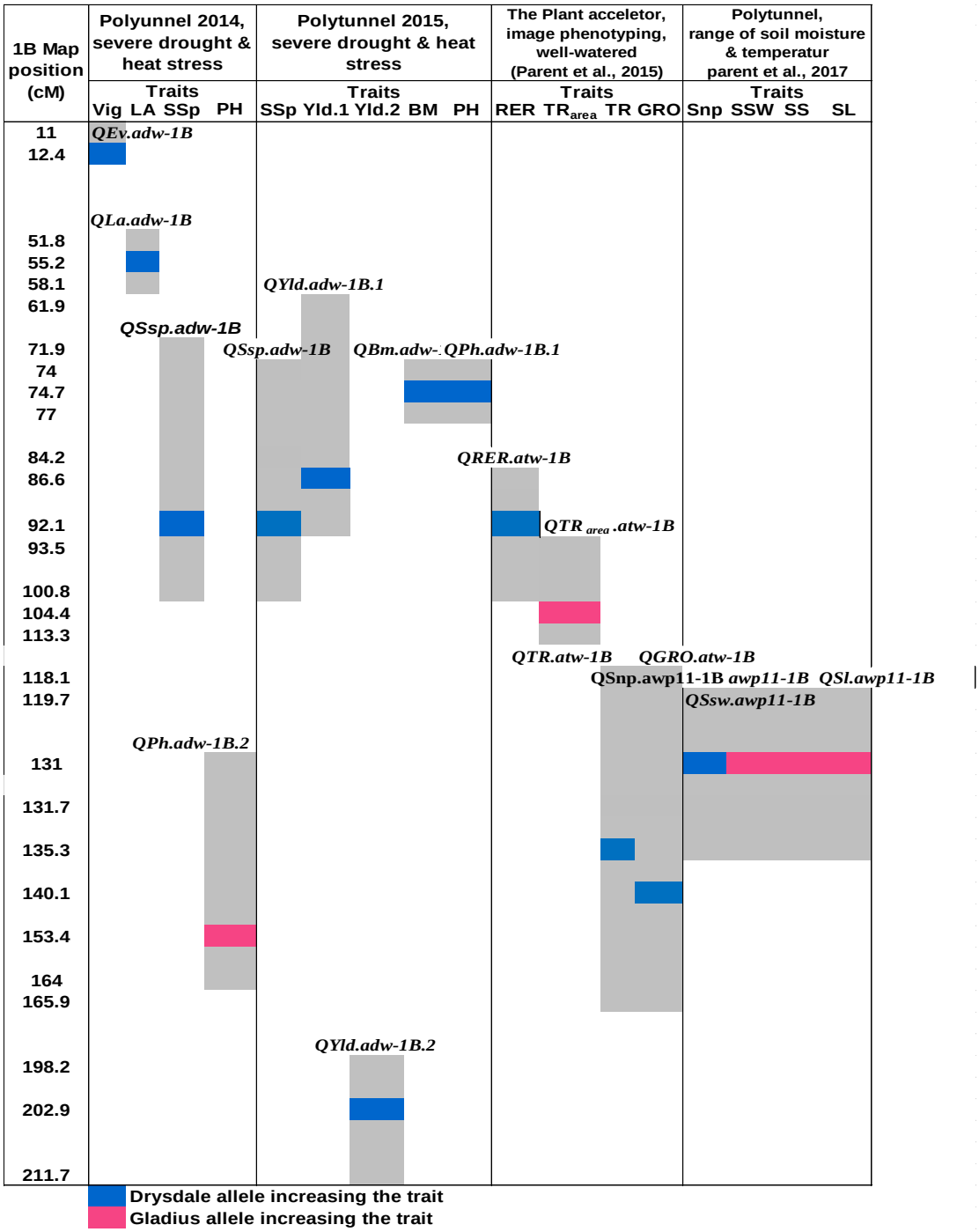
Vig = Early vigour, NDVI = normalize difference vegetative index, CT_v = canopy temperature at vegetative stage, CT_g = canopy temperature at grain filling, FT = fertile tillers, ST = sterile tillers, DTH = days to heading, DTA = days to anthesis, DTM= days to maturity, GFP = grain filling period, PH = plant height, SL = spike length, SpS = Spikelet/Spike, SSp = Seeds/Spikelet, SS = seed/spike, YLD = grain yield, BM = biomass, HI = harvest index and TGW = thousand grain weight. Values are Pearson correlation coefficients, with significance levels indicated by ***, **,* $p < 0.001$, $p < 0.01$ and $p < 0.05$

Table 4. QTL for yield components and growth traits in Drysdale/Gladius RIL under drought condition in the 2014 trial.

Trials	Traits	QTL name	Flanking markers (cM)	Peak position (cM)	LOD[a]	AE	R2 (%)	Allele increasing the traits
	Early vigour	*Qvig.adw-1B*	BS00029345 (11) - BS00043575 (12.4)	11.4	2.8	-0.3	11.3	Gladius
2014 (96 RIL)	Leaf area	*QLa.adw-1B*	adw638 (51.8) - adw357 (58.1)	55.2	2.6	2.9	11.4	Drysdale
Severe drought	Seeds/Spikelet	*QSsp.adw-1B*	adw580 (71.9) - adw582 (100.8)	92.1	4.2	0.3	16.2	Drysdale
	Plant height	*QPh.adw-1B.2*	adw510 (131) - BS00032039 (164)	153.4	4.2	-4.6	51	Gladius
	Grain yield	*QYld.adw-1B.1*	adw653 (61.2) – adw10 (92.1)	86.6	3	0.4	10	Drysdale
	Canopy temperature	*QCtv.adw-1B*	adw506 (74) - adw507 (77)	75.4	3.2	-6.7	8.9	Gladius
	Biomass	*QBm.adw-1B*	adw506 (74) - adw507 (77)	75.4	2.3	11	7	Drysdale
	Plant height	*QPh.adw-1B.1*	adw653 (61.2) – adw10 (92.1)	86.6	2.3	6.8	2.8	Drysdale
2015 (191 RIL)	Seeds/spikelet	*QSsp.adw-1B*	BS00032039 (164.2) – adw522 (192.5)	172.1	3.7	0.2	10.7	Drysdale
Severe drought	Seeds/spike	*QSs.adw-1B*	BS00032039 (164.2) – adw522 (192.5)	172.1	3	0.4	10	Drysdale
	Days to heading	*QDth.adw-1B.2*	BS00032039 (164.2) – adw522 (192.5)	172.1	2.8	0.4	9	Drysdale
	Days to Anthesis	*QDta.adw-1B*	BS00032039 (164.2) – adw522 (192.5)	172.1	2.2	0.6	6.8	Drysdale
	Days to maturity	*QDtm.adw-1B*	BS00032039 (164.2) – adw522 (192.5)	172.1	2.3	11	7	Drysdale
	Plant height	*QPh.adw-1B.2*	BS00032039 (164.2) – adw522 (192.5)	172.1	4.2	-4.6	51	Gladius
	Seeds/spikelet	*QSsp.adw-1B*	adw581 (74.7) – adw582(100.8)	92.1	3.7	0.2	10.7	Drysdale
	Grain yield	*QYld.adw-1B.1*	adw653 (61.9) – adwkasp10 (92.1)	86.6	3	0.4	10	Drysdale
2015 (148 RIL)	Grain yield	*QYld.adw-1B.2*	adw526 (198.2) - adwkasp37 (211.7)	202.9	2.8	0.4	9	Drysdale
Severe drought	Biomass	*QBm.adw-1B*	adw506 (74) - adw507 (77)	75.4	2.3	11	7	Drysdale
	Plant height	*QPh.adw-1B.1*	adw506 (74) - adw507 (77)	75.4	2.3	6.8	2.8	Drysdale
	Ave. relative leaf expansion rate	*QRERAVE.adw-1B*	adw582 (86.6) - adw8 (100.8)	92.1	4.5	3.6	16	Drysdale
TPA (135 RIL)			BS00042340 (118.1 cM)					
Well-watered	Ave.growth rate	*QGRO.atw-1B*	BS00032039 (164.2 cM)	135.3	3.4	0.03	43	Drysdale
			BS00042340 (118.1 cM)					
	Ave. transpiration rate	*QTR.atw-1B*	BS00032039 (164.2 cM)	140.1	6.5	15		Drysdale
	Ave. transpiration rate per unit of leaf area	*QTRarea.atw-1B*	adwkasp3 (93.5 cM) - adw590 (113.3 cM)	104.4	4.2	-0.3	12	Gladius

[a]The LOD threshold was estimated at $\alpha = 0.05$ from 1000 permutation tests for each trait. AE = allele e

Table 5. Co-location of QTL in Drysdale/Gladius RIL found under various moisture and temperature conditions.



Ev = early vigour, La = leaf area, Ssp = seeds/spikelet, Ph = plant height, Yld = grain yield, Bm= biomass, RER = average relative leaf area expansion rate, TRarea= average transpiration rate per unit leaf area, TR = transpiration rate, LER = average leaf area expansion, GRO = average growth rate, Snp = spike number/plant, Ssw = single seed weight, Ss = seeds number/spike and Sl = spike length.

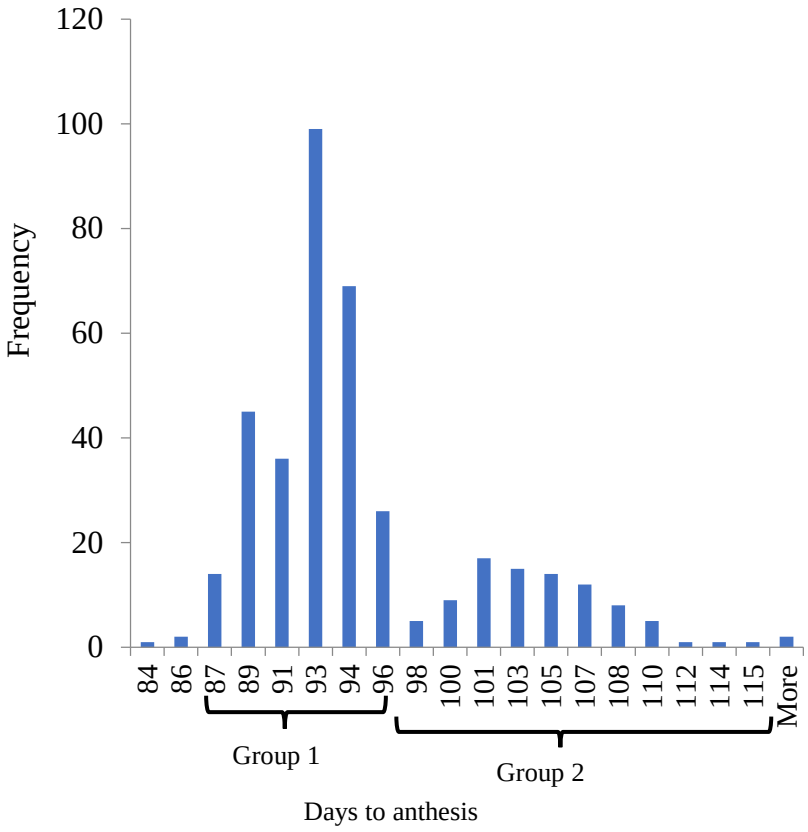


Figure 1. Frequency distribution of 191 RIL for days to anthesis.

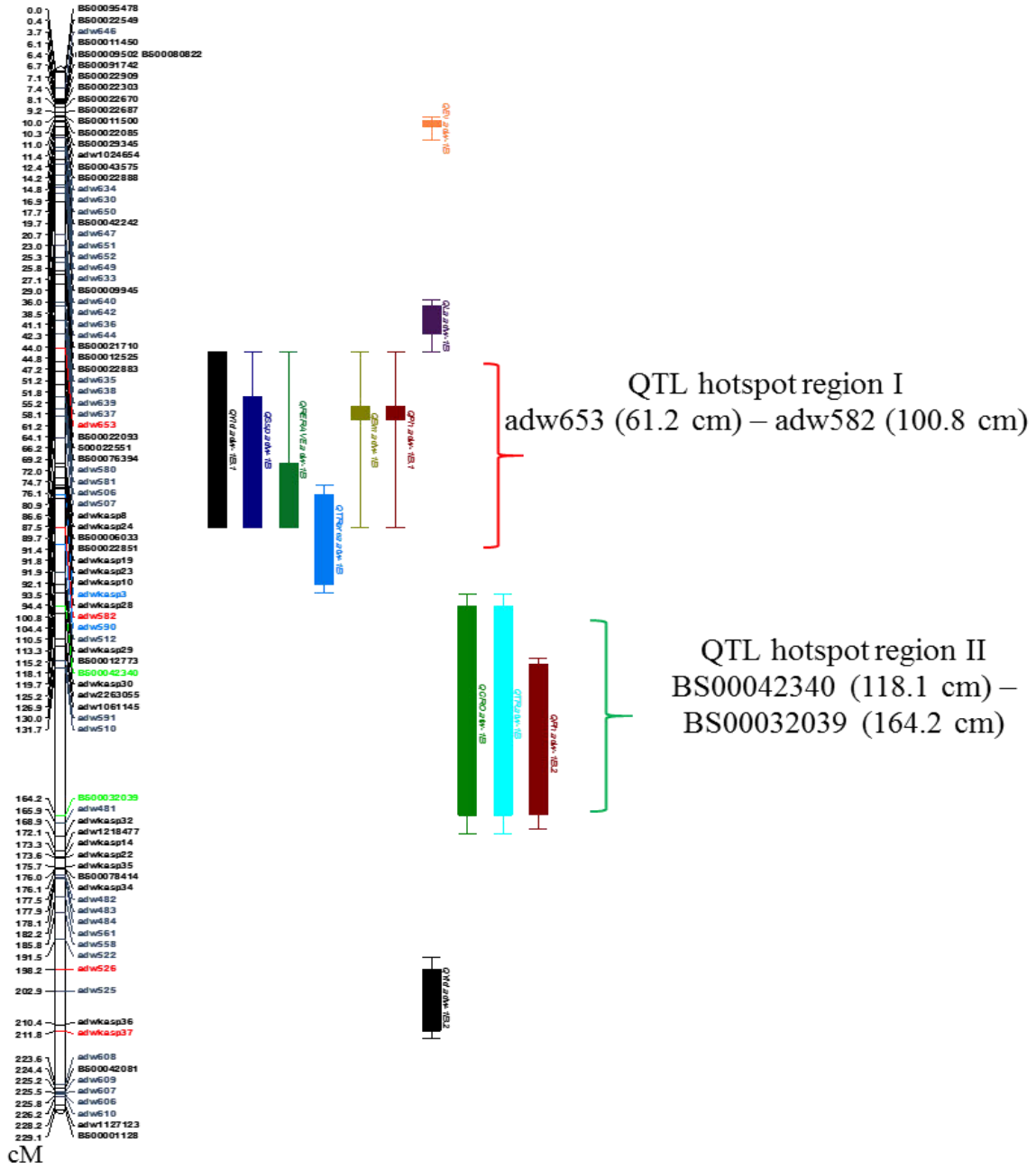


Figure 2. Positions of QTL on the new Drysdale/Gladius 1B genetic map (constructed from 371 RIL and 93 KASP™ markers) phenotypic data of 148 RIL in 2015 trial.

Ev = Early vigour, La = leaf area, Yld = grain yield, Ssp = seeds/spikelet, RER = average relative leaf area expansion rate, Bm = biomass, Ph = plant height, TRarea = average transpiration rate per unit leaf area, TR = transpiration rate, LER = average leaf area expansion, GRO = average growth rate

Supplementary Tables and Figures

Supplementary Table 1. Scaffolds from whole genome sequence assembly of Chinese Spring (IWGSC WGS v0.4) covering the region delineated by markers BS00022093 (56.3 cM) and adw2263055 (80.4 cM) of chromosome.

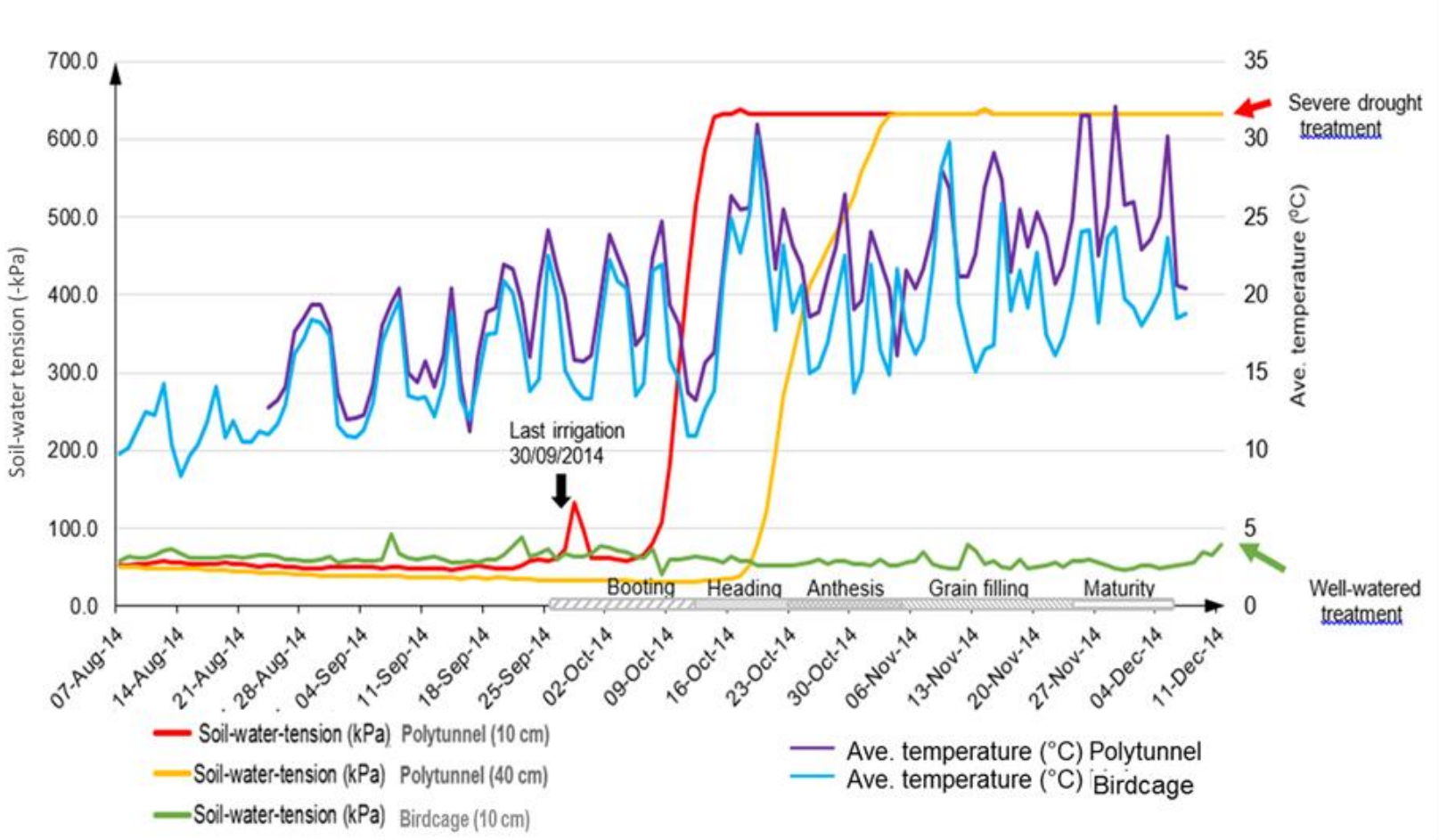
Marker ID	Position on Drysdale/Gladius map (cM)	Scaffold ID	Chromosome	Position on Chinese Spring POPSEQ map (cM)	Scaffold length (Mbp)	Coordinates on IWGSC WGA v0.4		Score	e-value
						Start (Mbp)	End (Mbp)		
BS00022093	57.9	scaffold68389	1B	47.8	11,686,755	27,940,852	539,627,606	141	2E-31
BS00086083	61.4	scaffold84115	1B	55.1	2,538,423	560,662,514	563,200,936	133	5.00E-29
BS0002551	64.2	scaffold8565	1B	57.8	,8642,656	566,366,724	575,009,379	141	2.00E-31
BS00076394	64.4	scaffold111335	1B	58.6	7,564,229	579,915,508	587,479,736	141	2.00E-31
1B_BS00006	68.8	scaffold143423	1B	66.1	22,153,207	602,310,145	624,463,351	133	5.00E-29
adwkasp29	74.1	scaffold106874	1B	72.2	3,130,190	624,463,452	627,593,641	380	e-103
BS00042340	74.0	scaffold71667	1B	74.5	4,718,469	627,593,742	632,312,210	141	2.00E-31
ADW226305	80.0	scaffold54766	1B	84.6	9,781,840	641,923,531	651,705,370	129	8.00E-28
		Total length			**70,215,769**				

Supplementary Table 2. Primer sequence of KASP™ markers added to the Drysdale/Gladius chromosome 1B genetic map.

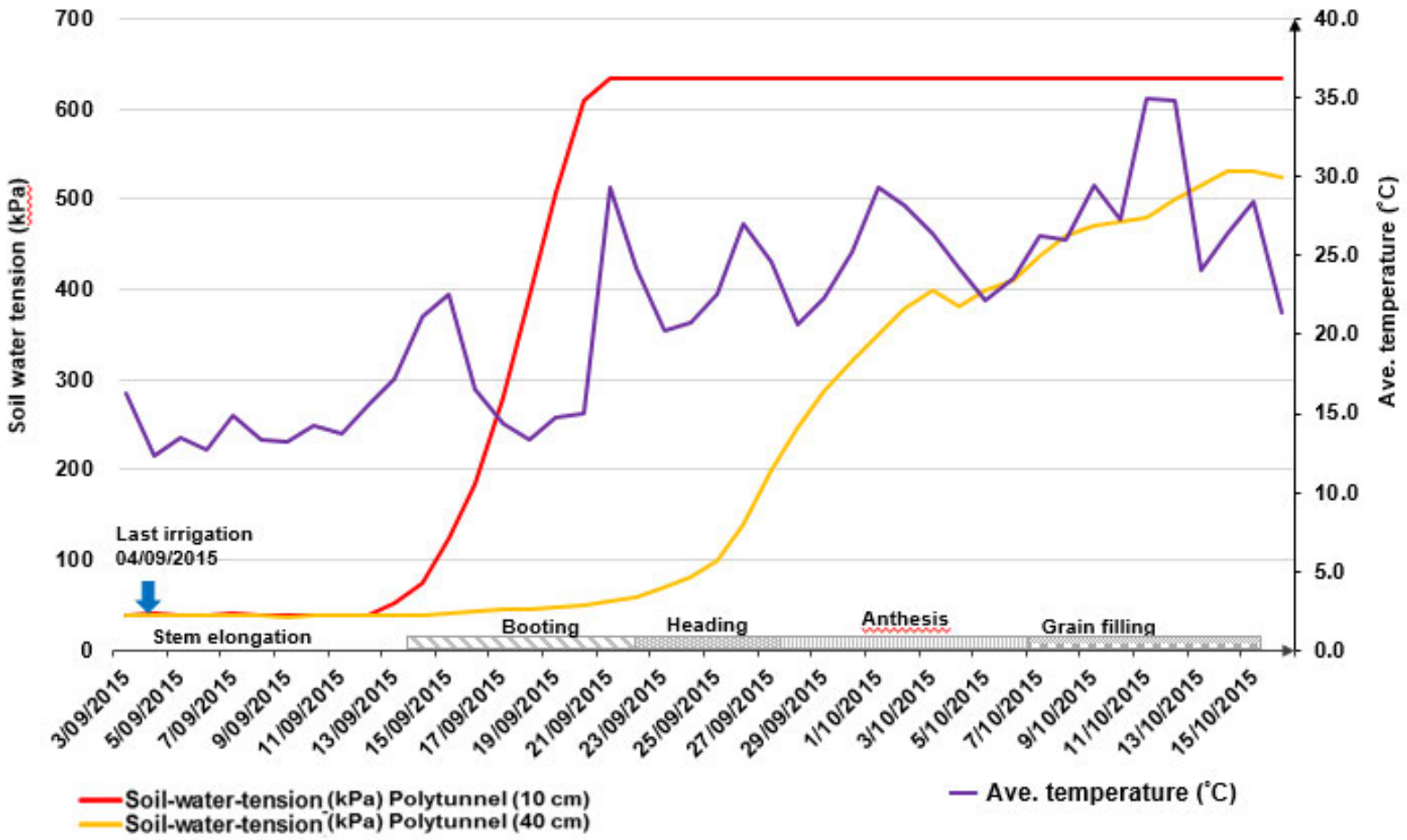
No.	SNP KASP™ marker ID	Primer_Allele X	Primer_Allele Y	Primer_Common	Allele X	Allele Y
1	adw481	GAAGGTGACCAAGTTCATGCTAGAGCCTTACGCACCTCGGTT	GAAGGTCGGAGTCAACGGATTGAGCCTTACGCACCTCGGTC	CGGGCATGGATATGGCTATGGAAAA	A	G
2	adw482	GAAGGTGACCAAGTTCATGCTTGAAATATCCCAATGATTAGTAAACTGCA	GAAGGTCGGAGTCAACGGATTGAAATATCCCAATGATTAGTAAACTGCG	CCCGCAATTAAAAGGTAGAATGTAGATGTA	A	G
3	adw483	GAAGGTGACCAAGTTCATGCTAACACTATCAATGTATACCAATAACAGCA	GAAGGTCGGAGTCAACGGATTCACTATCAATGTATACCAATAACAGCC	GTCTGTGCCATGATTGAATTTATGAGGAA	T	G
4	adw484	GAAGGTGACCAAGTTCATGCTGGAGCTGGCTATTGCGGCAG	GAAGGTCGGAGTCAACGGATTATGGAGCTGGCTATTGCGGCAA	CCAACGGTGTACTGTGTTTGGTAGTT	G	A
5	adw506	GAAGGTGACCAAGTTCATGCTACTTGCATTCCTACCTGAAACTGCA	GAAGGTCGGAGTCAACGGATTGCATTCCTACCTGAAACTGCC	GGTTGCACAACTAGATGGGCAGTAT	A	C
6	adw507	GAAGGTGACCAAGTTCATGCTGAGGCAGTCGTCATCTTTAGCC	GAAGGTCGGAGTCAACGGATTAATGAGGCAGTCGTCATCTTTAGCA	TCATCTCAAAACCAGAAGTTACGGTTGTT	C	A
7	adw510	GAAGGTGACCAAGTTCATGCTCAGCCCAAATAAATGAGTATGAGGAC	GAAGGTCGGAGTCAACGGATTACAGCCCAAATAAATGAGTATGAGGAT	AGGACACAGGCTAATCATGAAGAAATGAA	G	A
8	adw512	GAAGGTGACCAAGTTCATGCTCTGATGAATTCCTTGGTATGTGGC	GAAGGTCGGAGTCAACGGATTCTCTGATGAATTCCTTGGTATGTGGT	TGTCCCCAAGAAGGTTAACTGGGAA	G	A
9	adw522	GAAGGTGACCAAGTTCATGCTACCGTCCACCATGCCCCG	GAAGGTCGGAGTCAACGGATTACCGTCCACCATGCCCCC	GTCGGCCTCGTAGCTGGCGTT	G	C
10	adw525	GAAGGTGACCAAGTTCATGCTTCCAGTTCCATCGATCTATGCG	GAAGGTCGGAGTCAACGGATTCTTCCAGTTCCATCGATCTATGCA	GATCTAGGAAGCAATGGACAATGAATGAT	G	A
11	adw526	GAAGGTGACCAAGTTCATGCTACCGTTCCCCGTGCCCA	GAAGGTCGGAGTCAACGGATTACCGTTCCCCGTGCCCG	CGGCCTCGTAGCCGGCGTT	T	C
12	adw558	GAAGGTGACCAAGTTCATGCTCATCTCGGTGACGGCCGAC	GAAGGTCGGAGTCAACGGATTGACATCTCGGTGACGGCCGAT	CGGAGCTCAGGGCGCACGAA	C	T
13	adw561	GAAGGTGACCAAGTTCATGCTAGCAGTGCCGACTAAACTGCC	GAAGGTCGGAGTCAACGGATTGAGCAGTGCCGACTAAACTGCT	CATCAGTGTCTATTGCTTCCACTTGAAAT	G	A
14	adw580	GAAGGTGACCAAGTTCATGCTGGAACTCTATAGGATGCACGGGT	GAAGGTCGGAGTCAACGGATTGAACTCTATAGGATGCACGGGC	GTTTGCGAAAGTACGGTATAGAATATGCAT	A	G
15	adw581	GAAGGTGACCAAGTTCATGCTGCGTTCTGCAGCCACTGTCG	GAAGGTCGGAGTCAACGGATTAGCGTTCTGCAGCCACTGTCA	GTCAGGTACTTCAGATCTTAAATTCAACAA	C	T
16	adw582	GAAGGTGACCAAGTTCATGCTCGCTTCATGGTCAGAGAAGGCT	GAAGGTCGGAGTCAACGGATTGCTTCATGGTCAGAGAAGGCG	GACGATCTTTGTAGGACAGGAGCAT	A	C
17	adw590	GAAGGTGACCAAGTTCATGCTGGTTTTAGATACACAAATTGTGCTATTAG	GAAGGTCGGAGTCAACGGATTGGTTTTAGATACACAAATTGTGCTATTAC	TTGGCAAATCCCTAGTACCATGTATGATA	C	G
18	adw591	GAAGGTGACCAAGTTCATGCTAACTTGCTGCTTATAGAACACGCTC	GAAGGTCGGAGTCAACGGATTATAACTTGCTGCTTATAGAACACGCTT	TTGGAGAAACAATTGAAGATGAACGAGAAA	C	T
19	adw606	GAAGGTGACCAAGTTCATGCTTGCCATGTTTGCTTGATCCGCT	GAAGGTCGGAGTCAACGGATTGCCATGTTTGCTTGATCCGCA	GCTCACAGCCAGATATAGTTGCCAA	A	T
20	adw607	GAAGGTGACCAAGTTCATGCTCGGTAACGTGACCAGCTATGAG	GAAGGTCGGAGTCAACGGATTCGGTAACGTGACCAGCTATGAC	GTCCCCAGCTGACTCTAGCCTT	G	C
21	adw608	GAAGGTGACCAAGTTCATGCTCGCATATGAACCCATCCTCATCAT	GAAGGTCGGAGTCAACGGATTGCATATGAACCCATCCTCATCAC	GCTGGAGGAGGCCTTCTGTGTT	T	C

Supplementary Table 3. List of successful KASP™ markers designed from the QTL for seeds/spikelet interval and mapped on Drysdale/Gladius 1B map. Continued).

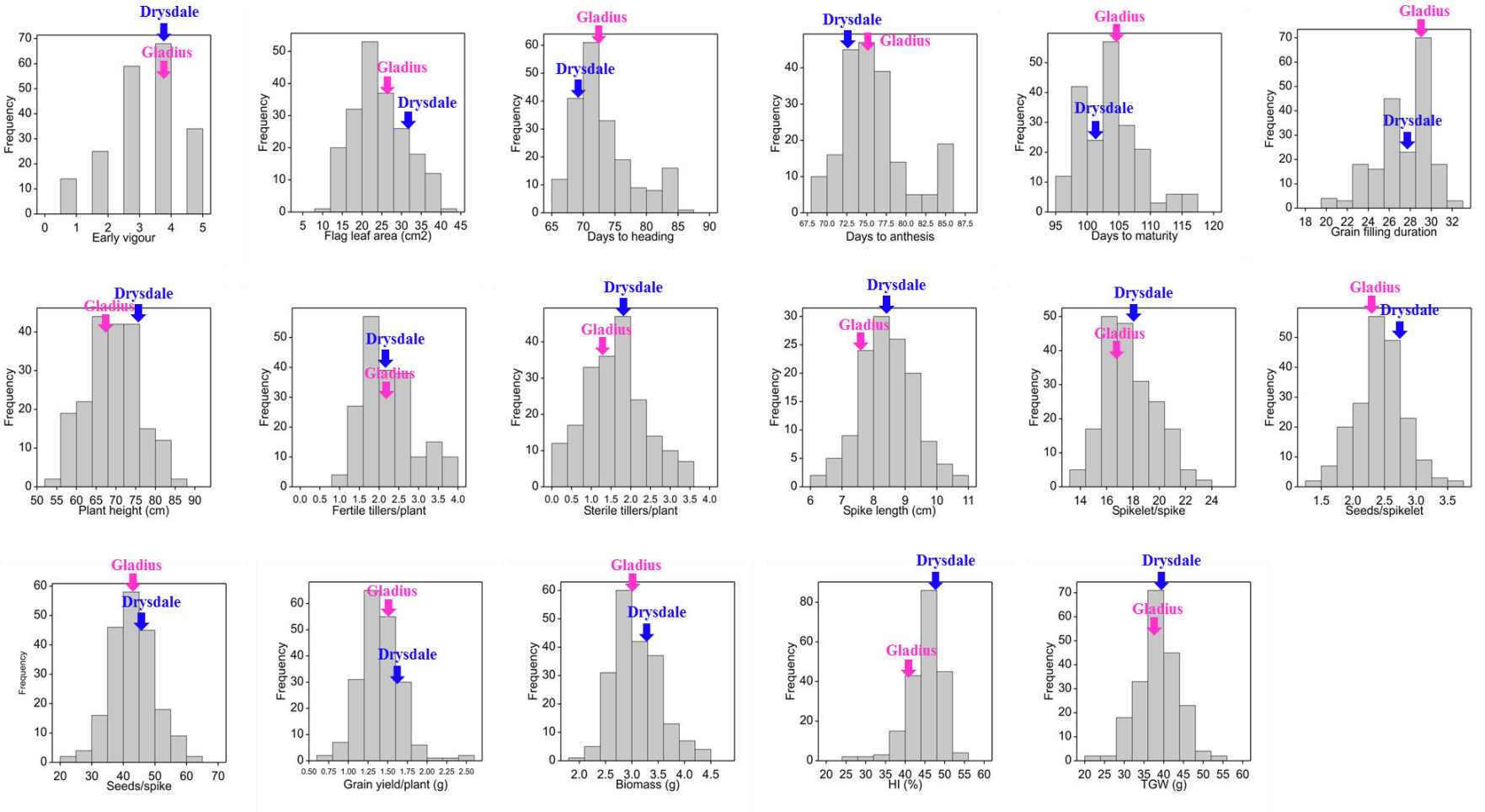
No.	SNP KASP™ marker ID	Primer_Allele X	Primer_Allele Y	Primer_Common	Allele X	Allele Y
22	adw609	GAAGGTGACCAAGTTCATGCTGGAAGAATCATAACCCGGTGATTC	GAAGGTCGGAGTCAACGGATTAATGGAAGAATCATAACCCGGTGATTT	GGGGAGGTAATTAATGCTCATACTAGATT	G	A
23	adw610	GAAGGTGACCAAGTTCATGCTGAATGTAACGCTAAAACGTGTCTAG	GAAGGTCGGAGTCAACGGATTGTGAATGTAACGCTAAAACGTGTCTAA	GCAATAGATTTGTCCAAATGCGGATGTAT	G	A
24	adw630	GAAGGTGACCAAGTTCATGCTGTAATTTGTCGAATTCCCAAGAACAAGT	GAAGGTCGGAGTCAACGGATTAATTTGTCGAATTCCCAAGAACAAGC	AAACTCACAAGCCACCAAATAGAATCCAA	T	C
25	adw633	GAAGGTGACCAAGTTCATGCTGGTTCTGACTCTCCTAGAGAAAACA	GAAGGTCGGAGTCAACGGATTGTTCTGACTCTCCTAGAGAAAACG	CTCGCTTCGTAGTTTTCGTCTAATGAATA	A	G
26	adw634	GAAGGTGACCAAGTTCATGCTACCAATGTGAGCTACTACAATTGAGT	GAAGGTCGGAGTCAACGGATTCCAATGTGAGCTACTACAATTGAGC	ACCCTCATTTTTGTTTGGACACCACAT	A	G
27	adw635	GAAGGTGACCAAGTTCATGCTAAATTATCTTACCAGGAAATATTTTGAATACAT	GAAGGTCGGAGTCAACGGATTAAATTATCTTACCAGGAAATATTTTGAATACAG	TGATTCTCCTTTGTTGAACCTTTGCAGAT	A	C
28	adw636	GAAGGTGACCAAGTTCATGCTTCGTCCTCCGCCTGTCAACA	GAAGGTCGGAGTCAACGGATTCGTCCTCCGCCTGTCAACG	GAGAGGAGGAGGAAACCTGTCAATT	T	C
29	adw637	GAAGGTGACCAAGTTCATGCTGGCAGGCAAATCAGAGACGTGAT	GAAGGTCGGAGTCAACGGATTGCAGGCAAATCAGAGACGTGAC	CTAGCCTCTTGTACTCTTCCAGCAT	T	C
30	adw638	GAAGGTGACCAAGTTCATGCTGACATTTTCACATGACCCACACTGAA	GAAGGTCGGAGTCAACGGATTACATTTTCACATGACCCACACTGAG	GATGAAAGTGAAAGTGAGAACAATGGATTT	T	C
31	adw639	GAAGGTGACCAAGTTCATGCTTGATTGCATCATTTCAATAACTTCGTCAA	GAAGGTCGGAGTCAACGGATTGCATCATTTCAATAACTTCGTCAG	CTGCGTACTCTGAGGATGATGACTA	A	G
32	adw640	GAAGGTGACCAAGTTCATGCTTCGCAGCAGAAACCGATCAATTT	GAAGGTCGGAGTCAACGGATTCGCAGCAGAAACCGATCAATTC	CAGTTGGACTGATCCAACATATCAACATT	T	C
33	adw642	GAAGGTGACCAAGTTCATGCTGGTGGCGCTTGGTTGCCAGT	GAAGGTCGGAGTCAACGGATTGTGGCGCTTGGTTGCCAGC	GGCAACTTTGGTCAGTTTCCACACAT	A	G
34	adw644	GAAGGTGACCAAGTTCATGCTCCAGATGGTGCTAATCTCCCCTT	GAAGGTCGGAGTCAACGGATTCAGATGGTGCTAATCTCCCCTC	CTTGCTGAATCCTTGCCAGACACAA	T	C
35	adw646	GAAGGTGACCAAGTTCATGCTGTCGAAACACTTGCTGGTGTA	GAAGGTCGGAGTCAACGGATTCTGTCGAAACACTTGCTGGTGTG	AAGGGCAATGAACTGCAATTTAGGCTTTT	T	C
36	adw647	GAAGGTGACCAAGTTCATGCTATAAAACACAAGTTTTGAAGAAAATAGGATATA	GAAGGTCGGAGTCAACGGATTATAAAACACAAGTTTTGAAGAAAATAGGATATC	TGGAATTTGCAGGCCATGTATATTCACAA	A	C
37	adw649	GAAGGTGACCAAGTTCATGCTGTGCATACTATCCAGAATCTGGTACT	GAAGGTCGGAGTCAACGGATTGCATACTATCCAGAATCTGGTACG	CTCCGTGCGGCGYTCTAGCAT	T	G
38	adw650	GAAGGTGACCAAGTTCATGCTAACATTAGATTATCGACGCGGCACA	GAAGGTCGGAGTCAACGGATTCATTAGATTATCGACGCGGCACG	GGAATTCTAGTAGGCTTCCGTGGAA	T	C
39	adw651	GAAGGTGACCAAGTTCATGCTTGAAATCTGTTCAGCAGTCAGTGAA	GAAGGTCGGAGTCAACGGATTGAAATCTGTTCAGCAGTCAGTGAG	AAATTCCATGAGGATTTGAGTACACCGTT	T	C
40	adw652	GAAGGTGACCAAGTTCATGCTCAACATGTTCTGAAATCTGGCGATCA	GAAGGTCGGAGTCAACGGATTAACATGTTCTGAAATCTGGCGATCG	TATTGTTCTTGATCAAATGGATGCTGAGAT	A	G
41	adw653	GAAGGTGACCAAGTTCATGCTTGCTATAACTGAAGCCGAAATCCTTA	GAAGGTCGGAGTCAACGGATTGCTATAACTGAAGCCGAAATCCTTG	GATGTGCTCGAGACTGTCAAATATCATAA	T	C



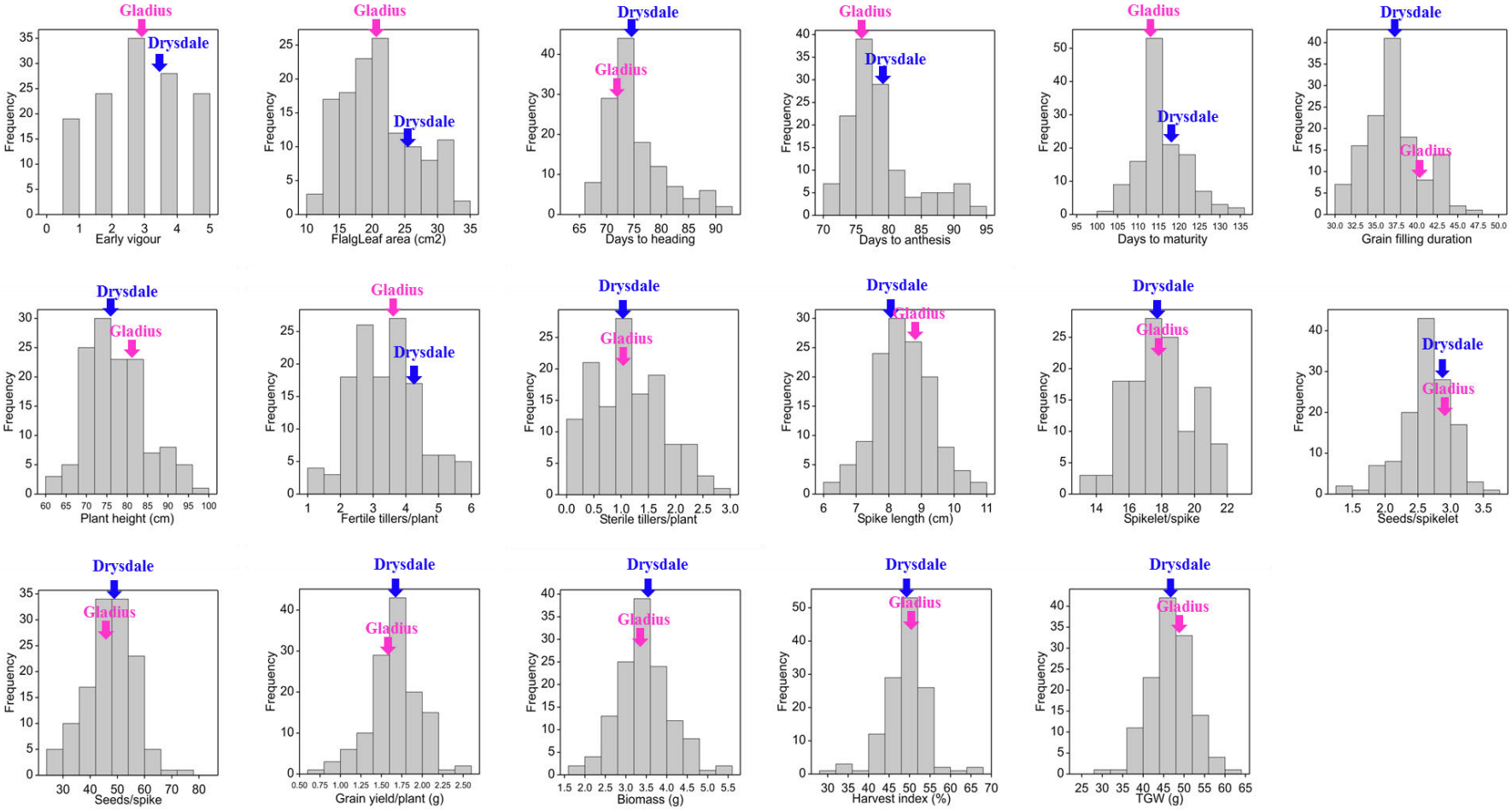
Supplementary Figure: 1. Soil moisture and temperature recorded under well-watered and drought conditions in the 2014 trial.



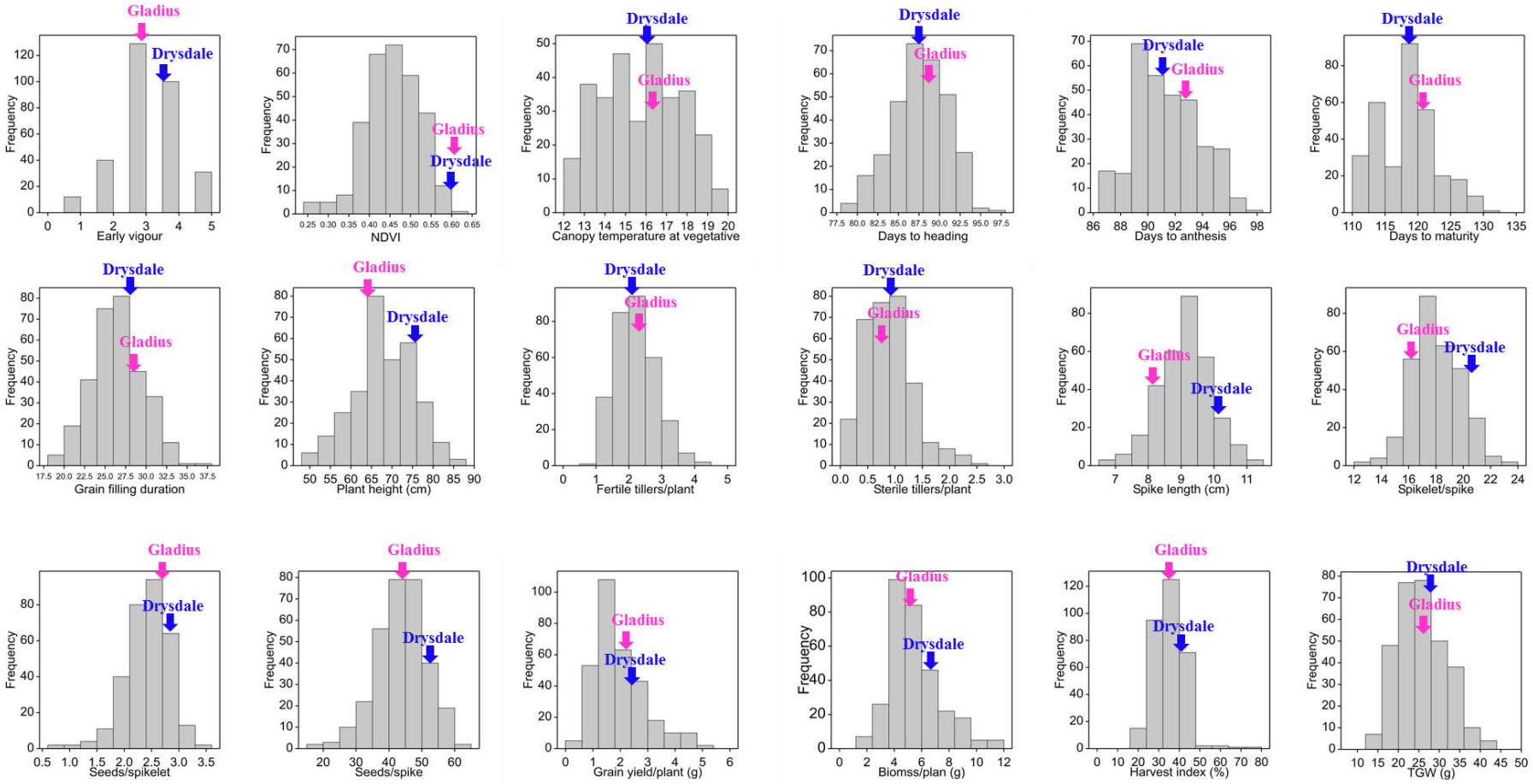
Supplementary Figure: 2. Soil water tension and average daily temperatures in the polytunnel in the 2015 trial.



Supplementary Figure: 3. Frequency distribution of traits measured on 96 Drysdale/Gladius RIL grown under drought condition in the 2014 trial.



Supplementary Figure: 4. Frequency distribution for traits measured on 96 Drysdale/Gladius RIL grown under well-watered condition in the 2014 trial.



Supplementary Figure: 5. Frequency distribution for traits measured on 191 Drysdale/Gladius RIL grown under severe drought and heat stress in 2015 trial.

Chapter 4: Fine mapping of a yield QTL on chromosome 1B in the spring wheat Excalibur/Kukri population

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Principal Author

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Contribution to the Paper	Generated a new genetic map of chromosome 1B in Excalibur/ Kukri DH, fine mapped the yield QTL, developed near-iso-genic lines ,run field trials, analysed data, narrowed down yield QTL and wrote the paper.
Overall percentage (%)	80
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 11/01/2018

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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Contribution to the Paper	Design the project, supervised Habtamu Tura and the overall experiments, reviewed and approved the paper.		
Signature		Date	12/1/2018

Chapter 4: Fine mapping of a yield QTL on chromosome 1B in the spring wheat Excalibur/Kukri population

4.1. Abstract

Grain yield is under the complex genetic control and highly influenced by the environmental conditions. Grain yield, grain weight, days to heading and grain filling duration under drought-stress and irrigated conditions were investigated in the Excalibur/Kukri doubled haploid mapping population in 28 field trials across the climatic zones of southern Australia, India and north western Mexico. Out of 48 QTL detected on the whole genome of Excalibur/Kukri DH, 19 QTL for yield or TGW showed significant G x E interactions. Eleven yield QTL had a significant main effect across environments. Among those, 1B increased both grain yield and TGW and did not co-located with any QTL for days to heading. The 1B yield QTL was highly expressed under severe drought and consistent in multiple South Australian field trials. The QTL was targeted for fine mapping to identify robust markers and candidate genes for marker-assisted breeding for drought tolerance in wheat. The 1B genetic map was improved with 142 markers and average distance of 1.6 cM between adjacent markers. Near iso-genic lines (NIL) were developed and evaluated under semi-controlled conditions to delimit the QTL interval and measure the effect of the QTL under dry and hot conditions. Four NIL pairs showed significant differences in grain yield or at least three yield components, confirming the positive effect of the Excalibur allele on yield, fertile tillers and biomass in the target interval found in DH. The Excalibur allele increased grain yield by 54.5%, biomass by 43%, and fertile tillers by 32.8% in four NIL pairs in which the traits were co-segregating. The 1B yield QTL was narrowed down to 2.9 cM which corresponded to 2.2 Mb region on the chromosome 1B genomic reference sequence of cv. Chinese Spring (IWGSC RefSeq v1.0). Forty two predicted candidate genes were identified in the QTL region for further expression analysis and characterization of the candidate gene/s responsible for grain yield under dry and hot environments.

4.2. Introduction

Improvement in grain yield is a primary target for wheat breeding programs globally and drought is an important abiotic factor affecting wheat production and productivity. The drought area coverage and its intensity are increasing as a result of climate change, population pressure and deforestation. It affects at least 60 million hectares of wheat grown in the drought prone areas every year (Monneveux et al. 2012) and can cause more than 50% of global wheat yield losses (Bray 2000; Kosina et al. 2007; Nezhad et al. 2012). In the last 15 years, more than 51% and 28% of the total area under cereal cultivation was allocated for wheat production in Australia and India, respectively (http://faostat3.fao.org/). Drought results in about 3.5 million tonnes annual yield losses in Australia and limits the country's average yield to 1.7 tonnes ha^{-1}, which is below the world minimum (Gavran 2012; Ray et al. 2013). In South Australia, cyclic drought, characterized by sporadic rainfall during anthesis and grain filling can cause total crop loss in extreme years (Izanloo et al. 2008; Reynolds et al. 2007).

Yield is the result of cumulative effects of many traits and processes in the plant that interact with each other and the environment throughout the plant growth period. The development of high yielding varieties under water limitation is hampered by the complex polygenic nature of the trait, its low heritability and large genotype x environment interactions (Blum 2011; Langridge and Reynolds 2015; Passioura 2012). Moreover, most traits associated with drought tolerance such as transpiration efficiency (Condon et al. 1993a; Nakhforoosh et al. 2016; Rebetzke et al. 2002b), leaf rolling, carbohydrate storage and remobilisation (Mphande et al. 2016a; Ovenden et al. 2017; Rebetzke et al. 2008b; Saint Pierre et al. 2010b; Zamani et al. 2014), osmotic adjustment (Abdolshahi et al. 2015; Budak et al. 2013; Merchuk-Ovnat et al. 2016; Morgan 2000) and canopy temperature (Blum et al. 1982; Reynolds et al. 1998), vary across sites, years and genetic material. Drought is often concomitant with heat stress in the field. Thus, it is essential to target a specific environmental scenario to improve yield in drought and heat stressed environments (Lopes et al. 2014; Tardieu 2011a, b).

Improvement of a complex trait such as yield in dry and hot environments can be facilitated through quantitative trait loci (QTL) mapping and QTL cloning techniques. This includes identifying a QTL for yield in targeted environments, fine mapping the QTL and identify molecular markers tightly linked to the trait for molecular breeding (Graziani et al. 2014; Langridge and Reynolds 2015; Soriano et al. 2017). Several studies have reported QTL for

grain yield and yield components in wheat under different drought and heat stressed conditions (Bennett et al. 2012a; Bennett et al. 2012b; Graziani et al. 2014; Maccaferri et al. 2010; Maphosa et al. 2014; McIntyre et al. 2010; Shukla et al. 2015), but to date, none of these QTL have been cloned in wheat (Fleury and Langridge 2014; Shukla et al. 2015). In addition to the complex quantitative nature of yield, lack of a completed wheat genome sequence information has also been an obstacle for QTL cloning up to now.

The current advancements in molecular markers technology such as SNP markers and high throughput genotyping platform enables the development of high density linkage maps which, in conjunction with multi-location field trials, become a crucial tool for QTL mapping (Colasuonno et al. 2014; Maccaferri et al. 2015; Marcotuli et al. 2017; Rustgi et al. 2013). Moreover, the International Wheat Genome Sequence Consortium (IWGSC) recently released the wheat genome reference sequence information along with annotated genes (IWGSC RefSeq v.1.0) which is publicly available at http://wheaturgi.versailles.inra.fr/Seq-Repository/. This information augmented with the sequence data of 16 Australian varieties (Edwards et al 2012) enable now identifying new SNP markers for QTL fine mapping and functional markers/genes for precise breeding for drought tolerance (Clavijo et al. 2017; International Wheat Genome Sequencing 2014; Staňková et al. 2016; Uauy 2017) .

QTL for yield, *QYld.aww-1B*, and yield components were identified on chromosome 1B under rain fed environments of South Australia and Mexico using double-haploid (DH) lines from a cross between Excalibur and Kukri (Edwards 2012c). The yield QTL on the 1B chromosome *QYld.aww-1B* was found to be stable across environments, independent of heading date and mainly adapted to severely drought-stressed environments of South Australia. The QTL covered a large interval of 40.0 cM and needed to be narrowed down using a positional cloning approach.

Positional cloning is a stepwise genomic approach to narrow down a wide QTL region to the shortest possible genetic interval with addition of new markers and phenotyping of recombinant inbred lines (RIL) and/or near iso-genic lines (NIL). NIL are homozygous lines that only differ in a specific DNA segment carrying a locus of interest. NIL are a powerful tool in searching the genes underlying the target QTL (Boopathi 2013). NIL can be developed as heterogeneous inbred family (Shiferaw et al.) (Tuinstra et al. 1997) from RIL that are heterozygous in the QTL region. The heterozygous plants are self-pollinated and the resulting

progeny families are then genotyped to identify those HIF families that are still segregating for the QTL. Sibling lines homozygous for contrasting alleles at the target locus are then phenotyped as NIL (Loudet *et al.*, 2002).

Therefore, the first objective of this study was to generate new SNP markers to construct a high resolution genetic map and fine map the 1B yield QTL in Excalibur/Kukri double haploid (DH) population. The second objective was to validate and fine map the 1B yield QTL under severe drought and heat stressed conditions using NIL.

4.3. Material and methods

4.3.1. Plant materials

A double haploid (DH) population, consisting of 233 lines derived from a cross between two Australian cultivars, Excalibur and Kukri was used in this study. The parental line Excalibur (RAC177/'Monoculm'//RAC311S) is a drought tolerance cultivar that was released from the University of Adelaide in 1991. Kukri (76ECN44/76ECN36//RAC549; MADDEN/6*RAC177) is a drought susceptible cultivar and was also released from the University of Adelaide in 1999. The cultivars were also contrasting in yellow rust resistance and end use quality. The two cultivars were selected for the *Rht-D1b* semi-dwarfing locus, similarities in phenology and their high yield potential in non-stressed environments (Izanloo et al. 2008).

4.3.2. Excalibur/Kukri DH field trials

A total of 32 field experiments were conducted at 10 locations over six seasons. An experiment is defined as a location by year combination. The field experiments are summarised in Supplementary Tables 1 and 2. Field trials in Australia and Mexico were detailed in Edwards (2012c). Briefly, three experiments were conducted in South Australia in 2006, and five experiments in South Australia and Victoria in 2007 under rain fed conditions. The southern Australian field experiments were designed in a nearest neighbour with two replications of 233 DH, two parental lines (checks) and 13 cultivars (controls). Each experimental plot at the Minnipa and the Piednippie sites were 1.8 meters wide and 7 meters long and had 8 rows, the plots at the other southern Australian sites were 1.25 metres wide and 5 metres long with 5 or

6 rows. The experiments were managed following the agronomic practices recommended for the region.

In 2007, two experiments were conducted at CIMMYT's (International Maize and Wheat Improvement Centre) drought evaluation site, Centro de Investigaciones Agricolas del Noroeste (CIANO), in Ciudad de Obregon (north-western Mexico). The Mexican field experiments were a two replicates alpha lattice design with 233 DH lines, two parental lines and one control variety (Sokoll). Drip and flood irrigation schemes were used to create two contrasting environments. Fifty mm of water was irrigated in three different periods: at sowing, 28 and 40 days thereafter in a drip irrigation scheme to simulate a southern Australian cyclical drought stress. In a flood irrigation scheme, the experimental field was irrigated to the field capacity in four different periods: at sowing, 48, 72 and 130 days thereafter to have a non-drought-stressed environment. The plot size for the drip irrigation was 0.8 metres wide by 3.5 metres long with 4 rows, but the flood irrigated plot was 0.4 metres wide by 2 metres long with 2 rows.

A total of 15 experiments were conducted in India in irrigated and rain fed environments over three years (2010-11, 2011-12 and 2012-13) at four locations, Hisar, Kanpur, Karnal, and Pune (Supp. Tables 1 and 2). The experiments were conducted in augmented block designs comprising 12 blocks with each block containing 19 DH lines and three check genotypes (NI5439, PBW175, and WH147). Each of the DH lines and the check genotypes in a block were evaluated in plots of 0.75 m^2 with three rows of 1.5 m length and row to row distance of 25 cm. In the irrigated experiments, flood irrigation was applied as per the requirements of raising an optimum crop. In the rain fed experiments, only one irrigation was applied at 21 days after sowing for establishment of the crop, then the crop was raised under rain fed conditions. Standard field management practices were followed for conducting the experiments.

4.3.3. Phenotyping of the Excalibur/Kukri DH population

Phenotyping was described in details in Edwards (2012c). Briefly, the population was phenotyped for grain yield (Yld), thousand grain weight (TGW), days to anthesis (DTA) and grain filling duration (GFD) in all trials. The plots were machine harvested and the grain cleaned. The cleaned samples were weighed to calculate Yld expressed in kg ha^{-1}. DTA was scored as the date at which anthers were visible from 50-75% of the heads in a plot. The data

on grain filling duration (GFD) was calculated as the difference in number of days between days to maturity (DTM) and DTA. TGW was recorded in grams by counting and weighing 1,000 grains.

4.3.4. DH genotyping and genetic map construction

Genomic DNA of the DH lines was purified using a method described by (Pallotta et al. 2000). DH lines were genotyped by for simple sequence repeat markers (SSR) and Diversity array technology (DArT) assays. Genotyping-by-sequencing (GBS), ISBP and SNP markers were added to this dataset. GBS were performed by Triticarte Pty Ltd (www.triticarte.com.au). Insertion site-based polymorphism markers (ISBP) (Paux et al. 2010) used the 'Multiplex-ready' PCR and a fluorescence-based DNA fragment analyser as described by Hayden et al. (2008)(www.genica.net.au). Capillary electrophoresis and visualization of PCR products were performed on an ABI3730 DNA analyser (Applied Biosystems), with semi-automated SSR allele sizing performed using GeneMapper 4.0 (Applied Biosystems).

The DH genotyping dataset was corrected by removing markers with >20 % missing values or a distorted segregation patterns (at alfa level=0.05), and lines with >100 observed count of crossovers. In the final step, poorly linked markers were removed based on double cross over counts and heatmap. The linkage map was then constructed using R/qtl with marker data for 155 lines and Kosambi mapping function (Kosambi 1944). The map included a total of 3502 markers including 174 SSR, 285 DArT markers, 2970 GBS markers, 51 SNP, 18 ISBP and 5 gene-based markers (glutenin *B1*, *TaGW2*, *Sr15*/*Lr20* and *Vrn-A1*). The markers were assembled into 26 linkage groups and assigned to 21 wheat chromosomes by BLASTN onto the reference wheat genome (IWGSC). The total length of the genetic map is 2864 cM, containing 2356 unique loci with an average distance of 1.23 cM (min=0.1 and max=48.1 cM) between two markers.

4.3.5. QTL analysis

The statistical analysis of the DH population followed the approach described in (Bonneau et al. 2013b). Four traits have been analysed in this study: days to heading (DTH) as well as three yield-related traits: yield kg/ha, thousand grain weight (TGW) and grain filling duration (GFD). In the first step univariable analysis were conducted for each trait. Genome-wide QTL scan

was conducted on marker-by-marker basis. Both the main effect of the marker and the environment by marker interaction (E x QTL interaction) were tested in the model. Based on the results of the univariable analysis of DTH, marker intervals *QDth.aww.5A* and *QDth.aww.7A* were selected as DTH-related covariates and univariable models for the three yield-related traits were re-fitted with DTH-related marker intervals included as covariates. This is to remove the effect of phenology genes from the yield QTL analysis. Edwards (2012c) reported the influence of phenology on yield QTL on some of the chromosomes in this population. For example, the 1B yield QTL was significant at four trials which was increased to six trials after the removal of the effect of phenology genes by covariance. In the next step, all markers significantly associated to the trait at p-value < 0.01 were selected and re-fitted in multivariable multi-environment trial (MET) QTL model (i.e. fitted simultaneously). The positions of these markers can be identified by the QTL peaks that exceed the 0.01 threshold. QTLs were selected as markers that tested significant at p-value < 0.01 in the multivariable model.

4.3.6. Scaffold assignment and SNP discovery

SNP located in the QTL region were identified using Diversity Aamong Wheat geNome (DAWN), an in-house wheat genomic platform (University of Adelaide) (Baumann *et al*., unpublished). DAWN runs on the Integrative Genomics Viewer (IGV) platform and combines the whole genome shotgun sequence assembly of Chinese Spring (IWGSC WGA v0.4 from https://wheat-urgi.versailles.inra.fr/), the whole genome shotgun sequences of 16 cultivars (also called BPA dataset, for Bioplatforms Australia) (Edwards et al. 2012), a RNA-Seq dataset (International Wheat Genome Sequencing 2014) and the Munich Information Centre for Protein Sequences (MIPS HCS) gene models (http://pgsb.helmholtz-muenchen.de/plant/wheat/index.jsp). The BPA reads of 16 Australian cultivars includes Excalibur and Kukri parental lines, with a 10X genomic sequence coverage and 100 bp paired-end Illumina HiSeq sequence reads (https://researchdata.ands.org.au/bpa-wheat-cultivars/2614). In DAWN, the BPA reads are aligned to the IWGSC WGA v0.4 assembly to identify SNP between the parental lines in specific regions. DAWN setting is highly specific and identified SNP are homeolog specific.

The sequences of all markers from the QTL interval were aligned to the chromosome 1B of Chinese Spring IWGSC WGA v0.4 using an in house BLASTN portal to retrieve scaffolds

matching the QTL interval. Polymorphic SNP were identified on these scaffolds between Excalibur and Kukri parental lines using DAWN. Only the SNP found within a sequence stretch of 200 bp, containing ≥ 50% GC and a depth of read coverage >10 were considered for selection. Using the nucleotide positions of the SNP in the scaffolds and the Fetch-Seq tool in DAWN, 100 bp sequence segments containing the SNP were retrieved. The 100 bp sequences were then used to design KASP™ markers (Semagn *et al.*, 2014) using the workflow management Kraken software (Supplementary Table 3).

4.3.7. KASP™genotyping and mapping onto chromosome 1B

In order to increase the marker density on the chromosome 1B genetic map, we used four different markers sequence sources: the 90K Wheat Illumina Infinium iSelect genotyping array (Wang et al. 2014), Genotype-by-sequencing (GBS) markers, the Avalon/Cadenza chromosome 1B genetic map (Allen et al. 2013), the Breeders' 35k Axiom® array (Wilkinson et al. 2012) from the CerealDB database (www.cerealsdb.uk.net), and new SNP from DAWN. SNP were converted to KBioscience Competitive Allele-Specific Polymerase chain reaction (KASP™) assays (He et al. 2014; Semagn et al. 2014). KASP™ primers were designed using the Kraken software from LGC genomics (www.lgcgroup.com) and assayed on parents and Excalibur/Kukri DH lines using a SNPLine (LGC genomics, www.lgcgroup.com/our-science/genomics-solutions/genotyping/kasp-genotyping-chemistry). or each KASP™ SNP, two allele specific forward primers (A1 and A2) and one common reverse primer (C1) were designed by importing SNP sequences into KrakenTM software (Version 13.4.18.11833) (LGC Genomics, Middlesex, United Kingdom). All the markers were assessed on parents and all the lines of the mapping population using the LGC genomics KASP™ assay. The KASP™ protocol used is available online from LGC genomics, Middlesex, United Kingdom (www.lgcgroup.com/our-science/genomics-solutions/genotyping/kaspgenotyping-chemistry). After genotyping, the SNP calls were converted to A or B in accordance to the genotypes of parents (Drysdale and Gladius, respectively) and "-" for missing data.

Markers were added to the existing chromosome 1B genetic map using ICiMapping v 4 (Meng et al. 2015) with linkage criterion set to LOD threshold >3. Recombination frequencies were converted to cM using the Kosambi mapping function (Kosambi, 1943) and the marker order was optimised using RECORD algorism. Double cross overs were manually curated and markers with high segregation distortion were discarded.

4.3.8. Development of near iso-genic line (NIL) and DNA extraction

NIL were developed from heterozygous inbred families (Shiferaw et al.) that were segregating for *QYld.aww-1B* following the method described by Tuinstra et al. (1997). For each generation, DNA was extracted from 8 weeks old leaves collected from individual plants using the modified freeze dry method (Dietrich et al. 2002).

Five Excalibur/Kukri RIL, heterozygous at the markers BS00066864 and adw572 (Fig 1a) were selected from a collection of 1500 Excalibur/Kuri RIL ($F_{2:5}$) using KASP assays. Eight seeds of each of the six RIL ($F_{2:6}$) were sown in September 2015 at Urrbrae, SA. Single plant DNA were assayed with four markers (BS00066864, adw1218477, BS00084985, adw572) and heterozygous plants $F_{2:6}$ were self-fertilized. Segregating progenies ($F_{2:7}$ plants) were genotyped with the four markers above; homozygous plants carrying Excalibur or Kukri allele were selected as NIL (Fig 1b). A total of six pairs of NIL (EK853, EK428_2, EK428_8, EK570, EK405 and EK664) were seed multiplied in green house from March to June 2016. The $F_{2:8}$ NIL and their parental lines (Excalibur and Kukri) were evaluated under severe drought and hot conditions in a semi-controlled rainout shelter (polyurethane) from August to December 2016.

4.3.9. Semi-controlled NIL trial

The 12 NIL and their parental lines (Excalibur and Kukri) were grown using a rainout shelter made of polyurethane and drip irrigation to evaluate their performance under severe drought and hot conditions in 2016 (Urrbrae, SA) (Supp. Fig 1). Forty-eight plants of each NIL were planted in 6 rows of 0.6 m by 0.8 m (mini-plots) with a space of 10 cm between plants within a row and 10 cm between rows. The experiment was arranged in a fully randomized complete block design with two replications. Two border rows were planted at first and last plots for each replication to avoid border effects. The trial was planted on August 10, 2016, relatively late compared to the farmers practice to expose the plants to severe drought and heat during flowering and grain filling stages. Drought treatment was induced by withholding irrigation at early booting stage (41 score on the Zadoks' scales) (Zadoks et al. 1974). Soil moisture status was measured using six gypsum blocks (MWS model, Hunter Industries, Australia) that were installed at three positions (3 m apart) of the trial and in two different soil depths (15 cm and

40 cm). Temperature and humidity data were recorded using mobile logger (KG100 model, Adelaide, Australia) positioned at three equal distance (3 m apart) in the field.

4.3.10. NIL phenotyping

NIL were phenotyped for days to heading (DTH), days to anthesis (DTA), days to maturity (DTM), grain filing duration (GFD), biomass (BM), grain yield (Yld), harvest index (HI), fertile tillers/plot (FT), screenings (Scr), total grains number/plot (TGN), thousand grain weight (TGW), and normalized differences in vegetative index (NDVI). DTH, DTA and DTM were the number of days from planting to the date to which 50% of the plants in a plot reached heading, anthesis and physiological maturity, respectively.

Canopy biomass was measured at vegetative stage (tillering to booting stage) using NDVI by capturing the reflectance spectra of the canopy with a portable spectroradiometer, GreenSeeker^{TM} (NTech Industries Ins, Ukiah, California, USA) as described by Gutiérrez-Rodríguez et al. (2004) . The GreenSeeker^{TM} senses was held at 0.4 to 0.6 m above the canopy to scan the 0.48 cm2 mini plot areas. Thin metal string with pointer at end was hung at the tip of the GreenSeeker to keep the constant distance above the canopy during scanning. NDVI data was measured from 10:00 am morning to 1:00 pm after noon.

All above ground biomass was harvested at maturity and weighted to measure the BM (g). Plant height (PH) was measured in meters on ten plants per mini-plot from ground to the top of the spike excluding awns. Ten spikes were randomly sampled from 10 plants from the middle of each mini-plot and threshed to measure seed/spike (SS), seed/spikelet (SSp) and spike length (SL). SL was measured from first floret to the tip of the last floret excluding the awn using a ruler.

We used a 2.0 mm sieve (Graintech scientific, Queensland, Australia) to screen the seeds and measure screenings (Scr, %). Scr is the ratio of the weight of the seeds passing through the screen to the weight of total seeds per plot and multiplied by 100 was reported as Scr%. TGW was measured in grams by weighing 500 randomly sampled seeds after screening. TGN per plot was counted with a seed counter (Pfueffer GmBH, Germany) after seed screening. HI was calculated as the ratio between Yld and BM.

4.4. Results

4.4.1. QTL in Excalibur/Kukri DH population

The broad range of environmental conditions across 28 experiments, provided diverse water conditions with growing season rainfalls ranging from 68 mm to 229 mm and average grain yields from 285 kg ha^{-1} to 2660 kg ha^{-1}. Heritabilities ranged from 0.21 to 0.85 (Supp. Table 4).

A total of 48 QTL were found over the whole genome for four traits analysed across 28 experiments (Table 1). Ten QTL for yield, 9 for TGW, 4 for days to heading and 2 for grain filling duration showed significant G x E interactions. Eleven yield QTL had a significant main effect across environments. Among those, two QTL were found on 1B chromosome in the region 149.5-182.2 cM that increased grain yield and TGW and did not co-locate with any QTL for days to heading (Supp Fig 2a and 2b). Excalibur was the source of favourable alleles for these two traits in this population, and contributed for the yield increment of 23.6 to 136.1 kg ha^{-1} (Table 1). These results match Edwards (2012) data on *QYld.aww-1B* (Supp. Table 5).

Re-analysis of the yield QTL with data from 28 experiments and the new high resolution 1B genetic map resulted in two yield QTL (Supp. Fig 2). *QYld.aww-1B-1* flanked with BS00004129 (149.5 cM) and ADW1061145 (150.1 cM) while *QYld.aww-1B.2* spanned from ADW121877 (168.5 cM) to adw490 (182.2 cM) with allelic effect 65 kg/ha and 123 kg/ha for the first and second QTL, respectively. Excalibur increased grain yield in both the QTL. We focused the on the fine mapping on the second QTL for NIL development (Fig 1).

4.4.2. Chromosome 1B yield QTL, *QYld.aww-1B.2*, fine mapping

The marker density of chromosome 1B was increased to develop a high resolution genetic map of *QYld.aww-1B* (Fig 2). A total of 79 KASP™ markers designed from four different marker sources were genotyped in the parents and population, and mapped on chromosome 1B. Nine GBS markers were converted to KASP™ markers (collaboration with Beata Sznajder) and prefixed 'ADW' followed with seven digits numbers. Twenty eight KASP™ markers prefixed 'BS' were sourced from chromosome 1B of the Avalon/Cadenza map and the Breeders' 35k Axiom® array (Wilkinson et al. 2012). We also designed and mapped 13 markers from 90K

iSelect SNP array (Wang et al. 2014) and prefixed them 'ADWKASP'. One gene-based marker, recently published as a new flowering gene in wheat (*TaTOE1-B1*) (Zikhali et al. 2017) was also mapped on 1B.

The remaining 28 KASP™ markers prefixed 'adw' were new SNP, designed using DAWN platform. DAWN setting is highly specific and identified SNP that are homeolog specific. KASP markers are also homeolog specific, as verified by segregation of each marker in the mapping population. The region of the yield QTL flanked by ADW1218477 and X1022619 markers (30.1 cM interval) was first anchored onto the wheat reference sequence WGA v 0.4 (Alaux et al. 2016) (Fig 2, Supp. Table 4). We found 17 scaffolds covering 24 Mbp (Supp. Table 6). Seventy-four SNP identified on these scaffolds were polymorphic between Excalibur and Kukri as visualized in the DAWN platform. Out of 74 new KASP™ assays that were tested on parental lines and population, 28 markers were mapped to 23 loci on chromosome 1B within and flanking the QTL interval (Supp. Table 3). Out of the 28 markers only nine markers mapped inside the yield QTL interval. As we narrowed down the initial QTL interval from 30.4 cM to 13.7 cM, 19 markers designed from the scaffolds in the QTL region were found to be outside the QTL fine interval (Fig 2). The final chromosome 1B genetic map included 142 markers covering 238.8 cM total length with 1.6 cM average distance between two adjacent markers (Fig 2).

The QTL for yield, TGW, DTH and GFD were then re-analysed on chromosome 1B using the updated high-resolution map and the MET model including 28 field trials on three continents (Australia, Mexico and India) (performed by Beata Sznajder). We detected two yield QTL peaks (Supp. Fig 3). *QYld.aww-1B.1* flanked with BS00004129 (149.5 cM) and ADW1061145 (150.1 cM) while *QYld.aww-1B.2* spanned from ADW121877 (168.5 cM) to adw490 (182.2 cM). *QYld.aww-1B.1* was expressed only at two Australian trials and one Indian trial. *QYld.aww-1B.2* showed significant effect at four South Australian environments (23.6 kg to 136.1 kg ha^{-1}) all contributed by Excalibur allele (Table 1) as previously found by Edwards (2012). This QTL showed a significant effect (249 kg ha^{-1}) at one Indian trial contributed by Kukri allele. We also found a partial co-location with a TGW QTL (Supp. Fig 2). As *QYld.aww-1B.2* was relatively stable in very dry southern Australian environments, we focused our attention to this QTL. The interval was narrowed down from 30.4 cM to 13.7 cM with new flanking markers ADW121877 (168.5 cM) and adw490 (182.2 cM) (Supp. Fig 3), which were used for NIL development, fine mapping and candidate gene identification.

4.4.3. NIL performance

Six pairs of NIL (EK853, EK570, EK428_2, EK428_8, EK405 and EK664) segregating for the AA allele donated from Excalibur (drought tolerant) and BB allele from Kukri (drought susceptible) were phenotyped in semi-controlled field conditions (Fig 1b). The trial was under severe drought (0.6 MPa) starting form early booting stage and heat stress starting from heading throughout the grain filling stage (Supp. Fig. 1). Maximum high temperatures ranged from 32.5 to 42.3 C during anthesis and grain filling stages. Thus, the trial was under a combination of severe drought and heat stress at critical crop growth stages.

As expected (Izanloo et al., 2008), Excalibur variety showed significantly higher values than Kukri for grain yield, total grain number/plot, number of fertile tillers, NDVI and biomass (Fig 3a-i), and Supp. Table 7). Kukri plants were taller with longer spikes, more seeds per spike, but smaller (high screenings) than Excalibur. Statistical analysis showed highly significant and strong positive correlations among grain yield, biomass, grains number, NDVI, fertile tillers, TGW and plant height (Supp. Table 8). Screenings was significantly and strongly negatively correlated to grain yield, grain number/plot and TGW. Significant negative correlation was also observed between seeds/spike and TGW.

Grain yield, grains number/plot, fertile tillers/plot, NDVI, biomass, plant height, seeds/spikelet, seeds/spike, spikelet/spike, spike length, screenings, showed significant variations at least in one NIL pair (Supp. Table 7). Maturity traits (days to heading, anthesis and maturity) and TGW did not show significant differences within NIL pair. All NIL pairs showed significant differences in yield or yield components between AA and BB alleles (Supp. Table 7).

Among them, four of the NIL pairs (EK570, EK428_2, EK428_8 and EK664) exhibited significant differences in grain yield, thus validating the effect of the 1B QTL under severe drought and heat stress conditions. The remaining two NIL pairs (EK853 and EK405) did not show significant differences for grain yield (Fig 3a). EK853 showed significant differences for seeds/spikelet, seeds/spike, and spikelet/spike, while EK405 was significant for total grains number/plot, NDVI, plant height and screenings. Kukri contributed higher allele effects for plant height in EK405 (Fig 3g-i, and Table 7).

Grain yield significantly co-segregated with grain number/plot, fertile tillers/plot and biomass in these four NIL pairs (EK428_2, EK428_8, EK570 and EK664) (Fig 3a). All NIL carrying the Excalibur allele showed a significant increase in grain yield and all other traits, except screenings, over their sibling line carrying the Kukri allele. Kukri contributed higher allele effects for seeds/spikelet in EK570 (Fig 3g) and seed screenings in all NIL pairs (Fig 3i). The high seed screenings contributed by the Kukri allele was characterized by a large number of shrivelled seeds resulted from severe drought and heat stress during grain filling stage.

The average values of grain yield, biomass and fertile tillers in these lines carrying the Excalibur allele were greater than the corresponding siblings NIL pair carrying the Kukri allele by 54.5%, 43.5% and 32%, respectively. Fertile tillers/plot consistently co-segregated with grain yield, total grain number/plot, and biomass in three NIL pairs (EK428_2, EK428_8 and EK664) and with grain yield and biomass in four NIL pairs (EK570, EK428_2, EK428_8 and EK664), with an average of 36.5% advantage of the Excalibur allele over the Kukri allele (Fig 3a-e). NDVI significantly co-segregated with grain yield, fertile tillers and biomass in EK570 and EK664 NIL.

By mapping the haplotype of the NIL (EK570, EK428_2, EK428_8 and EK664) against the DH genetic map, we delimited the Excalibur/Kukri yield QTL interval to 2.9 cM, flanked by the markers adw1218477 and BS00022342 (Fig 4). The delimited QTL interval corresponds to 2.2 Mbp on the 1B chromosome of Chinese Spring reference (IWGSC RefSeq v1.0).

4.5. Discussion

Yield is made of three major components: number of fertile spikes per area, number of grain per spike and TGW. Each component is controlled by multiple loci in complex interactions with each other and with the environment (Dhungana et al. 2007; Rustgi et al. 2013; Slafer et al. 1999b; Xing and Zhang 2010). Edwards (2012) previously found that *QYld.aww-1B* was co-located with QTL for floret fertility, grain m^{-2}, grain size and increased test weights in Excalibur/Kukri DH population. The QTL was more significant in South Australian sites with a severe drought stress (Yield MET < 500 kg/ ha), but not in Mexican and Indian trials. This study confirmed *QYld.aww-1B* and its colocation with grain number/plot, fertile tillers, early vigour and seed screenings QTL (Supp. Table 5). Excalibur contributed the positive allele for all these traits in both the DH and the NIL, except for screenings.

Grain yield QTL have been identified in this region of the long arm of chromosome 1B in a number of previous studies (Bennett et al. 2012a; Bennett et al. 2012b; Graziani et al. 2014; Maccaferri et al. 2010; McIntyre et al. 2010; Shukla et al. 2015). *QYld.aww-1B* in Excalibur/Kukri DH (*QYld.aww-1B*) also coincided with the yield QTL (*QYld.aww-1B*) in RAC875/Kukri DH found in multiple field trials of South Australian environments (Bennett et al. 2012b).

Quarrie *et al*. (2005b) found that the number of heads per plant, the number of grains per head and TGW were significantly associated with grain yield on chromosome 1B. Griffiths et al. (2015) also reported that the number of grains m^{-2} was significantly associated the grain yield locus on the chromosome 1B. Campbell et al. (1999) and Wu et al. (2015) identified a TGW QTL in this region. In some studies reported that the increase of number of grains per unit of land area was partially offset by a reduction in grain weight in different chromosomes (Slafer and Andrade 1993; Slafer et al. 1996).This negative relationship between grain number and grain weight increases the proportion of small grains at particular positions of the spikelet and/or spike (Acreche and Slafer 2006; Slafer 2003; Slafer et al. 1999b).

TGW was not significantly different within NIL pair in our study (Supp. Table 6). This indicates that there was no significant compensation between TGW and number of seeds in this study. Here, it is the number of fertile tillers that increased yield. There is a body of evidence suggesting that the negative relationship between grains number and TGW is largely independent of the competition between grains for metabolites, and an increase in number of grains may reduce average grain weight even if an excess assimilates are available for the sink (Acreche and Slafer 2006; Miralles and Slafer 1995; Richards 1996b; Slafer et al. 1996). Thus, grain weight (grain size) in wheat is generally sink rather than source limited and the number of grain m^{-2} is better related to grain yield than individual grain weight (Borrás et al. 2004; Estrada-Campuzano et al. 2012; Slafer et al. 1996). Despite to this general behaviour, Slafer and Savin (1994), Borrás et al. (2004) and Slafer and Miralles (1992) reported some responses of wheat grain size to co-limited post-anthesis source/sink ratios under stress conditions where small but significant source limitation was observed.

Water deficit in Mediterranean climate usually occurs from heading stage and continues during the grain filling period with episodes of heat stress which affects both grain number and grain

weight (Fleury et al. 2010; Ji et al. 2010). Thus, research aiming to maintain yield under drought and heat should focus on these developmental stages. In the NIL trial, a severe drought of 0.6 MPa was recorded at the top 10 cm soil profile starting from heading stage with a maximum temperature between 32.5 and 42.3°C throughout flowering and grain filling (Supp. Fig. 1). These conditions are similar to the South Australian severe drought stressed sites (yield MET < 500 kg/ha) where *QYld.aww-1B* effect was the strongest in Edwards' study (2012) on Excalibur/Kukri DH population.

In the NIL trial, the mean value of Excalibur showed significantly higher grain yield, biomass, grains number/plot, fertile tiller and NDVI when compared with Kukri (Fig 3a-d, Supp. Table 7). This finding is in agreement with Fleury et al. (2010), Hill et al. (2013a), Izanloo et al. (2008) reports on the higher performance of Excalibur under stressed environments compared to Kukri. Kukri produced higher seeds/spikelet, seeds/spike and plant height than Excalibur (Fig 3f-h), but the trend was not consistent in the NIL pairs where Excalibur was the positive allele contributor for these traits in most of the NIL pairs.

Our NIL study validates the co-location of grain yield QTL with grains number/plot, fertile tillers, early vigour and test weight reported in DH by Edwards (2012) in South Australian field trials. Moreover, six yield related traits such as biomass, NDVI, spikelet/spike, plant height and spike length which were not reported in DH analysis showed significant effect in NIL and co-segregated with grain yield. The 1B yield QTL in the DH was partly influenced by maturity in the study by Edwards (2012). Here, different NIL pairs showed differences in maturities; however, no maturity differences were observed within NIL pairs, demonstrating the effect of this QTL for enhancing yield *per se*. As breeders already select plants with a suited phenology for their target environment, a new QTL to be used in marker assisted selection should not affect phenology. *QYld.aww-1B* is therefore a good candidate for selection.

The co-segregation of grain yield with plant height and its significant strong positive correlation with plant height in two NIL pairs may be due to the contribution of plant height to water soluble carbohydrate that would maintain grain number and yield under terminal drought and stress (Austin et al. 1980; Ehdaie et al. 2006b; Rebetzke et al. 2008b). But, additional studies would be required to understand whether it is solely due to a source accumulation or due to the influence of dwarfing genes (Ehdaie et al. 2006b; Ruuska et al. 2006; Shakiba et al. 1996).

The co-segregation of grain yield with higher grain number/plot, fertile tillers, NDVI and biomass in four of the six NIL pairs (EK570, EK428_2, EK428_8 and EK664) (Fig 3a-f) and significant strong positive correlations among these traits (Supp. Fig 8) indicated that NDVI and fertile tillers might be responsible for the 1B yield QTL in Excalibur/Kukri. NDVI is a strong predictor of grain yield and highly correlated with canopy biomass and early vigour (Gutiérrez-Rodríguez et al. 2004; Lukina et al. 1999; Raun et al. 2001; Tucker 1979). Greater early vigour or NDVI contributes to a higher biomass accumulation and positively affects grain number m^{-2} and grain yield in wheat under terminal drought and heat stressed environments (Foulkes et al. 2002). This is an important trait in southern Australian environment where the availability of sufficient rainfall with cool winter season is favourable for fast early biomass accumulation that would be translocated to sink to maintain grain yield during terminal severe drought and high temperatures. Pre-anthesis assimilates not only contributes to grain m^{-2} but also to grain weight under terminal drought and heat stressed conditions (Rebetzke et al. 2008b; Richards 1996b; Savin and Slafer 1991; Yang et al. 2001).

(Pinto et al. 2010) also reported coincidence of QTL for NDVI, grain yield, grains m^{-2} and TGW on chromosome 1B under drought and heat stresses in the Seri/Babax population. Coincidence of QTL for NDVI with grain yield QTL was also reported in RAC875/Kukri by Bennett et al. (2012a) and Bennett et al. (2012b). It was suggested that QTL for NDVI was more closely associated with biomass production *per se* (Ehdaie et al. 2006a) and greatly contributed to stem water soluble carbohydrate (WSC) that would be remobilized to developing grains under terminal drought stress (Ehdaie et al. 2006a; Mphande et al. 2016b; Yáñez et al. 2017). WSC contribute from 37 to 65% of grain yield under severe drought (Ehdaie et al. 2008). Hill et al. (2013); Hill et al. (2015) reported a QTL for metabolite traits such as maltose and fructose in 1B in Excalibur/Kukri DH population under terminal drought and heat stress. These metabolites QTL were highly expressed in leaf under drought and heat stress and are the main components of WSC. Excalibur was the positive allele contributor for both traits. Interestingly, the maltose QTL (*QMal-1B*) (wPt0705-wPt2526) was partially co-located with *QYld.aww-1B* and test weight QTL (wPt9809-wPt2526) found by (Edward, 2012) in Excalibur/Kukri DH population. This indicates that *QYld.aww-1B* effects on yield might be due to the accumulation of the WSC maltose and fructose in leaves that would be translocated to grain during grain filling period.

The higher number of tillers exhibited in the NIL contributed to the grain number/plot by increasing fertile spikes/plot. de Oliveira et al. (2013) also reported an increase of grain yield under terminal drought and high temperature conditions with increased number of fertile tillers and grain number per unit area. Therefore, the result of this study and other studies elsewhere (Merchuk-Ovnat et al. 2016; Naruoka et al. 2011) indicate that plasticity in terms of fertile tillers per unit area is a very important attribute for yield under drought and temperature stress environments. Rapid ground cover with high tillering capacity enable cultivars to reduce soil water evaporation and increase light interception and assimilation capacity under pre-anthesis conditions (Asseng and Van Herwaarden 2003; Blum 1997; Giunta et al. 2003; Loss and Siddique 1994; Richards et al. 2002b). Thus, increasing fertile tiller per unit area would not necessarily reduce grain number and weight as the extra tillers would also increase stem carbohydrates and provide an extra source of assimilates for fertile spikes and grain weight during grain filling stage (Slafer et al. 1999a). This might explain why there was no significant reduction in TGW concomitant with the increased number of fertile spikes and grain number/plot in the NIL (Supp. Table 7). Further experiments will be required on source-link relations and WSC status of the NIL to validate this hypothesis.

Tillering is under genetic control with large variation in most cereals (Innes et al. 1981). Free tillering cultivars respond better to dynamic environments and maintain maximum plant density per unit area to increase grain yield under stressed conditions (Moeller et al. 2014; Zhu et al. 2013). Thus, significant yield penalty was reported in using reduced tillering lines (*tin* lines) which could not compensate with increased grain weight and reduced seed screenings over free tillering lines (Mitchell et al. 2012). Parent et al. (2017) also reported that the 1B QTL for number of tillers in Drysdale/Gladius population increases yield depending on the magnitude and compensation mechanism between number of grains, number of tillers and TGW. In this study, we found a QTL for TGW in the Excalibur/Kukri DH population (Supp. Table 7), which was not observed in the NIL. This might be due to the small QTL effect and the difference in statistical power between the two studies, with 32 trials for the DH enabling to detect small effects *versus* one trial for the NIL. The small effect on TGW might be compensated with fertile tillers and grain number/plot.

Our results are in line with Izanloo et al. (2008) in regards to Excalibur's tillering in response to drought stress. Excalibur is characterized by a rapid ground cover and recovery from stress under cyclic drought stress by aborting tillers to adapt to the available soil moisture. *QYld.aww-*

1B might be part of this responsive mechanism that ensures maximum fertile tillers/plot and reduced percentage of seed screenings.

Grain yield was not significantly different in two NIL pairs (EK853 and EK405) though they segregate for the QTL large interval between 160.4-171.4 cM and between 160.4-180.9, respectively. These NIL showed significant differences in seeds/spike with the favourable allele coming from Kukri but not for fertile tillers. A possible explanation is the effects of specific genetic background in these two NIL. Genotyping of the NIL with twenty-two KASP markers spread throughout the genome showed a background variation in EK405 NIL pair at one locus (Supp. Table 9). The NIL are being genotyped using a targeted genotyping by sequencing assay to verify the homogeneity of the genetic background between NIL in each pair.

Consequently, we used only the four NIL pair (EK570, EK428_1, EK428_8 and EK664) that showed co-segregated for grain yield to narrow down the 1B yield QTL. By mapping these four pair of NIL haplotypes on the high-resolution genetic map of Excalibur/Kukri DH, *QYld.aww-1B.2* interval was narrowed down to 2.9 cM (Fig 4) corresponding to 2.2 Mb in Chinese Spring (IWGSC RefSeq v1.0) (details in chapter 4).

4.6. Conclusion

The yield and yield components QTL reported on chromosome 1B in Excalibur/Kukri DH was validated in NIL analysis under severe drought and heat stressed conditions. The positive grain yield and yield components allele derived from Excalibur at the 1B QTL and contributes to Australian wheat adaptation to severe drought and heat stressed environments. The co-segregation of grain yield with higher grain number/plot, fertile tillers, NDVI and biomass in NIL and DH trials, indicates that the 1B yield QTL is primarily due to combined effect of fertile tillers and remobilized assimilates. The yield advantage of the Excalibur allele may be due its dynamic response to effectively utilize the available soil moisture to establish a high amount of biomass and to adjust tiller number at later stage when stress occurs. This has practical implications for South Australian wheat growing in environments where sufficient soil moisture is available during cool winter followed by terminal drought with sporadic heat waves. The NIL will be trialled in yield plots over different locations to test genotype x environment interaction and reproducibility of the yield effect. Further study is also required on the source-sink relation and WSC production in NIL to identify the essential mechanisms underlying the yield QTL. The molecular tools and information presented in this study could be exploited in breeding to develop cultivars with high tillering capacity and fast early growth stage biomass accumulation with the effective remobilization under Southern Australia terminal drought and heat scenarios.

4.7. References

Abdolshahi R, Nazari M, Safarian A, Sadathossini TS, Salarpour M, Amiri H (2015) Integrated selection criteria for drought tolerance in wheat (*Triticum aestivum* L.) breeding programs using discriminant analysis. Field Crops Research 174:20-29

Acreche MM, Slafer GA (2006) Grain weight response to increases in number of grains in wheat in a Mediterranean area. Field Crops Research 98:52-59

Alaux M, Letellier T, Alfama-Depauw F, Jamilloux V, Rogers J, Choulet F, Guerche C, Loaec M, Flores R-G, Michotey C (2016) IWGSC Sequence Repository: Moving towards tools to facilitate data integration for the reference sequence of wheat. PAG XXIV-Plant and Animal Genome Conference, p np

Allen AM, Barker GLA, Wilkinson P, Burridge A, Winfield M, Coghill J, Uauy C, Griffiths S, Jack P, Berry S (2013) Discovery and development of exome- based, co- dominant single nucleotide polymorphism markers in hexaploid wheat (*Triticum aestivum* L.). Plant Biotechnolgy Journal 11:279-295

Asseng S, Van Herwaarden AF (2003) Analysis of the benefits to wheat yield from assimilates stored prior to grain filling in a range of environments. Plant Soil 256:217-229

Austin RB, Morgan CL, Ford MA, Blackwell RD (1980) Contributions to grain yield from pre-anthesis assimilation in tall and dwarf barley phenotypes in two contrasting seasons. Annals of Botany 45:309-319

Bennett D, Izanloo A, Reynolds M, Kuchel H, Langridge P, Schnurbusch T (2012a) Genetic dissection of grain yield and physical grain quality in bread wheat (*Triticum aestivum* L.) under water-limited environments. Theoretical and Applied Genetics 125:255-271

Bennett D, Reynolds M, Mullan D, Izanloo A, Kuchel H, Langridge P, Schnurbusch T (2012b) Detection of two major grain yield QTL in bread wheat (Triticum aestivum L.) under heat, drought and high yield potential environments. Theoretical and Applied Genetics 125:1473-1485

Blum A (1997) Improving wheat grain filling under stress by stem reserve mobilisation. Wheat: Prospects for Global Improvement. Springer, pp 135-141

Blum A (2011) Drought resistance–is it really a complex trait? Funct Plant Biol 38:753-757

Blum A, Mayer J, Gozlan G (1982) Infrared thermal sensing of plant canopies as a screening technique for dehydration avoidance in wheat. Field Crops Research 5:137-146

Bonneau J, Taylor J, Parent B, Bennett D, Reynolds M, Feuillet C, Langridge P, Mather D (2013) Multi-environment analysis and improved mapping of a yield-related QTL on chromosome 3B of wheat. Theor Appl Genet 126:747-761

Boopathi NM (2013) Mapping Population Development. Genetic Mapping and Marker Assisted Selection. Springer, pp 23-37

Borrás L, Slafer GA, Otegui MaE (2004) Seed dry weight response to source–sink manipulations in wheat, maize and soybean: a quantitative reappraisal. Field Crops Res 86:131-146

Bray EA, Bailey-Serres, J., and Weretilnyk, E. (2000) Responses to abiotic stresses. In: B.B. Buchanan WG, and R.L. Jones (ed) Biochemistry and Molecular Biology of Plants. American Society of Plant Physiologists, Rockville, MD, pp 1158–1203

Budak H, Kantar M, Yucebilgili Kurtoglu K (2013) Drought tolerance in modern and wild wheat. The Scientific World Journal 2013

Campbell KG, Bergman CJ, Gualberto DG, Anderson JA, Giroux MJ, Hareland G, Fulcher RG, Sorrells ME, Finney PL (1999) Quantitative trait loci associated with kernel traits in a soft× hard wheat cross. Crop Sci 39:1184-1195

Clavijo BJ, Venturini L, Schudoma C, Accinelli GG, Kaithakottil G, Wright J, Borrill P, Kettleborough G, Heavens D, Chapman H (2017) An improved assembly and annotation of the allohexaploid wheat genome identifies complete families of agronomic genes and provides genomic evidence for chromosomal translocations. Genome Res 27:885-896

Colasuonno P, Gadaleta A, Giancaspro A, Nigro D, Giove S, Incerti O, Mangini G, Signorile A, Simeone R, Blanco A (2014) Development of a high-density SNP-based linkage map and detection of yellow pigment content QTLs in durum wheat. Mol Breed 34:1563-1578

Condon A, Richards R, Farquhar G (1993) Relationships between carbon isotope discrimination, water use efficiency and transpiration efficiency for dryland wheat. Australian Journal of Agricultural Research 44:1693-1711

de Oliveira ED, Bramley H, Siddique KHM, Henty S, Berger J, Palta JA (2013) Can elevated CO2 combined with high temperature ameliorate the effect of terminal drought in wheat? Funct Plant Biol 40:160-171

Dhungana P, Eskridge KM, Baenziger PS, Campbell BT, Gill KS, Dweikat I (2007) Analysis of genotype-by-environment interaction in wheat using a structural equation model and chromosome substitution lines. Crop Sci 47:477-484

Dietrich CR, Cui F, Packila ML, Li J, Ashlock DA, Nikolau BJ, Schnable PS (2002) Maize Mu transposons are targeted to the 5′ untranslated region of the gl8 gene and sequences flanking Mu target-site duplications exhibit nonrandom nucleotide composition throughout the genome. Genetics 160:697-716

Edwards D, Wilcox S, Barrero RA, Fleury D, Cavanagh CR, Forrest KL, Hayden MJ, Moolhuijzen P, Keeble-Gagnere G, Bellgard MI, Lorenc MT, Shang CA, Baumann U, Taylor JM, Morell MK, Langridge P, Appels R, Fitzgerald A (2012) Bread matters: a national initiative to profile the genetic diversity of Australian wheat. Plant Biotechnology Journal 10:703-708

Edwards J (2012) A genetic analysis of drought related traits in hexaploid wheat. The University of Adelaide, School of Agriculture. Food and Wine (PhD thesis)

Ehdaie B, Alloush G, Madore M, Waines J (2006a) Genotypic variation for stem reserves and mobilization in wheat. Crop Science 46:2093-2103

Ehdaie B, Alloush G, Waines J (2008) Genotypic variation in linear rate of grain growth and contribution of stem reserves to grain yield in wheat. Field Crops Research 106:34-43

Ehdaie B, Alloush GA, Madore MA, Waines JG (2006b) Genotypic variation for stem reserves and mobilization in wheat. Crop Sci 46:2093-2103

Estrada-Campuzano G, Slafer GA, Miralles DJ (2012) Differences in yield, biomass and their components between triticale and wheat grown under contrasting water and nitrogen environments. Field Crops Res 128:167-179

Fleury D, Jefferies S, Kuchel H, Langridge P (2010) Genetic and genomic tools to improve drought tolerance in wheat. Journal of Experimental Botany 61:3211-3222

Fleury D, Langridge P (2014) QTL and association mapping for plant abiotic stress tolerance. Plant Abiotic Stress, Second Edition:257-287

Foulkes MJ, Scott RK, Sylvester-Bradley R (2002) The ability of wheat cultivars to withstand drought in UK conditions: formation of grain yield. The Journal of Agricultural Science 138:153-169

Gavran M (2012) Australian plantation statistics 2012 update. ABARES

Giunta F, Motzo R, Pruneddu G (2003) Comparison of temperate cereals and grain legumes in a Mediterranean environment. Agricoltura mediterranea 133:234-248

Graziani M, Maccaferri M, Royo C, Salvatorelli F, Tuberosa R (2014) QTL dissection of yield components and morpho-physiological traits in a durum wheat elite population tested in contrasting thermo-pluviometric conditions. Crop and Pasture Science 65:80-95

Griffiths S, Wingen L, Pietragalla J, Garcia G, Hasan A, Miralles D, Calderini DF, Ankleshwaria JB, Waite ML, Simmonds J (2015) Genetic dissection of grain size and grain number trade-offs in CIMMYT wheat germplasm. PloS one 10:e0118847

Gutiérrez-Rodríguez M, Reynolds MP, Escalante-Estrada JA, Rodríguez-González MT (2004) Association between canopy reflectance indices and yield and physiological traits in bread wheat under drought and well-irrigated conditions. Aust J Agric Res 55:1139-1147

Hayden MJ, Nguyen TM, Waterman A, McMichael GL, Chalmers KJ (2008) Application of multiplex-ready PCR for fluorescence-based SSR genotyping in barley and wheat. Molecular Breeding 21:271-281

He C, Holme J, Anthony J (2014) SNP genotyping: the KASP assay. Crop Breeding: Methods and Protocols:75-86

Hill CB, Taylor JD, Edwards J, Mather D, Bacic A, Langridge P, Roessner U (2013a) Whole-genome mapping of agronomic and metabolic traits to identify novel quantitative trait loci in bread wheat grown in a water-limited environment. Plant Physiology 162:1266-1281

Hill CB, Taylor JD, Edwards J, Mather D, Bacic A, Langridge P, Roessner U (2013b) Whole-Genome Mapping of Agronomic and Metabolic Traits to Identify Novel Quantitative Trait Loci in Bread Wheat Grown in a Water-Limited Environment1.Plant Physiology: 162(3): 1266–1281

Hill CB, Taylor JD, Edwards J, Mather D, Langridge P, Bacic A, Roessner U (2015) Detection of QTL for metabolic and agronomic traits in wheat with adjustments for variation at genetic loci that affect plant phenology. Plant Science 233:143-154

Innes P, Blackwell RD, Austin RB, Ford MA (1981) The effects of selection for number of ears on the yield and water economy of winter wheat. The Journal of Agricultural Science 97:523-532

International Wheat Genome Sequencing C (2014) A chromosome-based draft sequence of the hexaploid bread wheat (Triticum aestivum) genome. Science 345:1251788

Izanloo A, Condon AG, Langridge P, Tester M, Schnurbusch T (2008) Different mechanisms of adaptation to cyclic water stress in two South Australian bread wheat cultivars. Journal of Experimental Botany 59:3327-3346

Ji X, Shiran B, Wan J, Lewis DC, Jenkins CL, Condon AG, Richards RA, Dolferus R (2010) Importance of pre- anthesis anther sink strength for maintenance of grain number during reproductive stage water stress in wheat. Plant, Cell & Environment 33:926-942

Kosina P, Reynolds M, Dixon J, Joshi A (2007) Stakeholder perception of wheat production constraints, capacity building needs, and research partnerships in developing countries. Euphytica 157:475-483

Langridge P, Reynolds MP (2015) Genomic tools to assist breeding for drought tolerance. Current Opinion Biotechnology 32:130-135

Lopes MS, Rebetzke GJ, Reynolds M (2014) Integration of phenotyping and genetic platforms for a better understanding of wheat performance under drought. Jounal of Experimental Botany 65:6167-6177

Loss SP, Siddique KHM (1994) Morphological and physiological traits associated with wheat yield increases in Mediterranean environments. Advances in Agronomy 52:229-276

Lukina EV, Stone ML, Raun WR (1999) Estimating vegetation coverage in wheat using digital images. Journal of Plant Nutrition 22:341-350

Maccaferri M, Ricci A, Salvi S, Milner SG, Noli E, Martelli PL, Casadio R, Akhunov E, Scalabrin S, Vendramin V (2015) A high- density, SNP- based consensus map of tetraploid wheat as a bridge to integrate durum and bread wheat genomics and breeding. Plant Biotechnology Journal 13:648-663

Maccaferri M, Sanguineti MC, Demontis A, El-Ahmed A, Garcia del Moral L, Maalouf F, Nachit M, Nserallah N, Ouabbou H, Rhouma S (2010) Association mapping in durum wheat grown across a broad range of water regimes. Journal of Experimental Botany 62:409-438

Maphosa L, Langridge P, Taylor H, Parent B, Emebiri LC, Kuchel H, Reynolds MP, Chalmers KJ, Okada A, Edwards J (2014) Genetic control of grain yield and grain physical characteristics in a bread wheat population grown under a range of environmental conditions. Theoretical and Applied Genetics 127:1607-1624

Marcotuli I, Gadaleta A, Mangini G, Signorile AM, Zacheo SA, Blanco A, Simeone R, Colasuonno P (2017) Development of a High-Density SNP-Based Linkage Map and Detection of QTL for β-Glucans, Protein Content, Grain Yield per Spike and Heading Time in Durum Wheat. International journal of Molecular Sciences 18:1329

McIntyre CL, Mathews KL, Rattey A, Chapman SC, Drenth J, Ghaderi M, Reynolds M, Shorter R (2010) Molecular detection of genomic regions associated with grain yield and yield-related components in an elite bread wheat cross evaluated under irrigated and rainfed conditions. Theoretical and Applied Genetics 120:527-541

Meng L, Li H, Zhang L, Wang J (2015) QTL IciMapping: Integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. The Crop Journal 3:269-283

Merchuk-Ovnat L, Barak V, Fahima T, Ordon F, Lidzbarsky GA, Krugman T, Saranga Y (2016) Ancestral QTL alleles from wild emmer wheat improve drought resistance and productivity in modern wheat cultivars. Frontiers in Plant Science 7:452.

Miralles DJ, Slafer GA (1995) Yield, biomass and yield components in dwarf, semi- dwarf and tall isogenic lines of spring wheat under recommended and late sowing dates. Plant Breeding 114:392-396

Mitchell JH, Chapman SC, Rebetzke GJ, Bonnett DG, Fukai S (2012) Evaluation of a reduced-tillering (tin) gene in wheat lines grown across different production environments. Crop and Pasture Science 63:128-141

Moeller C, Evers JB, Rebetzke G (2014) Canopy architectural and physiological characterization of near-isogenic wheat lines differing in the tiller inhibition gene tin. Frontiers in plant science 5

Monneveux P, Jing R, Misra SC (2012) Phenotyping for drought adaptation in wheat using physiological traits. Frontiers in Physiology 3:429.

Morgan JM (2000) Increases in grain yield of wheat by breeding for an osmoregulation gene: relationship to water supply and evaporative demand. Australian Journal Of Agricultural Research 51:971-978

Mphande W, Nicolas ME, Seneweera S, Bahrami H (2016a) Dynamics and contribution of stem water-soluble carbohydrates to grain yield in two wheat lines contrasted under Drought and elevated CO2 conditions. Journal of Plant Physiology 214:1037-1058

Mphande W, Nicolas ME, Seneweera S, Bahrami H, Bbebe N (2016b) Dynamics and contribution of stem water-soluble carbohydrates to grain yield in two wheat lines contrasted under Drought and elevated CO2 conditions. Journal of Plant Physiology 214:1037-1058

Nakhforoosh A, Bodewein T, Fiorani F, Bodner G (2016) Identification of water use strategies at early growth stages in durum wheat from shoot phenotyping and physiological measurements. Frontiers in Plant Science 7:1155

Naruoka Y, Talbert L, Lanning S, Blake N, Martin J, Sherman J (2011) Identification of quantitative trait loci for productive tiller number and its relationship to agronomic traits in spring wheat. Theoretical and Applied Genetics 123:1043

Nezhad KZ, Weber W, Röder M, Sharma S, Lohwasser U, Meyer R, Saal B, Börner A (2012) QTL analysis for thousand-grain weight under terminal drought stress in bread wheat (*Triticum aestivum* L.). Euphytica 186:127-138

Ovenden B, Milgate A, Lisle C, Wade LJ, Rebetzke GJ, Holland JB (2017) Selection for water-soluble carbohydrate accumulation and investigation of genetic× environment interactions in an elite wheat breeding population. Theoretical and Applied Genetics:1-17

Pallotta M, Graham R, Langridge P, Sparrow D, Barker S (2000) RFLP mapping of manganese efficiency in barley. Theoretical and Applied Genetics 101:1100-1108

Parent B, Bonneau J, Maphosa L, Kovalchuk A, Langridge P, Fleury D (2017) Quantifying wheat sensitivities to environmental constraints to dissect G x E in the field. Plant Physiology: 10:1104, pp17-00372

Passioura J (2012) Phenotyping for drought tolerance in grain crops: when is it useful to breeders? Functional Plant Biology 39:851-859

Paux E, Faure S, Choulet F, Roger D, Gauthier V, Martinant JP, Sourdille P, Balfourier F, Le Paslier MC, Chauveau A, Cakir M, Gandon B, Feuillet C (2010) Insertion site-based polymorphism markers open new perspectives for genome saturation and marker-assisted selection in wheat. Plant Biotechnology Journal 8:196-210

Pinto RS, Reynolds MP, Mathews KL, McIntyre CL, Olivares-Villegas J-J, Chapman SC (2010) Heat and drought adaptive QTL in a wheat population designed to minimize confounding agronomic effects. Theoretical and Applied Genetics 121:1001-1021

Quarrie SA, Steed A, Calestani C, Semikhodskii A, Lebreton C, Chinoy C, Steele N, Pljevljakusic D, Waterman E, Weyen J, Schondelmaier J, Habash DZ, Farmer P, Saker L, Clarkson DT, Abugalieva A, Yessimbekova M, Turuspekov Y, Abugalieva S, Tuberosa R, Sanguineti MC, Hollington PA, Aragues R, Royo A, Dodig D (2005) A high-density genetic map of hexaploid wheat (*Triticum aestivum* L.) from the cross Chinese Spring X SQ1 and its use to compare QTLs for grain yield across a range of environments. Theoretical and Applied Genetics 110:865-880

Raun WR, Solie JB, Johnson GV, Stone ML, Lukina EV, Thomason WE, Schepers JS (2001) In-season prediction of potential grain yield in winter wheat using canopy reflectance. Agron J 93:131-138

Ray DK, Mueller ND, West PC, Foley JA (2013) Yield trends are insufficient to double global crop production by 2050. PLoS One 8:e66428

Rebetzke G, Condon AG, Richards R, Farquhar G (2002) Selection for reduced carbon isotope discrimination increases aerial biomass and grain yield of rainfed bread wheat. Crop Science 42:739-745

Rebetzke GJ, Van Herwaarden AF, Jenkins C, Weiss M, Lewis D, Ruuska S, Tabe L, Fettell NA, Richards RA (2008) Quantitative trait loci for water-soluble carbohydrates and associations with agronomic traits in wheat. Australia Journal of Agricultural Research 59:891-905

Reynolds M, Dreccer F, Trethowan R (2007) Drought-adaptive traits derived from wheat wild relatives and landraces. Journal of Experimental Botany 58:177-186

Reynolds MP, Singh RP, Ibrahim A, Ageeb OAA, Larque-Saavedra A, Quick JS (1998) Evaluating physiological traits to complement empirical selection for wheat in warm environments (Reprinted from Wheat: Prospects for global improvement, 1998). Euphytica 100:85-94

Richards RA (1996) Increasing the yield potential of wheat: manipulating sources and sinks. Increasing yield potential in wheat: Breaking the Barriers:Mexico D.F: CIMMYT:pp134-149

Richards RA, Rebetzke GJ, Condon AG, Van Herwaarden AF (2002) Breeding opportunities for increasing the efficiency of water use and crop yield in temperate cereals. Crop Science 42:111-121

Rustgi S, Shafqat MN, Kumar N, Baenziger PS, Ali ML, Dweikat I, Campbell BT, Gill KS (2013) Genetic dissection of yield and its component traits using high-density composite map of wheat chromosome 3A: bridging gaps between QTLs and underlying genes. PLoS One 8:e70526

Ruuska SA, Rebetzke GJ, van Herwaarden AF, Richards RA, Fettell NA, Tabe L, Jenkins CLD (2006) Genotypic variation in water-soluble carbohydrate accumulation in wheat. Funct Plant Biol 33:799-809

Saint Pierre C, Trethowan R, Reynolds M (2010) Stem solidness and its relationship to water-soluble carbohydrates: association with wheat yield under water deficit. Functional Plant Biology 37:166-174

Savin R, Slafer GA (1991) Shading effects on the yield of an Argentinian wheat cultivar. The Journal of Agricultural Science 116:1-7

Semagn K, Babu R, Hearne S, Olsen M (2014) Single nucleotide polymorphism genotyping using Kompetitive Allele Specific PCR (KASP): overview of the technology and its application in crop improvement. Molecular Breeding 33:1-14

Shakiba MR, Ehdaie B, Madore MA, Waines JG (1996) Contribution of internode reserves to grain yield in a tall and semidwarf spring wheat [Triticum aestivum]. Journal of Genetics & Breeding (Italy)

Shukla S, Singh K, Patil RV, Kadam S, Bharti S, Prasad P, Singh NK, Khanna-Chopra R (2015) Genomic regions associated with grain yield under drought stress in wheat (*Triticum aestivum* L.). Euphytica 203:449-467

Slafer GA (2003) Genetic basis of yield as viewed from a crop physiologist's perspective. Annals of Applied Biology 142:117-128

Slafer GA, Andrade FH (1993) Physiological attributes related to the generation of grain yield in bread wheat cultivars released at different eras. Field Crops Research 31:351-367

Slafer GA, Araus JL, Richards RA (1999a) Physiological traits that increase the yield potential of wheat. Wheat: ecology and physiology of yield determination. Eds EH Satorre, GA Slafer: pp. 379–415. Food Products Press: New York

Slafer GA, Araus JL, Richards RA (1999b) Physiological traits that increase the yield potential of wheat. In 'Wheat: ecology and physiology of yield determination'.Eds EH Satorre, GA Slafer: pp. 379–415. Food Products Press: New York

Slafer GA, Calderini DF, Miralles DJ (1996) Yield components and compensation in wheat: opportunities for further increasing yield potential. Increasing yield potential in wheat: Breaking the Barriers.Processidning of the workshop held in ciudad, Obrgan, Sonora, MexicoM.P. Reynolds, S. Rajaram and A. McNab (edrs):pp101-133

Slafer GA, Miralles DJ (1992) Green area duration during the grain filling period of an Argentine wheat cultivar as influenced by sowing date, temperature and sink strength. Journal of Agronomy and Crop Science 168:191-200

Slafer GA, Savin R (1994) Source—sink relationships and grain mass at different positions within the spike in wheat. Field Crops Research 37:39-49

Soriano JM, Malosetti M, Roselló M, Sorrells ME, Royo C (2017) Dissecting the old Mediterranean durum wheat genetic architecture for phenology, biomass and yield formation by association mapping and QTL meta-analysis. PloS One 12:e0178290

Staňková H, Hastie AR, Chan S, Vrána J, Tulpová Z, Kubaláková M, Visendi P, Hayashi S, Luo M, Batley J (2016) BioNano genome mapping of individual chromosomes supports physical mapping and sequence assembly in complex plant genomes. Plant Biotechnology Journal 14:1523-1531

Tardieu F (2011a) Any trait or trait-related allele can confer drought tolerance: just design the right drought scenario. Journal of Experimental Botany:err269

Tardieu F (2011b) Any trait or trait-related allele can confer drought tolerance: just design the right drought scenario. Journal of Experimental Botany 63:25-31

Tucker CJ (1979) Red and photographic infrared linear combinations for monitoring vegetation. Remote Sens Environment 8:127-150

Tuinstra MR, Ejeta G, Goldsbrough PB (1997) Heterogeneous inbred family (HIF) analysis: a method for developing near-isogenic lines that differ at quantitative trait loci. TAG Theoretical and Applied Genetics 95:1005-1011

Uauy C (2017) Wheat genomics comes of age. Current Opinion Plant Biolology 36:142-148

Wang S, Wong D, Forrest K, Allen A, Chao S, Huang BE, Maccaferri M, Salvi S, Milner SG, Cattivelli L (2014) Characterization of polyploid wheat genomic diversity using a high- density 90 000 single nucleotide polymorphism array. Plant Biotechnology Journal 12:787-796

Wilkinson PA, Winfield MO, Barker GL, Allen AM, Burridge A, Coghill JA, Edwards KJ (2012) CerealsDB 2.0: an integrated resource for plant breeders and scientists. BMC Bioinformatics 13:219

Wu Q-H, Chen Y-X, Zhou S-H, Fu L, Chen J-J, Xiao Y, Zhang D, Ouyang S-H, Zhao X-J, Cui Y (2015) High-density genetic linkage map construction and QTL mapping of grain shape and size in the wheat population Yanda1817× Beinong6. PloS One 10:e0118144

Xing Y, Zhang Q (2010) Genetic and molecular bases of rice yield. Annu Rev Plant Biol 61:421-442

Yáñez A, Tapia G, Guerra F, del Pozo A (2017) Stem carbohydrate dynamics and expression of genes involved in fructan accumulation and remobilization during grain growth in wheat (Triticum aestivum L.) genotypes with contrasting tolerance to water stress. PloS One 12:e0177667

Yang J, Zhang J, Wang Z, Zhu Q, Liu L (2001) Water deficit–induced senescence and its relationship to the remobilization of pre-stored carbon in wheat during grain filling. Agronomy Journal 93:196-206

Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. Weed Research 14:415-421

Zamani MM, Nabipour M, Meskarbashee M (2014) Stem water soluble carbohydrate remobilization in wheat under heat stress during the grain filling. International Journal of Agriculture and Biology 16:401-405

Zhu J, Vos J, van der Werf W, van der Putten PE, Evers JB (2013) Early competition shapes maize whole-plant development in mixed stands. Journal of Experimental Botany 65:641-653

Zikhali M, Wingen LU, Leverington- Waite M, Specel S, Griffiths S (2017) The identification of new candidate genes Triticum aestivum FLOWERING LOCUS T3- B1 (TaFT3- B1) and TARGET OF EAT1 (TaTOE1- B1) controlling the short day photoperiod response in bread wheat. Plant, Cell & Environment

Tables and Figures

Table 1. QTL and QTL effect size for grain yield, thousand grain weight, days to heading and grain duration a cross 28 environments.

Trait	Chromosome	Flanking markers	QTL peak (cM)	P Q x E	P main effect	QTL effect
Grain yield	1A	X3021844 - gwm0558	45.1 - 46.8	ns	<0.001	43
	1B	adw1061145 - adw490	160.4 - 182.2	<0.05	<0.001	23.6 - 136.1
	2B.1	X3064619 - X100005743	9.0 - 16.4	<0.001	ns	126 - 250
	2B.2	X1075921 - X3021359	52.2 - 52.9	ns	<0.01	32 - 240
	2D	X2242328 - X991014	19.4 - 28.2	<0.05	<0.0001	38 - 93
	3A	X1026080 - 991604	157.5 - 158.8	ns	<0.001	-91
	4A.1	X1072050 - X979934	51.4 - 53.5	ns	<0.0001	152
	4A.2	X3064552 - X1125529	130.0 - 142.7	<0.01	<0.01	-560 to -46
	5A	X1135154 - Vrn1A	123.6 - 125.5	<0.0001	<0.05	-583 to -53
	5B.1	X1032121 - X3026027	115.1 - 115.9	<0.05	<0.0001	377
	5B.2	X3020443 - X2261812	139.8 - 143.1	<0.001	ns	-580 to -192
	6DLb	X2244522 - X990024	14.3 - 17.2	ns	<0.001	116
	7A.1	1246868 - X1228158	56.3 - 56.9	<0.0001	ns	-546 to 448
	7A.2	X2341144 - X987692	171.5 - 174.5	<0.0001	<0.01	-463 to 301
	7DS	WPT-2551 - WPT-0366	0.0 - 1.1	<0.05	ns	28
TGW	1B	adw1005607 - adw535	164.2 - 178.6	ns	<0.001	2.1
	2A	X1133336 - X1056356	24.9 - 30.5	<0.01	ns	-1.9 to -1.2
	2B	X1022997 - X1082017	67.2 - 67.8	<0.05	<0.0001	1 - 2.4
	2DS	X1162627 - X1128261	2.6 - 4.3	ns	<0.0001	-0.8
	3A	cfa2170 - X1205035	123.2 - 123.9	<0.001	<0.05	-1.3 to 3.8
	3DL	X1234793 - X3026282	1.3 - 1.9	<0.01	ns	-3.9 to -3.8
	3DSL	X1109431 - wPt-2313	0 - 7.4	<0.01	ns	1.2
	4B	ksm0154 - DuPw0036	45.4 - 72.7	<0.01	ns	-1.7 to 5.7
	5A	X1135154 - Vrn1A	123.6 - 125.5	<0.001	ns	-1.4 to 1.8
	5B	X3023130 - X1217242	128.2 - 130.2	<0.05	ns	0.8
	7A.1	1246868 - X1228158	56.3 - 56.9	<0.0001	<0.05	1.3 - 4.1
	7A.2	barc0195 - X1127751	95.5 - 97.4	<0.001	<0.001	1.6 - 2.5
Days to heading	1D	X2243386 - 1126683	70.3 - 84.1	ns	<0.01	1
	2A	X1008748 - X100003139	33.0 - 39.3	ns	<0.01	2
	2D	X1046316 - wPt-1991	70.5 - 74.4	ns	<0.001	-4
	3A.1	X1164647 - X3022477	45.8 - 48.0	ns	<0.01	2.3
	3A.2	X1121175 - cfa2170	114.2 - 123.2	ns	<0.01	0.9
	3D.1	wPt-2313 - 2245896	7.4 - 22.0	<0.01	ns	-1.8
	3D.2	wPt-6262 - X1125700	59.0 - 60.4	ns	<0.01	-2.6
	4A	X1130647 - X1242399	124.0 - 124.4	<0.05	<0.0001	2.4 - 4.2
	5A	X1135154 - Vrn1A	123.6 - 125.5	<0.0001	ns	2.9 - 7.1
	6A.1	X1128290 - X1247837	12.3 - 13.6	ns	<0.01	-2.5

	6A.2	X3023657 - X1090067	80.2 - 83.3	ns	<0.0001	1.5
	7A	1246868 - X1228158	56.3 - 56.9	<0.0001	<0.01	-4.3 to -11.3
	7B	X1008572 - X1712267	57.6 - 59.0	ns	<0.05	-1.9 to -3
Grain filling duration	1A	X100000274 - X1023638	0 - 0.6	ns	<0.01	-1
	1B	wmc0830 - psp3100	105.3 -110.5	ns	<0.01	-1
	2B	X1143915 - X1133994	85.5 - 87.4	ns	<0.01	-2
	3B	wPt-8206 - X100001613	194.6 - 200.6	ns	<0.001	1
	4B	gwm0495 - ksm0154	43.4 - 45.5	ns	<0.01	2
	5A	X1135154 - Vrn1A	123.6 -125.5	<0.0001	<0.05	-3 to -2
	7A.1	1246868 - X1228158	56.3 - 56.9	<0.0001	<0.001	3 - 4
	7A.2	barc0195 - X1127751	95.5 - 97.4	ns	<0.001	2

P are p-values for the test of QTL main effect (P main) and QTL by environment interaction (P QxE) from the Wald test. ns means not significant. * indicates estimates significantly different from zero (with confidence interval of the estimate calculated as CI = estimate +/- 10.96*SE). Positive number shows allelic effect from Excalibur, negative number from Kukri. The highlighted yield QTL is partially overlapped with TGW QTL on chromosome 1B and targeted for further fine mapping.

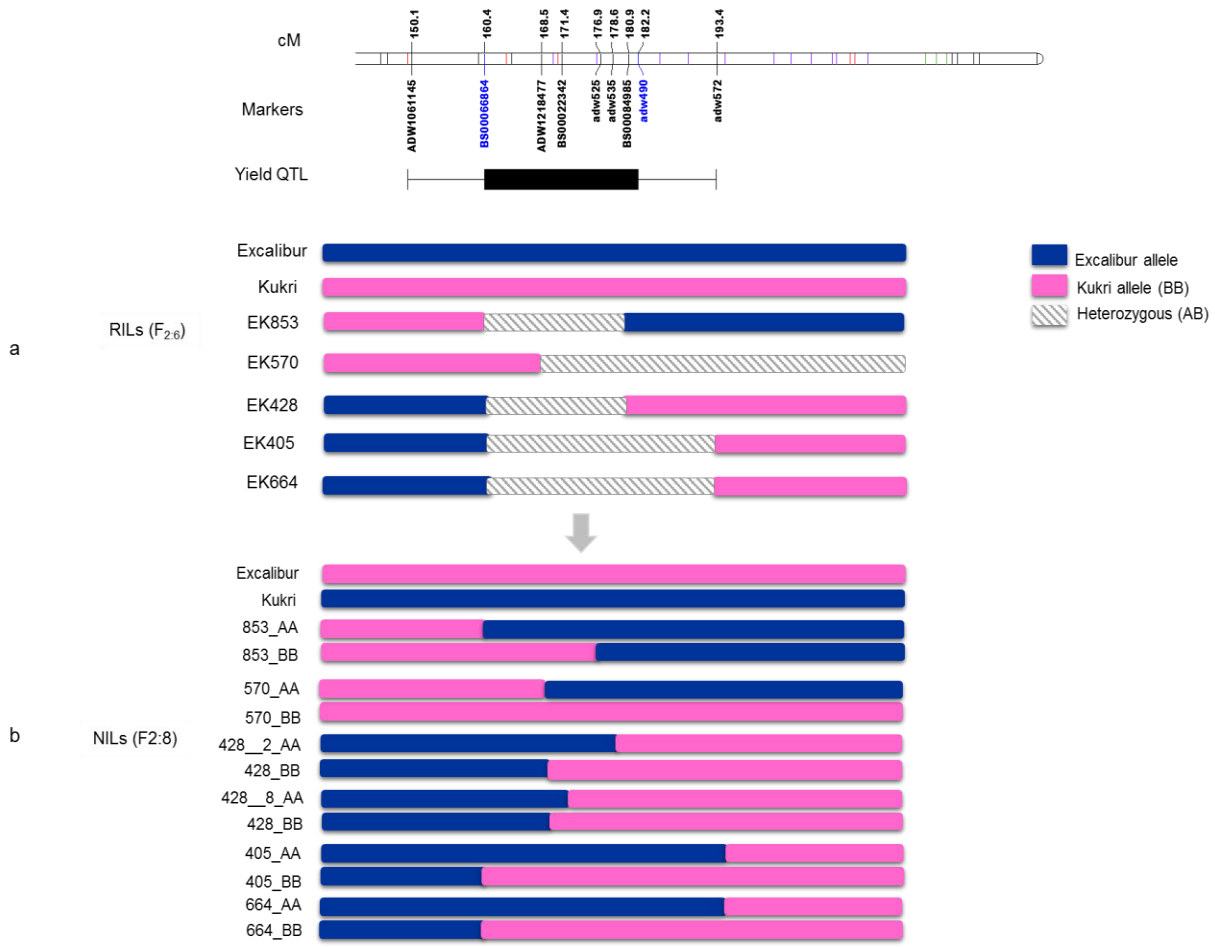


Figure. 1. Five heterozygous RIL (F2:6) contrasting at four markers under the QTL on chromosome 1B (a) and the six pairs of NIL haplotypes developed from these RIL (b).

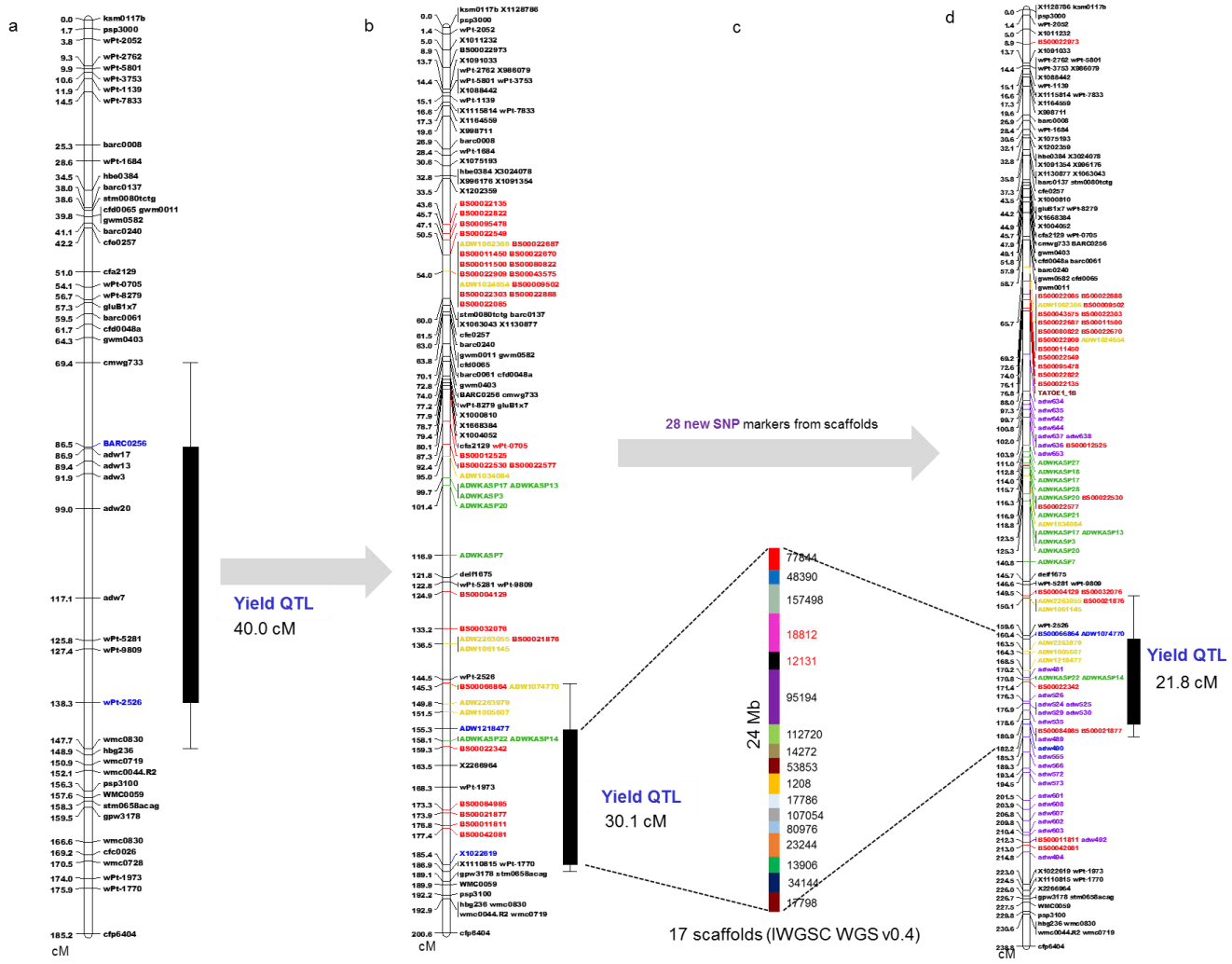


Figure. 2. Yield QTL position on chromosome 1B genetic map in Excalibur/Kukri DH initial map by Edward (2012) (a), its reduced interval on the updated map with KASP™ markers (b) its alignment onto 17 physical scaffolds (IWGSC WGS v0.4) (c) and high resolution genetic map with new SNP based KASP™ markers (purple), SSR-DArT and GBS markers (black), BS markers (red), GBS converted to KASP markers (yellow) and KASP markers from 90K Wheat Illumina Infinium iSelect (Comai et al.) (d).

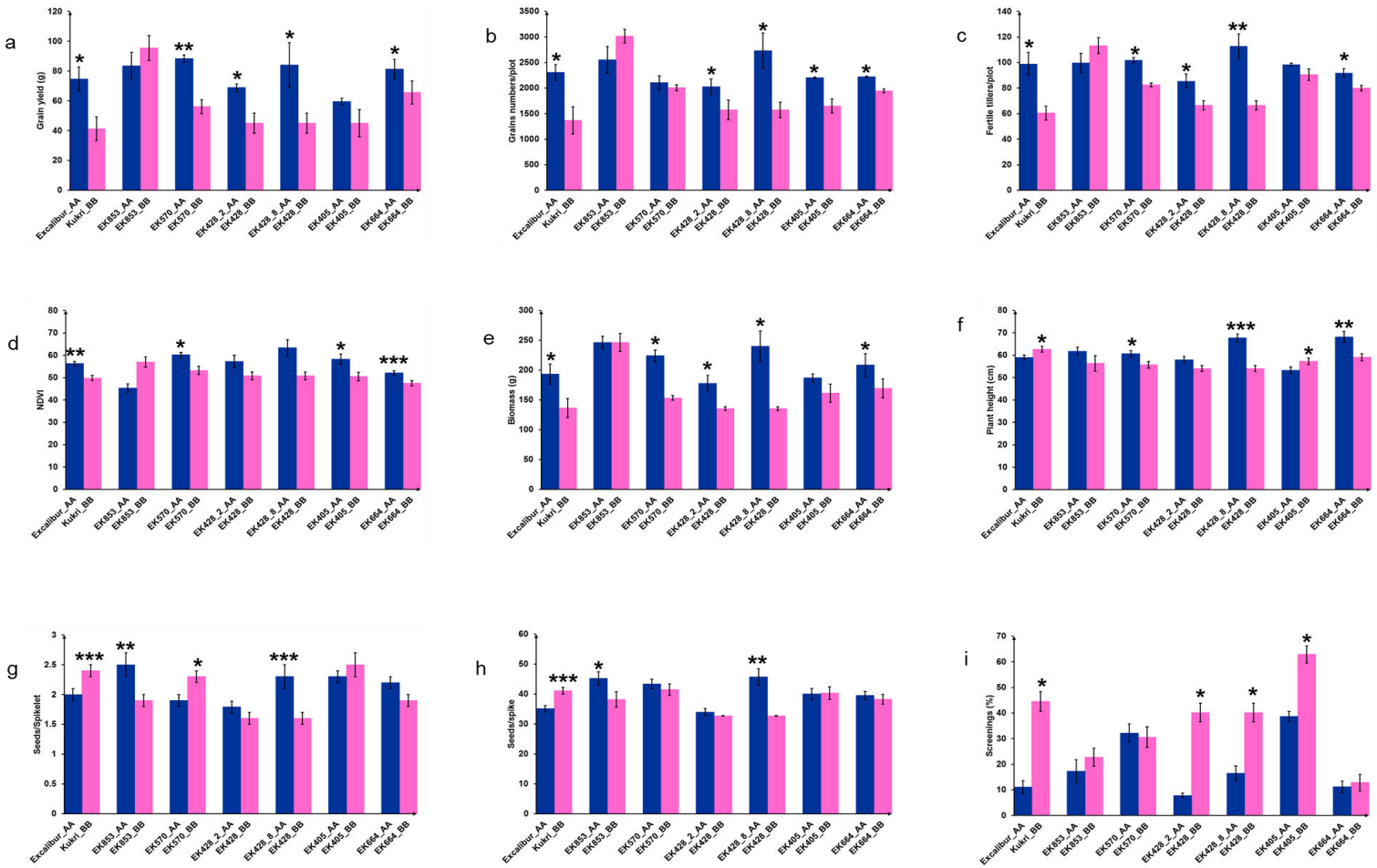


Figure. 3. Effect of six pairs of NIL on grain yield (a), total grains number/plot (b), fertile tillers (c), NDVI (d), biomass (e), plant height (f), seeds/spikelet (g), seeds/spike (h), and screenings (i) under severe drought and hot conditions (Urrbrae, SA, 2016). Differences between the mean of each NIL pair was evaluated at specific marker in the QTL region using *t-test*. *, **, *** significant at $p < 0.05$, 0.01 and 0.001 level, respectively.

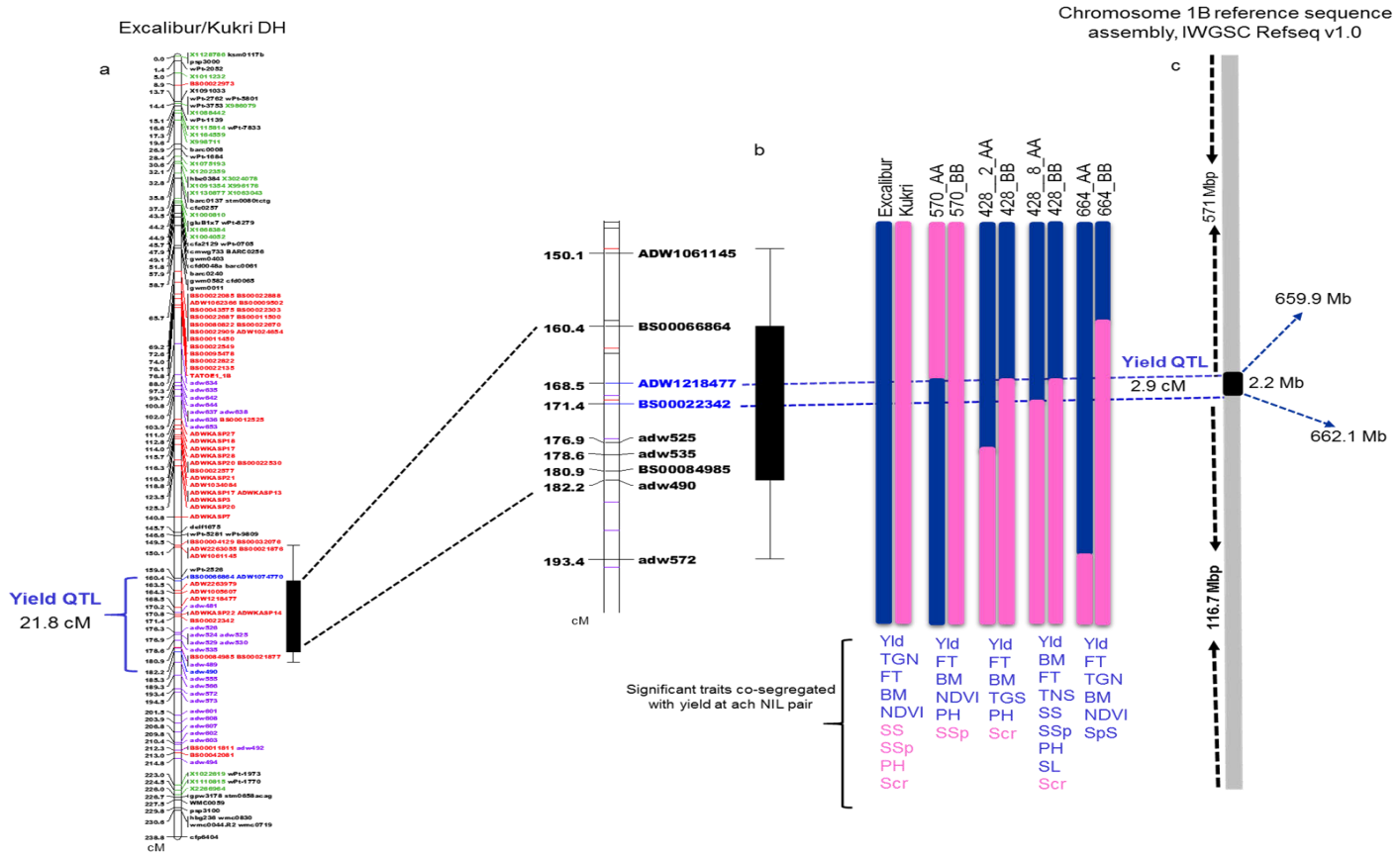


Figure. 4. High resolution genetic map of 1B QTL (a), schematic representation of 4 pairs of NIL haplotypes with significant traits co-segregating with grain yield aligned to Excalibur/Kukri DH yield QTL to delimit the QTL interval (blue line) (b) and position of QTL interval on the chromosome 1B Chinese Spring reference sequence assembly (IWSGC RefSeq V0.1). Traits in blue color indicating Excalibur allele, while red color is for Kukri allele. Yld = yield, TGN = total grains number, FT = fertile tillers, BM = biomass, NDVI, normalized difference vegetative index, SS = seeds/spike, SSp = seeds/spikelet, SpS = spikelet/spike, SL= spike length, PH = plant height, Scr = screenings.

Supplementary Tables and Figures

Supplementary Table 1. Growing season climatic data for 32 field experiments. Min: minimum; max: maximum.

Trial	Rainfall + irrigation	Number of days		Total daylight	Day length (hours)	
	mm	<10°C	>30°C	hours	min	max
Mex-Obr07-rf	150	98	10	1531	10.3	12.7
Mex-Obr07-ir	500[#]	106	38	1924	10.3	13.4
Aus-Boo06-rf	86	147	21	2018	9.9	14.0
Aus-Boo07-rf	191	158	16	2074	9.9	13.9
Aus-Min06-rf	68	116	10	1673	9.9	13.5
Aus-Min07-rf	74	120	12	1760	9.9	13.5
Aus-Min08-rf	114	112	8	1750	9.9	13.5
Aus-Pie07-rf	173	82	2	1585	9.9	12.9
Aus-Rac06-rf	131	154	12	1900	9.7	13.8
Aus-Rac07-rf	229	142	9	1873	9.7	13.7
Aus-Rac08-rf	222	144	9	1837	9.7	13.6
Aus-Rob07-rf	99	135	7	1821	9.7	13.6
Ind-His11-ir	254	100	27	1650	10.3	12.8
Ind-His11-rf	74	100	27	1650	10:3	12.8
Ind-His12-ir	352	97	16	1611	10:3	12.7
Ind-His12-rf	172	97	16	1611	10:3	12.7
Ind-Kan10-ir	282	52	23	1499	10:5	12.5
Ind-Kan10-rf	102	52	23	1499	10.5	12.5
Ind-Kan11-ir	282	48	25	1561	10.5	12.5
Ind-Kan11-rf	102	48	25	1561	10.5	12.5
Ind-Kan12-ir	367	73	32	1592	10.5	12.5
Ind-Kan12-rf	187	73	32	1592	10.5	12.5
Ind-Kar10-ir	251	84	20	1530	10.2	12.5
Ind-Kar10-rf	71	84	20	1530	10.2	12.5
Ind-Kar11-ir	264	91	15	1477	10.2	12:6
Ind-Kar11-rf	84	91	15	1477	10.2	12.6
Ind-Kar12-ir	440	87	16	1550	10.2	12.8
Ind-Kar12-rf	260	87	16	1550	10.2	12.8
Ind-Pun-10-ir	314	31	46	1476	11.0	11.7
Ind-Pun-10-rf	134	31	46	1476	11.0	11.7
Ind-Pun-11-ir	282	36	32	1535	11.0	11.8
Ind-Pun-11-rf	102	36	32	1535	11.0	11.8
Ind-Pun-12-ir	250	22	22	1547	11.0	11.5
Ind-Pun-12-rf	70	22	22	1547	11.0	11.5

An estimate of the water volume applied using flood irrigation.
The average vegetative temperature was calculated as the average daily temperature from sowing to first node. The average reproductive temperature was calculated as the average daily temperature from first node to physiological maturity.

Supplementary Table 2. Field experiment, location and abbreviation details.

Site	Address	Latitude	Longitude	Altitude (m)
Boo	Booleroo Centre, South Australia, Australia	32.88° S	138.35° E	390
Min	Minnipa Agricultural Research Centre, Minnipa, South Australia, Australia	32.86° S	135.14° E	139
Pie	Piednippie, South Australia, Australia	32.44° S	134.28° E	85
Ros	University of Adelaide, Roseworthy Campus, Roseworthy, South Australia, Australia	34.57° S	138.74° E	87
Rob	Robinvale, Victoria, Australia	34.59° S	142.78° E	58
Obr	International Maze and Wheat Improvement Centre, CIANO, Obregon, Mexico	27.20° N	109.56° W	38
His	Hissar, Haryana, India	29.10° N	75.46° E	215
Kan	CSAU, Kanpur, UP, India	26.28° N	80.24° E	125
Kar	Karnal, Haryana, India	29.43° N	70.58° E	245
Pun	Pune, India	18.04° N	74.21° E	548

Supplementary Table 3. List of successful KASP™ markers designed from the QTL interval and mapped on 1B map to fine map the QTL

SNP KASP™ marker ID	Primer_Allele X	Primer_Allele Y	Primer_Common	Allele X	Allele Y
adw481	GAAGGTGACCAAGTTCATGCTAGAGCCTTACGCACCTCGGTT	GAAGGTCGGAGTCAACGGATTGAGCCTTACGCACCTCGGTC	CGGGCATGGATATGGCTATGGAAAA	A	G
adw489	GAAGGTGACCAAGTTCATGCTATATCTCAAGTAAAGGAATGGAGTGC	GAAGGTCGGAGTCAACGGATTGATATATCTCAAGTAAAGGAATGGAGTGT	CAATAATACTCCCAGGAAAGGAAAACCTA	G	A
adw490	GAAGGTGACCAAGTTCATGCTGTTGGTGCAACTTACTGCCACAC	GAAGGTCGGAGTCAACGGATTGTTGGTGCAACTTACTGCCACAG	GTGCGGCGCCATGATACCCTT	G	C
adw492	GAAGGTGACCAAGTTCATGCTCAAATCGACGAGACAGTGGTGG	GAAGGTCGGAGTCAACGGATTCAAATCGACGAGACAGTGGTGA	CTATGCGGGGAGAACCTTATTCCAA	G	A
adw494	GAAGGTGACCAAGTTCATGCTACCGTGTTAATTTTCATTATCGGAGCT	GAAGGTCGGAGTCAACGGATTCCGTGTTAATTTTCATTATCGGAGCC	GGAGCATACTGCACACGGGGAA	A	G
adw524	GAAGGTGACCAAGTTCATGCTATCGCCCGGATCGAGGCCA	GAAGGTCGGAGTCAACGGATTCGCCCGGATCGAGGCCG	GGTGGACGGTAGGCGGGGAA	A	G
adw525	GAAGGTGACCAAGTTCATGCTTCCAGTTCCATCGATCTATGCG	GAAGGTCGGAGTCAACGGATTCTTCCAGTTCCATCGATCTATGCA	GATCTAGGAAGCAATGGACAATGAATGAT	G	A
adw526	GAAGGTGACCAAGTTCATGCTACCGTTCCCCGTGCCCA	GAAGGTCGGAGTCAACGGATTACCGTTCCCCGTGCCCG	GTCGTACCTGTCGGCCTCGTA	T	C
adw529	GAAGGTGACCAAGTTCATGCTCGCCACCGCCGAGGTCTG	GAAGGTCGGAGTCAACGGATTCGCCACCGCCGAGGTCTT	ACTGCGACAAAACATCGTGCACGAA	C	A
adw530	GAAGGTGACCAAGTTCATGCTCCTCGTCAGCGTTGTAGAGAC	GAAGGTCGGAGTCAACGGATTGCCTCGTCAGCGTTGTAGAGAT	GGCACATATCCCCTCTCAAGCAAAA	G	A
adw535	GAAGGTGACCAAGTTCATGCTCAGTACCTATACGTCCGCGCC	GAAGGTCGGAGTCAACGGATTCAGTACCTATACGTCCGCGCT	CGTGCACGCTCCACTACTCCAA	C	T
adw555	GAAGGTGACCAAGTTCATGCTAAGAATCCTCTGTTTCGGCGGC	GAAGGTCGGAGTCAACGGATTGAAGAATCCTCTGTTTCGGCGGT	GAACTCGCCATGCTTTCCCAAATCAA	G	A
adw566	GAAGGTGACCAAGTTCATGCTGCCGTTCTTCGGTGACATC	GAAGGTCGGAGTCAACGGATTGCTGCCGTTCTTCGGTGACATT	AAGGCGCCGACCACGCCCA	C	T
adw572	GAAGGTGACCAAGTTCATGCTATGCATCTTGATCCTTCGGATTCAC	GAAGGTCGGAGTCAACGGATTATGCATCTTGATCCTTCGGATTCAG	TTGGCCAACCTTCATCGCTCAGAA	C	G
adw573	GAAGGTGACCAAGTTCATGCTCGCTGAGTATGGAGGCGGCA	GAAGGTCGGAGTCAACGGATTGCTGAGTATGGAGGCGGCG	ATGGCTGTGAGGCCTCCACCAA	T	C
adw601	GAAGGTGACCAAGTTCATGCTCCTAATTGTGTCTCAACGGCG	GAAGGTCGGAGTCAACGGATTCTCCTAATTGTGTCTCAACGGCA	CAAGGCTGTGGCGGCAGCCAA	G	A
adw602	GAAGGTGACCAAGTTCATGCTCACCATACTTCAGGCAGGTTCTG	GAAGGTCGGAGTCAACGGATTCACCATACTTCAGGCAGGTTCTT	TATGGAATGAAGATGCTGACAAGTGGAAA	G	T
adw603	GAAGGTGACCAAGTTCATGCTCGTACTTGAACTCATCGAAGACATCA	GAAGGTCGGAGTCAACGGATTGTACTTGAACTCATCGAAGACATCG	GCCTGGCTCAAAGCGCTCAAGAA	A	G
adw607	GAAGGTGACCAAGTTCATGCTCGGTAACGTGACCAGCTATGAG	GAAGGTCGGAGTCAACGGATTCGGTAACGTGACCAGCTATGAC	GCTGCCAACCTCTTAAAATTTCCTTTGAA	G	C
adw608	GAAGGTGACCAAGTTCATGCTCGCATATGAACCCATCCTCATCAT	GAAGGTCGGAGTCAACGGATTGCATATGAACCCATCCTCATCAC	GCTGGAGGAGGCCTTCTGTGTT	T	C
adw634	GAAGGTGACCAAGTTCATGCTACCAATGTGAGCTACTACAATTGAGT	GAAGGTCGGAGTCAACGGATTCCAATGTGAGCTACTACAATTGAGC	GTATCTCAACAAAACAGGACCCTCATTTT	A	G
adw635	GAAGGTGACCAAGTTCATGCTAAATTATCTTACCAGGAAATATTTTGAATACAT	GAAGGTCGGAGTCAACGGATTAAATTATCTTACCAGGAAATATTTTGAATACAG	TGATTCTCCTTTGTTGAACCTTTGCAGAT	A	C
adw636	GAAGGTGACCAAGTTCATGCTTCGTCCTCCGCCTGTCAACA	GAAGGTCGGAGTCAACGGATTCGTCCTCCGCCTGTCAACG	TATATAAAAACTTAGGAGAGGAGGAGGAAA	T	C
adw637	GAAGGTGACCAAGTTCATGCTGGCAGGCAAATCAGAGACGTGAT	GAAGGTCGGAGTCAACGGATTGCAGGCAAATCAGAGACGTGAC	CTAGCCTCTTGTACTCTTCCAGCAT	T	C
adw638	GAAGGTGACCAAGTTCATGCTGACATTTTCACATGACCCACACTGAA	GAAGGTCGGAGTCAACGGATTACATTTTCACATGACCCACACTGAG	GGAGTTGTTAAGCACTTGTATATGGGAAT	T	C
adw642	GAAGGTGACCAAGTTCATGCTGGTGGCGCTTGGTTGCCAGT	GAAGGTCGGAGTCAACGGATTGTGGCGCTTGGTTGCCAGC	GGGCAAGGCAACTTTGGTCAGTT	A	G
adw644	GAAGGTGACCAAGTTCATGCTCCAGATGGTGCTAATCTCCCCTT	GAAGGTCGGAGTCAACGGATTCAGATGGTGCTAATCTCCCCTC	AACAACAATCGTTGGACTACTTGCTGAAT	T	C
adw653	GAAGGTGACCAAGTTCATGCTTGCTATAACTGAAGCCGAAATCCTTA	GAAGGTCGGAGTCAACGGATTGCTATAACTGAAGCCGAAATCCTTG	GATGTGCTCGAGACTGTCAAATATCATAA	T	C

Supplementary Table 4. Heritability estimates in each environment derived from MET of the Excalibur/Kukri DH population of four traits. Yield = yield kg/ha, TGW = thousand grain weight, DTH = days to heading, and GFD = grain filling duration, Aus = Australia, Mex = Mexico, Ind = India, rf rainfed, ir = irrigated trial-: trait not measured in the environment, ns: sites excluded from the final model due to very low estimated genetic variance.

Environment	Heritability			
	Yield	Thousand grain weight	Days to heading	Grain filling duration
Aus-Boo06-rf	0.69	0.81	0.90	-
Aus-Boo07-rf	0.68	0.86	-	-
Mex-Obr07-ir	0.78	0.83	0.90	0.83
Mex-Obr07-rf	0.80	0.68	0.90	0.75
Aus-Min06-rf	0.75	0.85	0.87	-
Aus-Min07-rf	0.82	0.83	0.90	-
Aus-Pie07-rf	0.81	0.85	0.90	-
Aus-Rob07-rf	0.82	0.76	-	-
Aus-Ros06-rf	0.85	0.81	0.90	-
Aus-Ros07-rf	0.79	0.85	0.91	0.77
Ind-His11-ir	ns	ns	0.27	-
Ind-His11-rf	0.30	ns	0.87	-
Ind-His12-ir	ns	0.71	-	-
Ind-His12-rf	ns	0.46	-	-
Ind-Kan10-ir	ns	-	-	0.28
Ind-Kan10-rf	0.21	-	-	0.14
Ind-Kan11-ir	-	0.25	-	0.51
Ind-Kan11-rf	-	0.70	-	0.51
Ind-Kan12-ir	0.34	0.18	-	ns
Ind-Kan12-rf	ns	ns	-	ns
Ind-Kar10-ir	0.49	0.69	0.78	0.69
Ind-Kar10-rf	ns	0.52	0.77	0.68
Ind-Kar11-ir	-	-	0.77	0.50
Ind-Kar11-rf	-	-	0.75	0.18
Ind-Kar12-ir	0.58	0.67	0.74	0.40
Ind-Kar12-rf	0.59	0.62	0.79	0.47
Ind-Pun10-ir	0.67	0.73	0.83	ns
Ind-Pun10-rf	0.40	0.73	0.82	0.64
Ind-Pun11-ir	ns	0.74	0.82	0.59
Ind-Pun11-rf	0.61	0.73	0.83	ns
Ind-Pun12-ir	0.30	0.74	0.83	0.69
Ind-Pun12-rf	ns	0.60	0.83	0.15

Supplementary Table 5. QTL originally found by Edwards (2012) on Excalibur/Kukri DH. Grain yield and co-located yield component QTL, marker interval, the number of experiments the QTL are significant in (P<0.05), heritability genotypic variance (σ^2g) and effect of the QTL and higher parental lines.

QTL name	Interval	Trait	Significant in # Environments	# MET analyses	Yield Kg ha^{-1}	Heritability	Variation (σ^2g)	Effect	Higher Parent
QYld.www-1B	wpt9809-wpt2526	Grain yield	6	2	290-2669	0.42-0.87	2.8-18.3	23-173	Excalibur
	wpt9809-wpt2526	Early vigour	2	–	–	0.65-0.76	3.0-9.0	< 0.5	Excalibur
	wpt9809-wpt2526	Sterility	3	–	–	0.52-0.76	5.0-8.0	< 0.5	Kukri
	wt9809-wpt2526	Screenings	2	–	–	0.77-0.95	2.0-3.0	< 2	Kukri
	wpt9809-wpt2526	TGW	2	–	–	0.92-0.95	1.0-2.0	< 2	Excalibur
	wpt9809-wpt2526	Test weight	4	–	–	0.62-0.92	5.0-12.0	4.0-13.0	Excalibur
	wpt9809-wpt2526	Grains m^2	4	–	–	0.48-0.78	4.0-9.0	101-325	Excalibur

Supplementary Table 6. Lists of 17 scaffolds retrieved from the yield QTL (*QYld.aww-1B.2*) interval between 155 and 185.4 cM in POPSEQ map using whole genome shotgun sequence assembly of Chinese Spring (IWGSC WGS v0.4).

						Coordinates on 1B IWGSC WGS v0.4	
Marker	**Excalibur/akukri DH map position (cM)**	**scaffold**	**chr**	**POPSEQ (cM)**	**scaffold_length (bp)**	**start (bp)**	**end (bp)**
ADW1218477	154.3	scaffold77844	1B	99.4	1,808,227	659,049,488	660,857,714
		scaffold48390	1B	100.1	327,306	660,857,815	661,185,120
BS00022342	159.3	scaffold157498	1B	99.8	2,249,376	661,185,221	663,434,596
		scaffold18812	1B	100.7	1,114,838	663,434,697	664,549,534
		scaffold12131	1B	102.8	3,106,187	664,549,635	667,655,821
X2266964	163.5	scaffold95194	1B	105.1	3,155,354	667,655,922	670,811,275
wPt-1770	168.5	scaffold112720	1B	106.1	1,779,378	670,811,376	672,590,753
BS00021877	173.9	scaffold14272	1B	108.1	2,952,914	672,590,854	675,543,767
		scaffold53853	1B	109.9	1,720,842	675,543,868	677,264,709
		scaffold1208	1B	110.0	544,619	677,264,810	677,809,428
		scaffold17786	1B	111.5	417,275	677,809,529	678,226,803
		scaffold107054	1B	111.5	186,836	678,226,904	678,413,739
		scaffold80976	1B	111.1	973,779	678,413,840	679,387,618
		scaffold23244	1B	110.3	739,720	679,387,719	680,127,438
BS00011811	176.8	scaffold13906	1B	111.9	1,193,198	680,127,539	681,320,736
		scaffold34144	1B	113.7	810,822	681,320,837	682,131,658
X1022619	185.4	scaffold17798	1B	112.4	959,100	682,131,759	683,090,858
					24,039,771		

Supplementary Table 7. Phenotypic performance of 6 NIL pair and two parental lines under severe drought and hot conditions, Urrbrae, SA, 2016.

NILs	BM (g)	TGN	Yld (g)	FT	SS	SSp	SpS	NDVI	PH (cm)	SL (cm)	Scr(%)	TGW (g)
Excalibur	193.3 ± 16.8*	2306 ± 154.5 *	74.6 ± 8.2*	99 ± 9*	35.1 ± 1.1	2.0 ± 0.1	17.6 ± 0.2	56.3 ± 1 **	59 ± 1.1	7.8 ±0.1	11.1 ± 0.5	32.9 ± 1.5
Kukri	136.6 ± 15.9	1368 ± 266	41.3 ± 7.9	60.5 ± 5.5	41.1 ± 1.1***	2.4 ± 0.1***	17.7 ± 0.3	49.8 ± 1.2	62.7 ± 1.3*	8.3 ± 0.4	44.5 ± 5.8*	30.8 ± 0.2
EK853_AA	246.5 ± 10.2	2554 ± 256.6	83.4 ± 9.3	99.8 ± 7.6	45.2 ± 2.2*	2.5 ± 0.2**	21.6 ± 0.7*	45.3 ± 1.9	61.8 ± 1.8	8.8 ± 0.1	17.3 ± 4.5	34 ± 3.8
EK853_BB	288.8 ± 15.3	3016 ± 136.4	95.5 ± 8.3	113.2 ± 6.2	38.2 ± 2.6	1.9 ± 0.1	20.7 ± 0.6	57 ± 2.3	56.4 ± 3.5	9.0 ± 0.1	22.8 ± 5.5	30.4 ± 1.6
EK570_AA	224.1 ± 9.8*	2104 ± 138.5	88.4 ± 2.5**	102 ± 2.0*	43.4 ± 1.6	1.9 ± 0.1	19.1 ± 0.2**	60.2 ± 0.2*	60.7 ± 1.4*	8.5 ± 0.1	32.2 ± 7.5	31.6 ± 2.6
EK570_BB	153.2 ± 3.9	2007 ± 56	56.2 ± 4.7	82.5 ± 1.5	41.5 ± 1.9	2.3 ± 0.1*	18.0 ± 0.2	53.2 ± 1.8	55.7 ± 1.5	8.3 ± 0.1	30.6 ± 4	27.8 ± 1.4
EK428_2_AA	177.7 ± 13.2*	2025 ±150*	68.9 ±2.5*	85.5 ±5.5*	34 ±1.2	1.8 ±0.1	19.1 ±0.3	57.2 ±2.8	58 ± 1.5*	8.8 ±0.1	7.8 ±0.9	35.2±1.9
EK428_BB	135.4 ± 3.2	1574 ± 192.5	45.1 ± 6.8	66.5 ± 3.5	32.7 ± 0.1	1.6 ± 0.1	19.7 ± 0.2	50.8 ± 1.8	54.1 ± 1.3	8.7 ± 0.1	40.2 ± 5.7 *	33.4±2.1
EK428_8_AA	240 ± 25.6*	2728 ± 350*	84.1 ± 15*	113 ± 9.5**	45.7 ± 2.8***	2.3 ± 0.2***	21.2 ± 0.3*	63.3 ± 3.7	67.8 ± 1.8***	9.3 ± 0.2*	16.5 ± 2.9	30.9 ±2.1
EK428_BB	135.4 ± 3.3	1574 ±150	45.1 ± 6.8	66.5 ± 3.5	32.7 ± 0.1	1.6 ± 0.1	19.7 ± 0. 2	50.8 ± 1.8	54.1 ± 1.3	8.7 ± 0.1	40.2 ± 5.7 *	33.4±2.1
EK405_AA	186.8 ± 7	2204 ± 14.5*	59.6 ± 2.2	98.5 ± 1	40 ± 1.9	2.2 ± 0.1	17.8 ± 0.3	58.3 ± 2.3*	53.3 ± 1.4	8.7 ± 0.1	38.7 ± 2.0	27.2 ± 0.6
EK405_BB	161.3 ±15.2	1650 ± 135.4	45.1 ± 9.1	90.5 ± 4.5	40.3 ± 2.1	2.5 ± 0.2	17.4 ± 0.4	50.5 ± 2	57.3 ± 1.5*	8.7 ± 0.2	62.9 ± 3.3*	27.6 ± 1.2
EK664_AA	208.3 ± 18.9*	2220 ± 15.6*	81.3 ± 6.6*	92 ± 3.3*	39.5 ± 1.4	2.2 ± 0.1	20.2 ± 0.1*	37.3 ± 1***	68.2 ± 2.5**	8.1 ± 0.1	11.2 ± 2.2	32.5 ± 0.8
EK664_BB	169.3 ± 16.1	1946 ± 39.5	65.6 ± 7.8	80 ± 2.1	38.3 ± 1.6	1.9 ± 0.1	19.4 ± 0.1	32.5 ± 1.2	59.1 ± 1.6	7.8 ± 0.1	12.9 ± 5.2	30.6 ± 1.2

BM = biomass, TGN = total grains number, Yld = yield, FT = fertile tillers, SS = seed/spike, SSp = seed/spikelet, SpS = spikelet/sike, NDVI = normalized difference vegetative index, PH = plant height, SL = spike length, Scr = screenings and TGW = thousand grains weight Significant mean difference of NIL pair tested using two sample t-test at $P < 0.001$,***,$P < 0.01$,** and $P < 0.05$, * , level of probability.

Supplementary Table 8. Correlations among between yield and yield components in Excalibur/Kukri NIL under severe drought and hot stress condition, Urrbrae, SA, 2016.

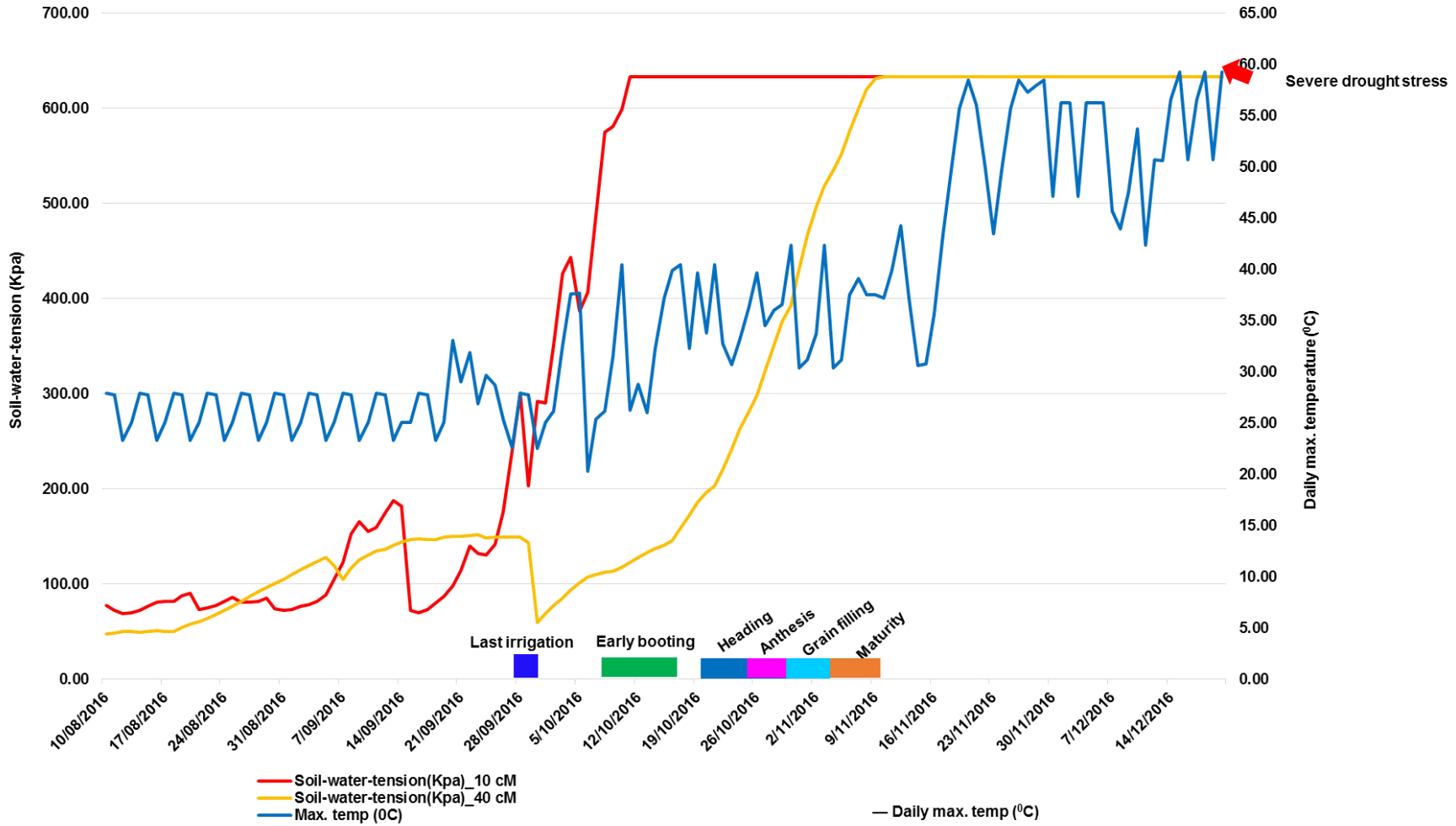
	BM	TSN	Yld	FT	SS	SSp	TKW	SpS	NDVI	PH	SL
BM	-										
TSN	0.8602	-									
Yld	0.9191	0.9244	-								
FT	0.9269	0.812	0.8573	-							
SS	0.4057	0.3285	0.3707	0.2769	-						
SSp	0.1218	0.1297	0.1501	0.132	0.8086	-					
TKW	0.4353	0.4833	0.602	0.3407	-0.0377	-0.1592	-				
SpS	0.3985	0.2492	0.2786	0.1873	0.193	-0.4153	0.1803	-			
NDVI	0.6939	0.5256	0.5344	0.7347	0.2136	0.0017	0.0846	0.338	-		
PH	0.5962	0.5783	0.6321	0.5723	0.3651	0.3373	0.4003	-0.043	0.2043	-	
SL	0.3095	0.1833	0.1808	0.2347	0.3018	0.0816	0.0169	0.3609	0.5158	0.1406	-
Scr (%)	-0.2682	-0.3723	-0.4546	-0.1948	0.0521	0.2251	-0.7761	-0.277	0.069	-0.2023	0.0989

BM = biomass, TSN = total seed number, Yld = grain yield FT = fertile tillers, SS = seed/spike, SSp = Seeds/Spikelet, TKW = thousand kernel weight, SpS = Spikelet/Spike, NDVI = Normalize difference vegetative index, PH = plant height, SL = spike length, Scr = Screening (%).Values are Pearson correlation coefficients, with significance levels indicated by $p < 0.01$ and $p < 0.001$

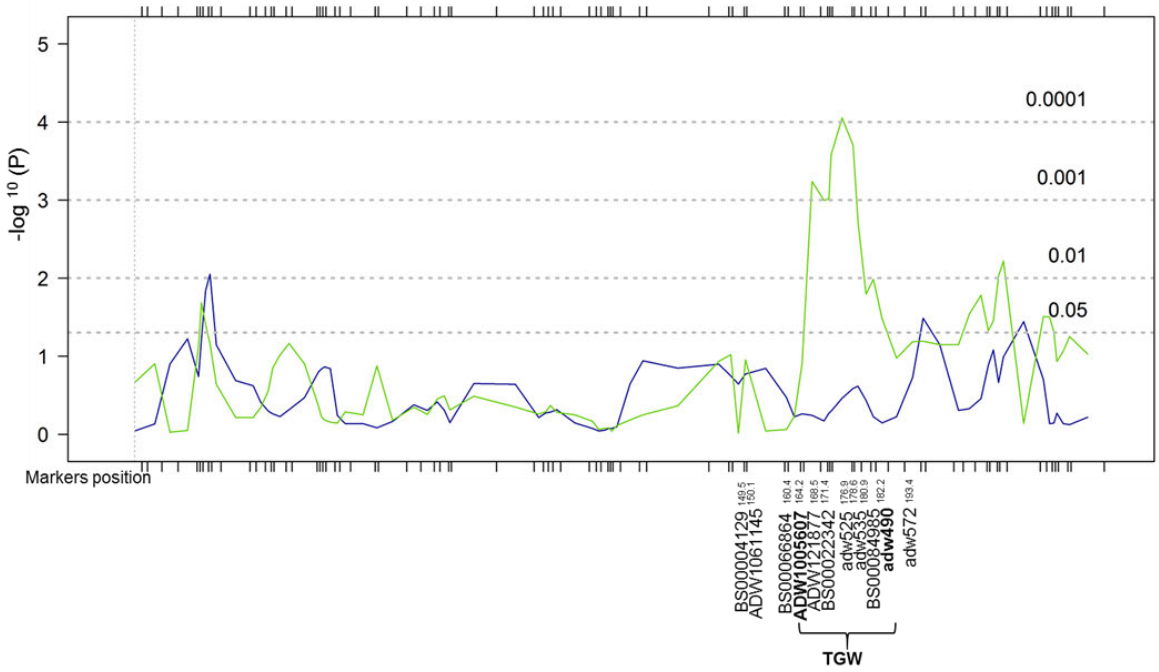
Supplementary Table 9. Genotypic profile of the six NIL pairs genotyped with 22 SNP KASP™ markers distributed across 17 chromosomes.

BS Markers on each chromosome	EK405 AA	EK405 BB	EK428 2AA	EK428 5BB	EK428 8AA	EK570 AA	EK570 BB	EK664 AA	EK664 BB	EK853 AA	EK853 BB	Excalibur	Kukri
1A_BS00005272	AA	AA	AA	AA	AA	BB	BB	BB	BB	BB	BB	AA	BB
1A_BS00012226	AA	AA	AA	AA	AA	BB	BB	BB	BB	BB	BB	AA	BB
2A_BS00022707	AA	AA	BB	BB	BB	AA	AA	AA	AA	BB	BB	AA	BB
2A_BS00023317	AA	AA	BB	BB	BB	BB	BB	BB	BB	AA	AA	AA	BB
3A_BS00022401	AA	AA	BB	BB	BB	AA	AA	AA	AA	BB	BB	AA	BB
3A_BS00025739	AA	AA	AA	AA	AA	BB	BB	BB	BB	AA	AA	AA	BB
3B_BS00076816	AA	AA	AA	AA	AA	AA	AA	BB	BB	BB	BB	AA	BB
3D_BS00067015	BB	BB	BB	BB	BB	BB	BB	BB	BB	AA	AA	AA	BB
4A_BS00021727	AA	AA	AA	AA	AA	AA	AA	BB	BB	AA	AA	AA	BB
4A_BS00021738	BB	BB	AA	AA	AA	AA	AA	BB	BB	AA	AA	AA	BB
4A_BS00022125	AA	AA	AA	AA	AA	AA	AA	BB	BB	AA	AA	AA	BB
4B_BS00011510	BB	BB	BB	BB	x	AA	AA	BB	BB	BB	BB	AA	BB
5B_BS00016003	AA	AA	AA	AA	AA	BB	BB	AA	AA	BB	BB	AA	BB
5D_BS00009821	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	BB	AA	BB
6A_BS00021982	x	x	AA	AA	AA	BB	BB	BB	BB	AA	AA	AA	BB
6B_BS00181492	AA	AA	AA	AA	AA	BB	BB	BB	BB	BB	BB	AA	BB
6D_BS00022204	AA	AA	BB	BB	BB	AA	AA	T:G	AA	AA	AA	AA	BB
7A_BS00089134	AA	AA	AA	AA	AA	AA	AA	BB	BB	BB	BB	AA	BB
7B_BS00009290	AA	AA	BB	BB	BB	BB	BB	BB	BB	BB	BB	AA	BB
2D_BS00072058	BB	AA	AA	AA	AA	BB	BB	BB	BB	BB	BB	AA	BB
3B_BS00089651	AA	AA	AA	AA	AA	BB	BB	BB	BB	BB	BB	AA	BB
5A_BS00021955	BB	BB	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB

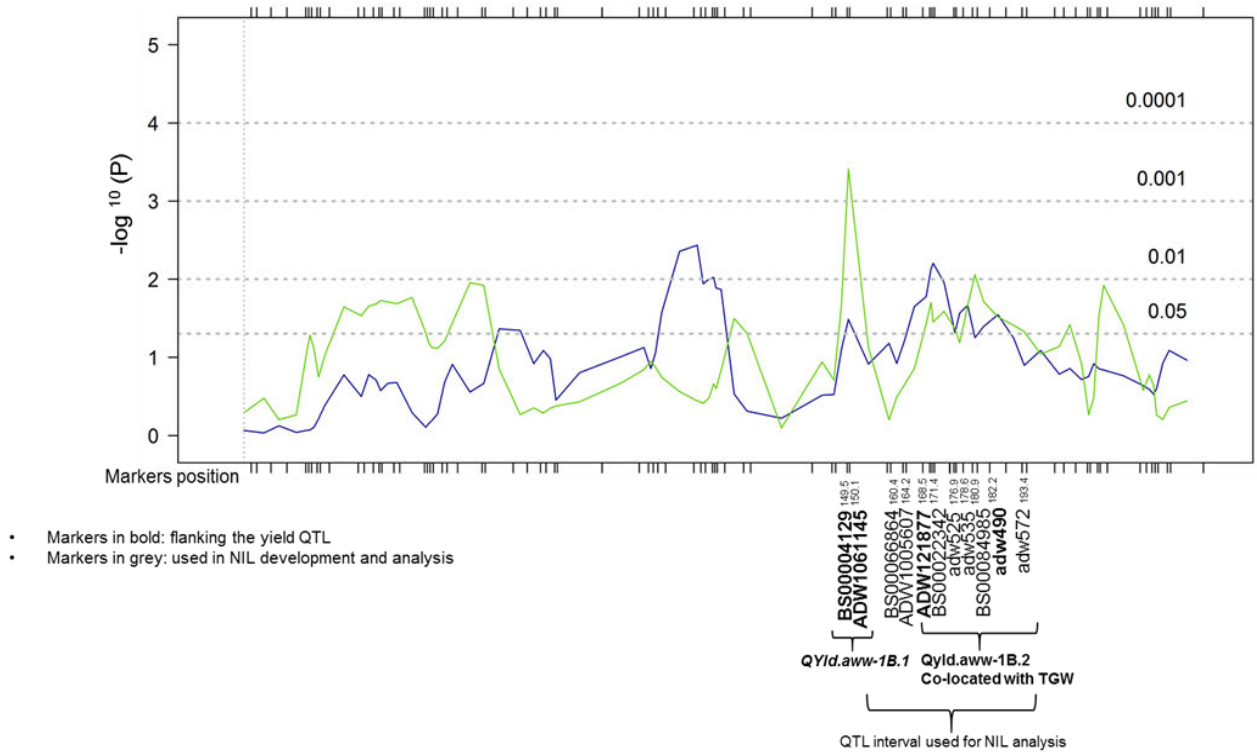
AA= excalibur allele
BB= Kukri allele
X = Missing value
T:G = heterozygous



Supplementary Figure 1. Soil water tension and temperature recorded during the NIL field trial, Urrbrae, SA, 2016.



Supplementary Figure 2 Yield QTL mapped on the updated high resolution map of chromosome 1B using MET model. P are the p-values for the test of QTL main effect (green colour) and QTL by environment interactions (green colour) from the Wald test across 28 trials.



Supplementary Figure 3. TGW QTL mapped on the updated high resolution map of chromosome 1B using MET model. P are the p-values for the test of QTL main effect (green colour) and QTL by environment interactions (blue colour) from the Wald test across 28 trials.

Chapter 5: Identifying candidate genes underlying yield QTL from three mapping populations in dry and hot environments

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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 11/01/2018

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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Chapter 5: Identifying candidate genes underlying yield QTL from three mapping populations

5.1. Abstract

Even though a number of yield QTL have been identified in wheat under drought-stressed environments, none have been cloned to date. A total of 14 QTL for yield, yield components and physiological traits were previously identified on chromosome 1B in three populations under dry and hot environments. The QTL were aligned to the genomic reference sequence on chromosome 1B (RefSeq v1.0) and three genomic regions were identified with a minimum of four overlapping QTL at each region. The QTL hotspot region spans from 531.2 Mbp to 668.7 Mbp. In the first QTL hotspot region (531.2 Mbp to 626.7 Mbp), the Drysdale/Gladius yield QTL (*QYld.adw-1B.1*) partially overlaps with QTL for seeds/spikelet (*QSSp.adw-1B*), seeds/spike (*QSS.adw-1B*) and average relative leaf expansion rate ($QRER_{AVE}$.atw-1B), all from the Drysdale/Gladius RIL population. The Drysdale/Gladius yield QTL (*QYld.adw-1B.1*) also overlaps with a yield QTL from the RAC875/Kukri DH population (*QYld.aww-1B.1*). In the second region (627.9 Mbp to 657.1 Mbp), QTL for growth rate (*QGRO.atw-1B*), transpiration rate (*QTR.atw-1B*), and average leaf expansion rate (*QLER.$_{AVE}$.atw-1B.1* and *QLER.$_{AVE}$.atw-1B.2*) from Drysdale/Gladius RIL fully co-locate and share 15.3 Mbp with a yield QTL from RAC875/Kukri. The yield QTL in Excalibur/Kukri (*QYld.aww-1B.1*) fully overlapped with all QTL found in the region. The colocation of QTL for physiological traits with yield QTL in this region may contribute towards studying the physiological mechanisms underlying wheat yield QTL under drought and heat stress. In the third region (657.1 Mbp to 688.7 Mbp), QTL for yield in Excalibur/Kukri (*QYld.aww-1B.2*), RAC875/Kukri (*QYld.awww-1B.2*), and Drysdale/Gladius (*QYld.adw-1B.2*) co-locate with QTL for spike number/plant (*QSnp.awp11-1B*) in Drysdale/Gladius, sharing 17.0 Mbp. Since yield and yield components QTL from the three mapping populations were co-located at the third region, this region was targeted for fine mapping. The initial 17.0 Mbp interval was reduced to 2.2 Mbp using Excalibur/Kukri near isogenic lines (NIL). We targeted Excalibur/Kukri 1B yield QTL (*QYld.aww.1B.2*) because of its strong effect in dry and hot climate. Seven putative candidate genes were identified in this region based on their differential expression in Chinese spring, Excalibur and RAC875 under drought treatment, suggesting that these genes may be functionally associated with yield under drought and heat stressed environments and are recommended for further gene expression analysis.

5.2. Introduction

Bread wheat (*Triticum aestivum* L.) is an allo-hexaploid (2n = 6x = AABBDD) derived from natural hybridizations of three ancestral genomes: *Triticum urartu* (2n = 2x = 14, AA), *Aegilops speltoides* (2n = 2x = 14, SS) and *Aegilops tauschii* (2n = 2x = 14, DD) (Petersen et al. (2006). The wheat genome is complex and large (17 Gbp) with highly repetitive DNA (>80%) and many transposable elements (Choulet et al. 2010; Mayer et al. 2014). Because of its large size and its complex properties, the wheat genome has been difficult to sequence (Paux et al. 2006), especially the non-genic regions, and *de novo* assembly was assumed to be problematic until recently (Wicker et al. 2006). However, comprehensive and complete wheat genome sequencing data are useful in SNP identification to design new marker and discover new genes for wheat improvement programs. In more recent years, genome sequencing techniques and platforms have progressed tremendously enabling sequencing of any genome.

The International Wheat Genome Sequencing Consortium (IWGSC) was organized in 2005 from a group of seed growers, scientists and breeders from different countries to overcome the difficulties in wheat genome sequencing (Doležel et al. 2007; Feuillet et al. 2012). IWGSC aimed to provide a complete high quality wheat genome sequence (Feuillet and Eversole 2007), using a chromosome-based sequencing strategy called BAC-by-BAC sequencing of flow-sorted chromosome arms. Individual chromosomes were sorted using a nullisomic collection of Chinese Spring lines (Gill et al. 2004) by flow cytometry (Doležel et al. 2007) to construct bacterial artificial chromosome (BAC) libraries (Šafář et al. 2010). The physical map was constructed from BAC clones through various fingerprinting technologies (Doležel et al. 2007; Doležel et al. 2012; Luo et al. 2003; Philippe et al. 2012). Overlapping BAC clones (a BAC is usually 100-150 kbp long) representing the minimal tiling path (MTP) were identified and sequenced as a pool (Engler et al. 2003). Then the adjacent BAC clones from the MTP were sequenced and aligned in order to obtain a series of continuous stretches of DNA made of partially overlapping fragments called contigs (Batzoglou et al. 1999). The chromosome based strategy is a useful resource to establish effective relationship between genetic and physical distance during positional cloning using the advantage of common markers deployed in the process of fine mapping.

The second method used in wheat was whole genome shotgun (WGS) sequence. WGS is based on generating large amounts of sequence from short reads and assembling them to complete

whole genome sequence using next generation sequencing technologies (Adams et al. 2000; Brenchley et al. 2012; Zhang et al. 2011). Roche/454 pyrosequencer and IIIumina HISeq2000 have been the most frequently used NGS technologies in wheat sequencing (Brenchley et al. 2012; Consortium 2014). For example, IIIumina HISeq2000 is currently used in large and complex genome sequencing such as wheat genome to overcome the problem of repetitive sequences in the genic region and increasing sequence quality with deep coverage (Muthamilarasan and Prasad 2014; Zhang et al. 2011). It is producing single reads of 2 × 100 base pairs, and 200 Gbp of short reads per run with > 99.5% accuracy and can assemble large genomes of up to 80 Gbp.

Even though its complex genomic nature and large size reduced the quality of wheat WGS sequence data (Wicker et al. 2010), significant data on gene content (Venter et al. 2001; Yu et al. 2002), its compositional gradients (Wong et al. 2002), and SNP (Goff et al. 2002) can be obtained from the WGS sequence assembly and used in gene identification and map-based cloning activities. Using whole shotgun sequencing of Chinese Spring, more than 132,000 SNP were identified within genic sequences between the three diploid wild ancestors of wheat (Brenchley et al. 2012). For example, a draft whole genome sequence of *Aegilops tauschii* (2n = 14; DD) has revealed genes related to stress tolerance, and grain quality (Jia et al. 2013). Similarly, the *Triticum urartu* (2n = 14; AA) genome sequence has provided useful information on the AA genome, on agronomically important genes and molecular markers (Ling et al. 2013).

POPSEQ is another approach for ordering NGS wheat data of bi-parental mapping population (Edae et al. 2015; Mascher et al. 2013). This method orders contigs in genetic map order using shallow coverage sequencing of a bi-parental mapping population initially developed for barley.

The IWGSC has released different versions of wheat genome sequence data for public use. Survey sequences of the 21 wheat chromosomes was completed in 2014 (IWGSC-CSS_assembly, https://urgi.versailles.inra.fr/) (Eversole et al. 2014). The physical maps for all chromosomes were completed by the end of 2015. More recently, NRGene and IWGSC established a collaboration for a de novo WGS sequencing of Chinese Spring. They have used the NRGene's DeNovoMAGIC™ software to assemble Illumina short sequence reads and released the whole genome shotgun assembly (IWGSC WGA v0.4) publically in June 2016 via

the IWGSC wheat sequence repository at URGI-INRA-Versailles, France (https://wheat-urgi.versailles.inra.fr/). The whole genome assembly was subsequently integrated with physical maps and other chromosome-based sequence resources to generate the first version of the chromosome-based reference sequence (IWGSC RefSeq v1.0) in January 2017.

The whole genome shotgun sequencing approach was also used for sequencing wheat cultivars that have an economic importance for Australia. A consortium of Australian researchers supported by BioPlatforms Australia (BPA) and the Australian Genome Research Facility (AGRF) developed Illumina whole genome shotgun sequencing of 16 wheat accessions at 5x to 12x coverage (Edwards et al. 2012). This dataset includes the five parental lines (Drysdale, Gladius, Excalibur, RAC875 and Kukri) used in this project (Rustenholz et al. 2011). Although the genome could not be assembled due to the low sequencing coverage, this dataset enables SNP discovery which can be exploited by breeders and researchers.

In addition to the IWGSC efforts, Zimin et al. (2017b) reported high quality and deep sequence coverage with the combination of the short reads of Illumina sequence with long Pacific Biosciences sequence reads. They developed a new hybrid sequence assembly platform and technique that has a capacity to produce highly contiguous assemblies (N50) with high quality coverage (error rate average <1%) using a *mega-reads* algorithm in the Maryland Super-Read Celera Assembler (MaSuRCA) pipeline. With a 15 Gbp assembly and an average (N50) contig size of 232 Mbp, the first bread wheat (*Tritium aestivum*) genome is now nearly completed (Zimin et al. 2017a).

Chromosome 1BL and 1BS physical maps (Philippe et al. 2013a; Raats et al. 2013) and reference sequences (IWGSC RefSeq v1.0) have been used for this chapter. The first objective of this study was to physically map yield and yield components QTL on chromosome 1B from Drysdale/Gladius, Excalibur/Kukri and RAC875/Kukri onto the reference sequence assembly for chromosome 1B. The second objective was to identify candidate genes under the yield QTL in Excalibur/Kukri NIL using the available expression data of candidate genes.

5.3. Materials and methods

5.3.1. Genetic linkage maps

We used the Drysdale/Gladius RIL described in Chapter 3 and the Excalibur/Kukri DH map described in Chapter 4. The Australian Research Council Wheat Hub constructed a new high-resolution genetic map of RAC875/Kukri DH population using modified R/ASMap (Taylor, 2015) with MSTmap function from the WGAIM package (http://www.cran.r-project.org)(performed by Paul Eckermann). Three sets of markers were combined: 238 SSR and 251 DArT from (Bennett et al. 2012a), 10,333 SNP markers scored on a subset of 180 lines at Australian Grain Technologies (AGT) and 17,830 SNP from 90K iSelect SNP array on 322 lines (Shahinnia et al 2016). The combined set of markers was first curated: lines were checked for missing data and clones. The RAC875/Kukri DH lines showing similarity for a large proportion of the genome (80 and 90%) were considered as partial clones and removed from the map using the fixClones function in R. The MSTmap Kosambi function ($p < 0.05$) was used to combine the markers and reorder the map. The final map included lines that were present in all three marker sets and had less than 5% missing markers. Poor quality markers and markers containing at least two double crossovers were removed. The final combined map ordering was checked using ASMap and consisted of a total of 6,247 markers comprised of 238 SSR, 224 DArT, 2,828 SNP markers from AGT and 2,957 SNP markers from the 90K iSelect SNP markers. The assembly contains 24 linkage groups assigned to 21 wheat chromosomes in the RAC875/Kukri mapping population. The total length of the genetic map is 3993.5 cM, containing 4,312 unique loci with an average distance of 0.93 cM (min = 0.63 and max = 41.15 cM) between markers and was used for QTL remapping.

5.3.2. Target QTL for physical mapping

QTL analysis of the RAC875/Kukri DH population was performed on phenotypic data collected from 24 field trials in Australia and Mexico between 2006 and 2013 using the WGAIM package (Taylor and Verbyla, 2011) in R (R Core Team, 2015) as described in (Bonneau et al. 2013a) . Trait were checked for outliers, and spatial trends were identified using the ASReml package in R, using the method of Gilmour et al. (1997). An exclusion window of 20 cM was used, with genetic data fitted at markers with the random method. Zadoks score was used as a covariate in the model to correct for the effect of maturity.

In this study, we used QTL for yield and yield components in Drysdale/Gladius population (described in Chapter 2), in Excalibur/Kukri populations (Chapter 3) and RAC875/Kukri, and QTL for growth, leaf expansion, transpiration rate and spike number/plant in Drysdale/Gladius (Parent et al. 2015). The QTL interval was defined by the markers indirectly flanking the peak. Detailed description of these QTL is in Table 1.

5.3.3. Alignment of the 1B QTL to the wheat reference sequence

The sequences of all markers found in the QTL regions (Table 1) were aligned to the chromosome 1B Chinese Spring reference sequence (IWGSC RefSeq v1.0) (http://wheaturgi.versailles.inra.fr/Seq-Repository/) using an in-house BLASTN portal (University of Adelaide). BLASTN results with > 99 % identity and e-value ≥ e-28 were kept. For all QTL aligned on the physical map, marker orders on the genetic map were also compared with the order of sequence coordinates on the physical map. We retained only the markers for which physical and genetic map locations matched. Only flanking markers are shown in the Fig 1.

5.3.4. Identification of candidate genes from the Excalibur/Kukri yield QTL interval

The candidate gene study was focused on the yield QTL interval which was found in Excalibur/Kukri NIL between markers adw1218477 and BS00022342 (Chapter 4). The sequences of the QTL interval (2.2 Mpb) were annotated using the TriAnnot pipeline (https://urgi.versailles.inra.fr/triannot/) (Leroy et al. 2012). TriAnnot is an online automated tool for annotation of structural, and functional protein-coding genes specifically designed to deal with Triticeae complex genomes, including high occurrence of gene copies and repeat elements. It allows the user to retrieve individual features such as genes, mRNA, protein-coding sequences (CDS) and protein sequences for further analysis. Firstly, exon-intron structures and CDS are predicted by BLASTN and BLASTPX sequence similarity within transcript and protein databanks, respectively (Altschul et al. 1997). Then, the sequences of the BLAST hit are recovered and aligned against the sequence data (Slater and Birney 2005). The outputs of the similarity search are then used to perform gene modelling (Amano et al. 2010). Protein sequence similarity search (BLASTP) against several available protein databanks was used to determine putative functions of the gene models (Finn et al. 2013). Finally, the identified genes were classified according to sequence homology and coverage compared to

grass genes (Quevillon et al. 2005). In this study, we considered only the high confidence (HC) gene class that was assigned based on the completeness and similarity to genes characterized in protein and DNA databases.

The protein sequences of the genes that were identified by TriAnnot were used for homology searches (BLASTP) to find the expressed genes in rice (*O. sativa*) and Brachypodium (*B. distachyon*) using Phytozome V12.1, the Plant Comparative Genomics portal, Department of Energy's Joint Genome Institute (https://phytozome.jgi.doe.gov/pz/portal.html). POPSEQ Ordered *Triticum aestivum* Gene Expression (POTAGE) (Suchecki et al. 2017) (http://crobiad.agwine.adelaide.edu.au/potage), an in house web based platform, was used to visualize the expression profile of the genes with a putative function retrieved from Phytozome. Candidate genes were selected based on their expression profile in wheat cultivars in POTAGE. POTAGE is a web based tool that aggregates several data sets commonly used in wheat search such as the Chromosome Survey Sequences, their order along the chromosomes is determined by the population sequencing (POPSEQ) approach, the gene predictions using MIPS gene model (Mewes et al. 2002) and RNA-Seq expression data.

We first used the public RNA-seq dataset of Chinese Spring described by Choulet et al. (2014) and available at wheat-urgi.versailles.inra.fr/Seq_Repository/RNA-seq. The dataset included RNA from five organs (leaf, stem, root, anther and grain) at three different developmental stages and in two replications: root tissues at Zadoks'scores 10-13-39, leaf at Zadoks'scores 10-23-71, stem at Zadoks'scores 30-32-65, spike at Zadoks'scores 32-39-65 and grain at Zadoks'scores 71-75 and 85.

We also used an in-house RNAseq dataset generated by ACPFG on Excalibur and RAC875 for previous projects. The RNA were purified by Dr Bao-Lam Huynh as followed. Excalibur and RAC875 were grown in a 2 x 3 factorial of three watering treatments (well-watered, mild drought and severe drought) in a split-plot design. The two varieties were grown together in the same pot receiving each of the three water treatments. Soil was a mix of South Australia field soil combined with sand, coco peat and slow-release fertilizer. The same amount of the well-mixed soil (5 kg) was filled in each pot supported by a plastic layer inside to prevent leaking when watering. To determine the soil water content, soils from three pots were oven-dried at 90^{o}C for a week and dry soil measured. Based on this dry soil value, water was added at sowing and treatment based on a calibration test. Soil moisture was kept at 30% at sowing

and then 25% during the seedling stage. Drought treatment was applied from the booting stage to -0.3 MPa for mild drought treatment and -0.6 MPa for severe drought based on the calibration curve. The well-watered and stable drought treatments were maintained by watering daily at 2 pm until harvesting. Plants were grown in a growth chamber under 12 h day/12 h night regime with 17,608 lux at day. A datalogger recorded the conditions: temperature was 26 ^{o}C ± 1.0 at day and 14 ^{o}C ± 0.3 at night, and humidity was 59 ± 3.7 % at day and 72 ± 7.4 % at night. Each main tiller was tagged at anthesis when first anthers became visible. Samples were harvested right after watering around 2 pm. Grain and flag leaf were harvested at three time points (3, 5 and 9 after anthesis) for cDNA synthesis. Ten grains were sampled in the middle of the spike and frozen immediately in liquid nitrogen. Flag leaf was harvested for the same tiller and frozen. Samples were ground in fine powder in liquid nitrogen. The RNA was cleaned up with QIAGEN RNA clean-up kit. RNA quality/integrity was analysed on a BioAnalyser. We synthesized the library for sequencing using the TruSeq RNA Library Prep Kit. Sequencing was done for 100 bp paired end on a HISEQ 2000 machine at the Australian Cancer Research Foundation - Cancer Genomics Facility (Adelaide). The RNA-Seq data was expressed in FPKMs (fragments per kilobase of exon per million fragments mapped) per gene.

5.4. Results

5.4.1. QTL physical mapping

Analysis of the multilocation field data of RAC875/Kukri DH population revealed a total of 134 QTL with LOD scores above 3.0. Two yield QTL were detected on chromosome 1B (Table 1). *QYld.aww-1B.2* was not previously reported by Bennett et al. (2012a). *QYld.aww-1B.1* flanked with CAP8_c4595_252 (110.0 cM) and CAP11_rep_c4760_280 (136.6 cM) markers. The QYld.aww-1B.1 was detected after removal of maturity effect using Zadoks data as a covariate in the QTL model. RAC875 provided the desirable alleles for both the yield QTL with allelic effect of 28 kg/ha and 11 kg/ha and LOD 5.8 and 5.2, respectively (Table 1).

We aligned 14 QTL for yield, yield components and physiological traits that were detected on chromosome 1B in three mapping populations (Drysdale/Gladius RIL, Excalibur/Kukri DH and RAC875/Kukri DH) (Table 1) onto the wheat reference sequence, RefSeq v1.0. The QTL covered the interval from 531.2 Mbp to 688.7 Mbp between the marker BS00022093 (47.8 cM on POPSEQ) and the marker Ku_c11537_539 (113.3 cM on POPSEQ) (Fig 1).

We divided the interval into three QTL hotspot regions, each harbouring at least four different traits (Fig 1). The three QTL hotspot regions were:

- Region I, from 531.2 Mbp to 626.7 Mpb, corresponds to the region between the markers BS00022093 (47.8 cM) and adw582 (72.2 cM) and favourable allele was from Drysdale for all traits.
- Region II, from 627.9 Mbp to 657.1 Mpb, corresponds to the interval between markers adw582 (72.2 cM) and CAP7_c4778_2155754 (92 cM) and favourable alleles were from Drysdale for all physiological traits, Excalibur and RAC875 for yield QTL in Excalibur/Kukri (*QYld.aww-1B.1*) and RAC875/Kukri (*QYld.aww-1B.1*), respectively.
- Region III, from 657.1 to 688.7 Mpb, covers the interval from marker CAP7_c4778_2155754 (92 cM) to marker Ku_c11537_539 (113.3 cM) and favourable alleles were from Drysdale for spike number and yield QTL in Drysdale/Gladius, Excalibur and RAC875 for yield QTL in Excalibur/Kukri (QYld.aww-1B.2) and RAC875/Kukri (QYld.aww-1B.2), respectively.

In Region I, the Drysdale/Gladius yield QTL (*QYld.aww-1B.1*) shared 31 Mbp with QTL for seeds/spikelet (*QSSp.aww-1B*) and 22.1 Mb with QTL for seeds/spike (*QSS.aww-1B*) (Fig 1 and Table 1) in the same mapping population. *QSSp.aww-1B* and *QSS.aww-1B* found under severe drought stress in semi-controlled field conditions, completely overlapped with a QTL found in glasshouse and well-watered conditions for average relative leaf expansion rate (*$QRER_{AVE}$.atw-1B*). *QYld.aww-1B.1* in Drysdale/Gladius also co-located with *$QRER_{AVE}$.atw-1B*, with a small overlap of 16.0 Mb sequence (Fig 1 and Table 1).

The QTL hotspot Region II showed the highest number of co-located QTL (Fig 1). The RAC875/Kukri yield QTL (*QYld.aww-1B.1*) coincided with four Drysdale/Gladius QTL for physiological traits (*QGRO.atw-1B*, *$QLER_{AVE}$.atw-1B.1*, *$QLER_{AVE}$.atw-1B.2* and *QTR_{area}.atw-1B*), with complete overlap in the interval between 627.9 Mbp and 643.2 Mbp. The QTL in this region shared a common sequence of 15.3 Mbp. The Excalibur/Kukri yield QTL (*QYld.aww-1B.1*) fully overlapped with the yield QTL in RAC875/Kukri (*QYld.aww-1B.1*) and all the QTL for physiological traits in Drysdale/Gladius. The Excalibur/Kukri QTL spanned only 0.6 cM in Excalibur/Kukri DH map, which corresponds to 3.0 Mbp (643.2 Mbp to 646.2 Mbp) on the Chinese Spring RefSeq v1.0. (Fig 1 and Table 1).

In Region III, four QTL for yield and yield component from three mapping populations were co-located (Fig 1). *QYld.aww-1B.2* in Excalibur/Kukri shared about 17 Mbp sequence (656.9 Mbp - 673.9 Mbp) (accounting for 90% of the entire QTL interval) with the yield QTL in RAC875/Kukri (*QYld.aww-1B.2*) and QTL for spike number/plant in Drysdale/Gladius (*Snp.apw11-1B*). *QYld.aww-1B.2* of Excalibur/Kukri also shared 5.9 Mbp with the yield QTL in Drysdale/Gladius, *QYld.aww-1B.2*. *QYld.aww-1B.2* was fine mapped in the Excalibur/Kukri NIL (QYld.aww-1B.2_NIL) to the 2.2 Mbp interval ranged from 659.9 Mbp to 662.1 Mbp (Fig 1 and Table 1). This QTL showed an average yield increase of 123 kg/ha in Excalibur/Kukri DH population and 285 kg/ha in NIL trials under southern Australian conditions. As this QTL was highly expressed under severe drought trials of south Australian environment (yield < 150 kg/ha) compared to higher yielding trials, we looked for candidate genes in this region.

5.4.2. Candidate genes underlying grain yield QTL under severe drought and heat stress

We focused on the yield QTL QYld.aww-1B_NIL between 659.9 Mbp and 662.1 Mbp to identify candidate genes that might be increasing yield in dry and hot environments. Forty-two candidate genes were predicted in the interval by the TriAnnot pipeline. Out of these 42 genes, 38 genes are homologous to rice and brachypodium genes (Table 2, Fig 2). Among the 38 candidate genes, 23 genes had their expression confirmed in at least one RNA dataset: five different organs (root, leaf, stem, spike and grain) of Chinese Spring, flag leaf and developing grains of Excalibur and RAC875 grown under well-watered, mild drought and severe drought (Table 2, Fig 2). Ten genes expressed in both Excalibur and RAC875 at least in one of the tissues were selected for detailed discussion (green rows in Table 3, Fig 2). All of the ten selected genes were expressed in the same tissues in the drought tolerant lines Excalibur and RAC875 (Fig 3a, b, c and d). Genes were considered highly expressed when FPKM > 10, moderately expressed, FPKM > 5 and < 10 and poorly expressed, FPKM < 5. The classification is only used for this study. Schematic representation of the candidate genes selection strategy followed in this study is presented in Fig 2.

Based on the candidate genes expression profile in the three cultivars (Chinese Spring, Excalibur and RAC875) found in POTAGE, we elaborated response of the 10 selected candidate genes across the three cultivars, tissues and stage of plant development and drought scenarios. For example, Gene 01 was highly expressed in RAC875 leaf tissue collected from well-watered treatment (FPKM > 35) and also in Excalibur leaf tissue (FPKM > 15) under well-watered condition (Fig 3a). While, in Chinese Spring, it was more highly expressed in stem at Z65 and also moderately expressed in spike, leaf and root at Z65, Z23 and Z10. Gene 09 was moderately expressed in Chinese Spring leaf (Z23 & Z71), spike (Z32, Z39 & Z65) and grain (Z85), and in Excalibur and RAC875 leaves in all water treatments. It was poorly expressed in grains of Excalibur and RAC875 in all watering regimes. Gene 10 showed the highest expression in Chinese Spring grains (Z65 & Z71) and Excalibur and RAC875 grains in severe and moderate drought conditions. Gene 10 was also highly expressed in Chinese Spring leaf (Z71) while moderately expressed in other tissues of Chinese Spring and in leaf tissues of Excalibur and RAC875 in all watering regimes. Gene 12 was similar to Gene 13 in rice and Brachypodium but different in wheat and less expressed in similar tissues and cultivars than Gene 13. The gene was poorly expressed in

all organs in Chinese Spring and only in grains of Excalibur and RAC875 under mild and severe drought conditions, while Gene 13 was moderately expressed in stem (Z30) and spike (Z32) in Chinese Spring and only in grains of Excalibur and RAC875 under all water treatments. Genes 29 and 31 were highly expressed in spike (Z32) and grain (Z85) of Chinese Spring, the latter was also highly expressed in root, leaf and stem. Genes 29 and 35 were moderately expressed in grains of Excalibur and RAC875 under all watering regimes. Gene 32 was moderately expressed through all cultivars, tissues, growth stages and water treatments.

5.5. Discussion

5.5.1. Co-location of QTL on the physical map and their practical implication for QTL cloning and yield improvement

The 14 QTL identified on chromosome 1B in Excalibur/Kukri, RAC875/Kukri and Drysdale/Gladius populations were clustered in three QTL hotspot regions on the physical map where at least four co-located QTL localized at each region (Fig 1). An overlap between the yield and yield components QTL across the three mapping populations at each QTL, suggesting the same drought and heat stress tolerance gene/s on chromosome 1B may be found in multiple cultivars. The co-location of yield and yield component QTL with QTL for physiological traits in similar genomic regions suggests that a single gene with pleiotropic effects or closely linked genes might control plant growth and contribute to drought tolerance. There are several research reports on the co-location of grain yield QTL with secondary traits and the contribution of positive pleiotropic effects for rapid yield improvement (Cai and Morishima 2002; Collins et al. 2008; Osman et al. 2013; Swamy et al. 2011). However, simultaneous selection for beneficial alleles at all loci might be hampered with the negative pleiotropic effect of the same gene or negative interactions from tightly linked genes (Osman et al. 2013).

In this study, the Drysdale allele increased the majority of traits (9 out 14 traits mapped) for the three regions (Fig 1). Drysdale carries the same allele across the three regions; the Drysdale allele increased yield and physiological traits at these three QTL (Table 4). Similarly, Excalibur shows the same allele in the regions II and III increasing yield at these QTL. The QTL for yield and yield component were drought specific while all the physiological traits in Regions I and II contributed by Drysdale allele seemed to be constitutive. Thus, this result will enable

breeders to use Drysdale and Excalibur haplotypes to improve most of the co-located traits. Alternatively, the KASP^{TM} markers linked to these haplotypes could also be added to genomic selection program using large breeding populations or diversity panel to improve yield under drought and heat stress. The co-location of yield and yield component QTL with QTL for physiological traits in similar genomic regions suggests that the pleiotropic effects of single or closely linked genes might control plant growth and contribute to drought tolerance. In addition, physiological traits could be used as proxy traits for the positional cloning of yield QTL in region II. Several physiological traits such as stay green, early vigour could also be used as a proxy trait for indirect selection to improve yield and yield components as it was done in maize under drought stress (Almeida et al. 2014; Osman et al. 2013; Wang et al. 2012a; Ziyomo and Bernardo 2013).

We calculated the recombination rate in each region based on their genetic to physical map ratio (cM/Mbp) and found the largest rate of recombination in Region III (0.74 Mbp) followed by Region II (0.67 Mbp) and Region I (0.25 Mbp). This result indicates that Region III would be a target for the breeders to select separate alleles that are linked to the yield QTL. Eventually, the yield QTL in Excalibur/Kukri was narrowed down using NIL (cM/Mbp = 1.3 recombination rate) and linked markers flanking the yield QTL were identified that can be used as molecular markers to assist breeding for yield improvement.

The co-localized yield and yield component QTL in the three mapping populations in Region III, and yield QTL and QTL for physiological traits in Region II could be potentially used in marker assisted selection (MAS) for QTL pyramiding to improve wheat yield under drought and heat stress. In this study, the co-located yield and growth QTL found in Region II (627.9 Mbp to 657.1 Mbp) explained from minor to large average phenotypic variations across the field trials could be used in MAS QTL pyramiding to accumulate the minor QTL effect into a single genotype to increase the overall gains. For example, RAC875/Kukri yield QTL (*QYld.aww-1B.1*), Excalibur/Kukri yield QTL (*QYld.aww-1B.1*), *QGRO.atw-1B, QLER.atw-1B.1, QLER.atw-1B.2* and *QTR.atw-1B* that accounted for 18%, 15%,46%, 26%, 16%, and 15%, of the phenotypic variation, respectively (Bennett et al. 2012b; Edwards 2012c; Parent et al. 2015). Interestingly, the yield QTL in RAC875/Kukri (*QYld.aww-1B.2*) co-located with the narrowed down yield QTL in Excalibur/Kukri NIL (*QYld.aww.2*) and spikes number/plant in Drysdale/Gladius in Region III (657.1 Mbp to 688.7 Mbp) can be potentially considered for MAS pyramiding using the already fine mapped yield QTL in Excalibur/Kukri NIL as a

background. The linked markers can be used in pre-breeding activities to pyramid the QTL alleles into the Excalibur cultivar. Several examples of successful QTL pyramiding for major gene traits such as disease resistant, grain quality, maturity traits and dwarfing genes in wheat are described in the literature. For example, QTL pyramiding has been applied for crown rot (Bovill et al. 2010), leaf rust, stripe rust, protein content (Gupta et al. 2010; Schnurbusch et al. 2004; Tyagi et al. 2014), heading date, plant height and fusarium (Klahr et al. 2007) More recently, a promising MAS pyramiding yield QTL under drought stress is reported in wheat (Gahlaut et al. 2017) and in rice (Ashikari and Matsuoka 2006; Wang et al. 2012c).

Regions I and II (Fig 1) covered yield and yield component QTL and growth-related QTL with positive favourable allele effects contributed from the Drysdale parent. This might be due to the Drysdale potential to exploit underground moisture during drought stress. Drysdale is characterized by a high transpiration efficiency (low carbon isotope discrimination), and high yield and biomass under water-limited environments (Condon et al. 2002b, 2004b; Rebetzke et al. 2002c). Moreover, the coincidence of the QTL may be valuable for the simultaneous improvement of multiple traits, as the favourable allele at these loci was from the Drysdale parent for all the QTL detected in the Drysdale/Gladius RIL.

5.5.2. Candidate genes of QTL in Region III

Though several genes were identified by positional cloning in wheat (Akhtar et al. 2012; Bahieldin et al. 2005; Shavrukov et al. 2016; Singh and Singh 2015), genes controlling grain yield under dry and hot environment have not yet been cloned and characterised. In this study, we identified 42 candidate genes spanning the 2.2 Mbp sequence of the co-located QTL in Excalibur/Kukri (*QYld.aww-1B*), RAC875/Kukri (*QYld.aww-1B.2*) and Drysdale/Gladius (*QSnp.apw11-1B*) (Fig 1).

We first search for evidence of expression of these genes in wheat. As TriAnnot is a bioinformatic pipeline, it sometime annotates genes as pseudogenes and vice-versa. The protein sequences of the 42 genes retrieved from TriAnnot were blasted on phytozome online platform. Out of the 42 genes, 38 were found to be homologous with rice and/or Brachypodium. Pseudogenes often don't have homologous across grasses, so we excluded the 4 predicted genes not found in rice and brachypodium. Among the 38 genes homologous to rice and/or Brachypodium, we found 25 genes in POTAGE that were expressed either in Chinese Spring, Excalibur and RAC875 wheat cultivars. As FKPM values <1 are under the threshold of

detection of gene expression, we focused on 11 genes with FKPM > 1 in at least one cultivar. Because RAC875 and Excalibur carry the positive allele at *QYld.aww-1B.2*, we would expect the candidate gene to have similar level of expression in both parents. We then focus on the 11 remaining genes and compared expression among various plant organs (Fig 2, Table 3).

Gene_01. DUF1645 domain containing protein

OsSGL gene was a novel DUF1645 domain-containing protein identified in rice. Its overexpression under drought results in higher accumulation of osmolytes such as proline and soluble sugars. The gene promoted cell division and grain filling suggesting that *OsSGL* may regulate stress-tolerance and cell growth acting on a cytokinin signalling pathway. Enlarged root systems were also observed and conferred a significantly improved drought tolerance in transgenic rice and *Arabidopsis thaliana* (Cui et al. 2016). *OsSGL* was located on rice chromosome 2 which is not syntenic to wheat chromosome 1B (Philippe et al. 2013b). Rice DUF1645 proteins are relatively closely related to their homologs in maize, *Triticum aestivum*, *Setaria italica*, and *Sorghum bicolor*, but OsSGL and other rice DUF1645 proteins are more divergent. POTAGE expression profile showed that DUF1645 domain-containing protein is highly expressed (FPKM >15) in Excalibur and RAC875 leaf tissue under well-watered conditions (Fig 6). It was also moderately expressed in leaf at Zadoks' growth stage Z10, root at Z23, stems and spike at Z65 of Chinese Spring. The gene may be acting in downstream pathways to maintain cell growth and plant development under drought and heat stress in wheat.

Gene_09. Suppressor of phytochrome A

Plant *phytochromes A* and *B* regulate many aspects of plant development and diverse physiological processes (Smith 2000). Hoecker et al. (1998) reported a mutant *suppressor phytochrome a (spa1*) gene as a singling intermediate specific to *phytochrome A* that enhanced photoresponsiveness and photomorphogenesis in *Arabidopsis*. *Phytochromes A, B* and *E* were proposed to be involved in plant responses to drought stress by regulating stomatal conductance (Rushton et al. 2012) and abscisic acid levels (Boggs et al. 2010). Liu et al. (2012) reported that *phytochrome B* mutation enhance drought tolerance in rice by regulating stomata mechanisms while it is regulating ABA in Arabidopsis (GonzáLez et al. 2012). POTAGE expression profiles indicated that s*uppressor of phytochrome A* was highly expressed in leaf of all of the three wheat cultivars and most highly expressed at early plant growth stage (Zadoks 23) in Chinese Spring (Supplementary Figure 5). The gene expressed similarly under well-

watered and drought conditions and seems to be involved in early seedling establishment, ground cover and leaf greenness that would contribute to high NDVI values observed at the early vegetative stage in the Excalibur/Kukri NIL. The gene function seems to phenotypically match our observation on NIL: wild type *Phytochrome B* in Arabidopsis and rice increased leaf area and photosynthesis under well-watered conditions and were only responsive to drought in the mutant state. Thus, the gene is a potential candidate for its role in early vigour, but not due for its responsiveness to drought.

Gene_10. DnaK family protein

An important class of the Dnak family protein is heat-shock proteins which were reported repeatedly as responsive to heat, drought, cold and salt stresses, and upregulated in most cases (Al-Whaibi 2011; Rizhsky et al. 2004; Sarkar et al. 2013). Several genes were found to encode for the DnaK/Hsp70 protein family in different crops. Expression data revealed that a large number of heat-shock factors (***Hsfs)*** and heat-shock protein 70s (***Hsp70s)*** respond to heat stress in both upregulated and downregulated mechanisms in Brachypodium (Wen et al. 2017). Duan et al. (2011) reported that the rice *Hsp70* homolog gene in wheat, designated as *TaHSC70 (Triticum aestivum heat-shock cognate protein 70*), plays an important role in heat stress and stripe rust tolerance. Wheat seedlings with high expression of the *TaHSC70* gene may tolerate high temperatures, up to 40 °C. Many of the 56 members of *T. aestivum* heat shock factor family (*TaHsf*) identified in wheat were constitutively expressed and upregulated under heat, drought and salt stress (Xue et al. 2013). The expression result from POTAGE indicated that the *Hsp70/DnaK* gene family was highly expressed in all of the three cultivars in grains than other tissues (Fig 3a). It is also highly expressed in Chinese Spring leaf and grains at later growth stages (Zadoks 65 and 71) under mild and severe drought conditions, suggesting that the gene is drought-inducible (Supplementary Figure 5) in agreement with findings by several authors (Al-Whaibi 2011; Rousch et al. 2004; Su and Li 2008). The DnaK family comprises many genes and thus needs further study to identify the correct candidate gene among DnaK protein family.

Genes_12 and 13. Timeless protein

Timeless (tim) encodes for the TIMELESS protein essential for circadian rhythm in *Drosophila* (Hunter-Ensor et al. 1996; Myers et al. 1995) and involved in animal cellular development and cell cycles (Ünsal-Kaçmaz et al. 2005; Yoshizawa-Sugata and Masai 2007). Very recently, a homolog of FLOWERING LOCUS *T* was identified in switchgrass (*Panicum virgatum*) in a

locus containing homology to *tim* in animals (Grabowski et al. 2017). The gene would affect cell growth and development coupled with the circadian cycle, as the circadian clocks allow organisms to adjust physiological processes to daily changes in environmental conditions. POTAGE indicated that *tim* gene has two copies in wheat that are differently expressed. Genes 12 and 13 showed higher expression in the grain in both Excalibur and RAC875 in all watering conditions (Table 3). As Genes 12 and 13 showed similar expression pattern, we need to verify if they are indeed different genes or two splicing variants of the same gene. We proposed this gene as a candidate that could be involved in spike and grain development in wheat under severe terminal drought and heat stress. It will also be important to study its interaction with the circadian clock, known to affect maturity genes and then drought and heat stress responses.

Gene_18. LRR protein

Disease resistance genes (R) in plants involving allele specific genetic interactions between the host and pathogen that activated by protein interaction and regulatory activities upon infection (Flor 1971). The resistance proteins composed of leucine-rich repeats (LRR), a nucleotide-binding site, and amino-terminal which are genetically response specific involving hypersensitive reactions. About 149 disease resistance genes were identified in Arabidopsis expressed based on the specific stimuli signals (Belkhadir et al. 2004). Several studies have been conducted on the interrelationship between biotic and abiotic stress responses and reported a significant synchronized signalling network between disease resistance and drought tolerance in plants (Akhtar et al. 2012; Atkinson et al. 2015; Atkinson et al. 2013; O'Leary et al. 2017; Qiu and Yu 2009). For example, Chini et al. (2004) reported that Arabidopsis mutants activated disease resistance as the result of modification on the coiled-coil (CC) R protein domain simultaneously showed an increase in drought tolerance. They also reported that the mutation is conserved in higher cultivated crops. Noutoshi et al. (2005) reported that a change in single amino acid of the *WRKY* domain of the *TIR–NBS–LRR–WRKY* disease resistance protein in Arabidopsis activated both drought tolerance and disease resistance. In our current study the resistance protein showed strong expression in root in CS, moderately expressed in other organs of CS and Excalibur under both water regimes. In RAC875, it only expressed in leaf under well-watered and drought conditions. The gene could also be considered as a candidate gene for both disease resistance and drought tolerance in wheat.

Gene_24. OsFBLD6 F-box domain containing protein

F-box domain proteins (*F-box genes*) are one of the largest gene families in plants and are involved in various developmental processes and abiotic stress responses (Dreher and Callis 2007; Zhang et al. 2008). For example, overexpression of abiotic stress induced F-box gene **(*MAIF1*)** promotes root growth and reduces abiotic stress tolerance and ABA sensitivity (Yan et al. 2011). *T. aestivum F-box* (*TaFBA1*) is one of the F-box protein encoding genes identified in bread wheat under drought stress (Zhou et al. 2014). *TaFBA1* expression was upregulated in oxidative stress in wheat leaves and enhanced drought tolerance (Kong et al. 2016; Zhou et al. 2015). It was associated with seed germination and early growth in plants under drought stress. Gene_24 similar to *OsFBLD6 F-box domain containing protein* was highly expressed in grains of the three wheat cultivars across all watering regimes in POTAGE indicating there may be one or more genes in the group which would potentially contribute to drought tolerance in wheat (Supplementary Figure 5). Thus, *TAFBA1* and/or other F-box protein encoding gene could be a potential candidate contributing to early growth biomass and spike fertility in Excalibur and RAC875 cultivars.

Gene_29. Aldose 1-epimerase

Aldose 1-epimerase is an enzyme that catalyses the chemical reaction of alpha-D-glucose to beta-D-glucose and is involved in glycolysis and gluconeogenesis. Mofatto et al. (2016) reported *Aldose 1-epimerase* as a putative gene for drought tolerance in coffee as it was up-regulated under drought in the tolerant coffee cultivar while it was down-regulated in the drought susceptible one. Wang et al. (2007) reported that *Aldose 1-epimerase* upregulated the glycolysis pathway to produce pyruvate during sucrose starvation in rice. *Glucose-6-phosphate 1-epimerase,* a homolog of *Aldose 1-epimerase* in Brachypodium, was upregulated during grain filling in wheat under terminal drought and heat (Yang et al. 2016). POTAGE expression profile showed that *Aldose 1-epimerase* was highly expressed in all the three cultivars in the grain. Moreover, the gene was also expressed in the spike in Chinese Spring (Supplementary Figure 5) and was highly responsive to severe drought. The gene may regulate the translocation of stored carbohydrates from stem to grains, and carbohydrate metabolism more generally during grain filling under terminal severe drought and heat in wheat.

Gene_31. Transcription factor

Transcription factors (TFs) are also major gene families regulating gene networks in response to abiotic stresses. Several (TFs) have been reported in response to drought and other abiotic

stresses in Arabidopsis and many cereal crops including wheat (Budak et al. 2013; Ghatak et al. 2017; Yoshida et al. 2014). For example, TFs such as WRKY family, dehydration-responsive element binding protein (DREB) (NIU et al. 2012a), NAC *(NAM/ATAM/CUC)* (Tang et al. 2012), myeloblastosis (MYB) (Qin et al. 2012) were frequently reported to be involved in drought tolerance in cereals. From the cultivar expression profile in the POTAGE in this study, *Transcription factor* was highly expressed in all tissues in Chinese Spring but poorly expressed in grains of Excalibur and RAC875 under all water treatments. This gene wasn't annotated as a known TF gene by TriAnnot, so it might be a new TF that needs to be manually annotated.

Gene_32. WRKY19

WRKY is a large family of transcription factors involved in multiple plant functions mainly in plant growth, development and biotic and abiotic stress tolerance (Chen et al. 2012; Niu et al. 2012b; Rushton et al. 2010). More specifically, about 43 putative *WRKY* transcription factor type genes were identified in wheat and recently associated with several abiotic stresses tolerances including drought and heat stress. Transgenic studies in Arabidopsis on two wheat *WRKY* genes (*TaWRKY2* and *TaWRKY19*) indicated that overexpression of the two genes conferred drought and salt tolerance in Arabidopsis (Niu et al. 2012b) . It was also reported that *TaWRKY2* and *TaWRKY19* may conferred drought and salt tolerance by direct binding and activation of the *Cys2/His2-type zinc-finger transcriptional repressor* (*STZ*) and *dehydration-responsive element-binding protein 2A (DREB2A*) gene pathway, respectively (Niu et al. 2012b). Overexpression of *STZ* and *DREB2A* genes in transgenic Arabidopsis and rice increased stress tolerance (Sakuma et al. 2006; Xiao et al. 2009). In our expression profile analysis, *WRKY19* was poorly expressed in Excalibur and RAC875, while highly expressed in CS spike at Z32 (Figure 3).Thus, further expression analysis including Kukri may be required to determine if the gene is down regulated in Excalibur and RAC875 while upregulated in Kukri. This gene may be a candidate gene for early growth and development that contributed to the yield effect in Excalibur allele.

Gene_35. Mitochondrial transcription termination factor (mTERF) family protein

Plant mitochondrial transcription termination factor **(**mTERF**)** genes is a large family with important roles in regulating organelle gene expression. In *Arabidopsis*, the *mTERF* genes was shown to have multiple functions such as coordinating gene expression in the chloroplast and nucleus in response to environmental changes (Babiychuk et al. 2011; Kleine 2012). For

example, Quesada et al. (2011) reported that *Arabidopsis* RUG2 regulated proper plant development and the function of mitochondria and chloroplast. *RUG2* is the nuclear gene homologous of the metazoan *mTERFs,* which encodes a protein involved in the maintenance of transcripts level in both organelles. Most mitochondrial genes were downregulated, whereas the majority of the chloroplast genes were upregulated in *Arabidopsis.* In POTAGE, *mTERF* is highly expressed in grains of Excalibur and RAC875 under all the three watering regimes, while mainly expressed in spikes and early grain filling stage in Chinese Spring (Fig 3). This gene may be involved in maintaining the chloroplast function and sustain spike and flag leaf photosynthesis that would contribute to grain filling and development under stress.

5.5.3. Potential limitations on candidate gene identification and future strategies to select the best candidate gene

The 11 candidate genes were selected based on their expression profiles in CS, Excalibur and RAC875 and the functions reported in the literature combined with suggestions for candidates underlying the yield QTL in the Excalibur/Kukri NIL study (fined mapped in Chapter 4). However, there may be some limitations in our selection strategy to effectively target the gene responsible for the yield QTL. These limitations include:

1. Only expression data of Excalibur and RAC875, drought tolerant cultivars were considered, but not the response of the counterpart drought susceptible Kukri allele. Therefore, a comparison with the response of susceptible Kukri allele would be advantageous.
2. The causal variation might not affect the gene expression but the protein expression.
3. The interval may not be sufficiently accurate and the sequences flanking the selected region may be important.
4. We focused on annotated genes by TriAnnot. We might find more genes from manual annotation of the whole interval. There may be additional genes in the region that were not annotated in Chinese Spring as the reference sequence has not be exhaustively annotated in the region and the relevant sequences could be missing from Chinese Spring.
5. The phenotype could be under the control of multiple linked genes.
6. Our focus has been on variation in genes expression data but the key difference may lie in variation in the coding region,

7. The phenotypic variation may due to structural, miRNA or other factors rather than due to a gene.

Thus, to overcome potential risks listed above, the following strategies/molecular techniques can be used to select the best candidate genes from the list or other new one:

1. The first step would be to manually annotate the interval and identify sequence variations in the QTL interval among the parental lines. We will look for variants that may have an effect mRNA or protein: truncated domain, alternate splicing, stop codon changes, amino acid changes, or in the promoter region (differences in domain that could affect the gene expression). Those sequence variants should match the QTL alleles with Excalibur = RAC875 = Drysdale allele versus Kukri = Gladius.
2. qPCR expression analysis should be run on tissues collected from Excalibur/Kukri NIL at various growth stages under different drought treatments. This would help us to find contrast of gene expression between parents that have different sequences variants, and between NIL carrying contrasted alleles. For the differential expression analysis, tissue should be sampled at specific growth stages corresponding to the traits co-segregating with yield (NIL results in Chapter 4). For example, leaf, stem, root, spike and grains should be sampled starting from early growth stages (from seedling establishment to maturity) under various drought and heat treatments.
3. Functional studies will then be necessary to validate the gene function by complementation using transgenesis or genome editing. For example, Excalibur/Kukri NIL carrying Kukri allele could be complemented by editing the sequence to mimic the Kukri allele and observe if we can recover the Excalibur effects at the QTL.
4. Find TILLING lines mutated in these genes or using genome editing to create new alleles or null lines and observe the phenotype in the new alleles. This would extend our understanding of the specific role of the gene in abiotic stress responses.

5.6. Conclusion

We identified multiple QTL controlling yield, yield components and physiological traits on chromosome 1B and positioned them on the physical map (RefSeq v1.0). The QTL were initially detected in three mapping populations (Excalibur/Kukri DH, RAC875/Kukri DH and Drysdale/Gladius RIL) using different phenotyping platforms under drought and heat stress. The QTL partially/fully co-located and clustered into three QTL hotspot regions on the 1B physical map. Co-location of the yield QTL with QTL for physiological traits in Region II indicates the potential of this region to elucidate the genetic mechanisms and new candidate genes underlying yield QTL under dry and hot environments. It may also be a useful approach to exploit the advantage of positive pleiotropic effects of the co-located traits for simultaneous improvement. The co-located yield QTL from the three populations in Region III was delimited to 2.2 Mb using Excalibur/Kukri NIL that corresponds to 42 candidate genes. With integrative analysis of genomic data from different platforms and different data sets such as proteomics data and RNA-Seq data of three cultivars, seven putative genes were finally selected to be fully described and recommended for further gene characterization and complementation studies. Although the function of these genes needs to be confirmed, the strategy used in the current study is a good starting point for the discovery and mapping of drought and heat responsive genes in wheat. The research results may provide new insights into the molecular basis of the drought and heat stress tolerance in wheat and useful molecular markers for MAS.

5.7. References

Adams MD, Celniker SE, Holt RA, Evans CA, Gocayne JD, Amanatides PG, Scherer SE, Li PW, Hoskins RA, Galle RF (2000) The genome sequence of Drosophila melanogaster. Science 287:2185-2195

Al-Whaibi MH (2011) Plant heat-shock proteins: a mini review. Journal of King Saud University-Science 23:139-150

Alamillo J, Almoguera C, Bartels D, Jordano J (1995) Constitutive expression of small heat shock proteins in vegetative tissues of the resurrection plant Craterostigma plantagineum. Plant Molecular Biology 29:1093-1099

Almeida GD, Nair S, Borém A, Cairns J, Trachsel S, Ribaut J-M, Bänziger M, Prasanna BM, Crossa J, Babu R (2014) Molecular mapping across three populations reveals a QTL hotspot region on chromosome 3 for secondary traits associated with drought tolerance in tropical maize. Molecular Breeding 34:701-715

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research 25:3389-3402

Alvarez S, Marsh EL, Schroeder SG, Schachtman DP (2008) Metabolomic and proteomic changes in the xylem sap of maize under drought. Plant, Cell & Environment 31:325-340

Amano N, Tanaka T, Numa H, Sakai H, Itoh T (2010) Efficient plant gene identification based on interspecies mapping of full-length cDNAs. DNA Research 17:271-279

Ashikari M, Matsuoka M (2006) Identification, isolation and pyramiding of quantitative trait loci for rice breeding. Trends in Plant Science 11:344-350

Babiychuk E, Vandepoele K, Wissing J, Garcia-Diaz M, De Rycke R, Akbari H, Joubès J, Beeckman T, Jänsch L, Frentzen M (2011) Plastid gene expression and plant development require a plastidic protein of the mitochondrial transcription termination factor family. Proceedings of the National Academy of Sciences 108:6674-6679

Bennett D, Izanloo A, Reynolds M, Kuchel H, Langridge P, Schnurbusch T (2012a) Genetic dissection of grain yield and physical grain quality in bread wheat (Triticum aestivum L.) under water-limited environments. Theoretical and Applied Genetics 125:255-271

Bennett D, Reynolds M, Mullan D, Izanloo A, Kuchel H, Langridge P, Schnurbusch T (2012b) Detection of two major grain yield QTL in bread wheat (*Triticum aestivum* L.) under heat, drought and high yield potential environments. Theoretical and Applied Genetics 125:1473-1485

Boccalandro HE, Rugnone ML, Moreno JE, Ploschuk EL, Serna L, Yanovsky MJ, Casal JJ (2009) Phytochrome B enhances photosynthesis at the expense of water-use efficiency in Arabidopsis. Plant Physiology 150:1083-1092

Boggs JZ, Loewy K, Bibee K, Heschel MS (2010) Phytochromes influence stomatal conductance plasticity in Arabidopsis thaliana. Plant Growth Regulation 60:77-81

Bonneau J, Taylor J, Parent B, Bennett D, Reynolds M, Feuillet C, Langridge P, Mather D (2013) Multi-environment analysis and improved mapping of a yield-related QTL on chromosome 3B of wheat. Theoretical and Applied Genetics 126:747-761

Bovill W, Horne M, Herde D, Davis M, Wildermuth G, Sutherland M (2010) Pyramiding QTL increases seedling resistance to crown rot (*Fusarium pseudograminearum*) of wheat (*Triticum aestivum*). Theoretical and Applied Genetics 121:127-136

Brenchley R, Spannagl M, Pfeifer M, Barker GL, D'Amore R, Allen AM, McKenzie N, Kramer M, Kerhornou A, Bolser D (2012) Analysis of the bread wheat genome using whole-genome shotgun sequencing. Nature 491:705-710

Buchanan- Wollaston V, Page T, Harrison E, Breeze E, Lim PO, Nam HG, Lin JF, Wu SH, Swidzinski J, Ishizaki K (2005) Comparative transcriptome analysis reveals significant differences in gene expression and signalling pathways between developmental and dark/starvation- induced senescence in Arabidopsis. The Plant Journal 42:567-585

Budak H, Kantar M, Yucebilgili Kurtoglu K (2013) Drought tolerance in modern and wild wheat. The Scientific World Journal 2013

Cai H, Morishima H (2002) QTL clusters reflect character associations in wild and cultivated rice. Theoretical and Applied Genetics 104:1217-1228

Casal JJ (2013) Photoreceptor signaling networks in plant responses to shade. Annual Review of Plant Biology 64:403-427

Casson SA, Franklin KA, Gray JE, Grierson CS, Whitelam GC, Hetherington AM (2009) Phytochrome B and PIF4 regulate stomatal development in response to light quantity. Current Biology 19:229-234

Chen H, Lai Z, Shi J, Xiao Y, Chen Z, Xu X (2010) Roles of Arabidopsis WRKY18, WRKY40 and WRKY60 transcription factors in plant responses to abscisic acid and abiotic stress. BMC Plant Biology 10:281

Chen L, Song Y, Li S, Zhang L, Zou C, Yu D (2012) The role of WRKY transcription factors in plant abiotic stresses. Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms 1819:120-128

Choulet F, Alberti A, Theil S, Glover N, Barbe V, Daron J, Pingault L, Sourdille P, Couloux A, Paux E (2014) Structural and functional partitioning of bread wheat chromosome 3B. Science 345:1249721

Choulet F, Wicker T, Rustenholz C, Paux E, Salse J, Leroy P, Schlub S, Le Paslier M-C, Magdelenat G, Gonthier C (2010) Megabase level sequencing reveals contrasted organization and evolution patterns of the wheat gene and transposable element spaces. Plant Cell 22:1686-1701

Ciolkowski I, Wanke D, Birkenbihl RP, Somssich IE (2008) Studies on DNA-binding selectivity of WRKY transcription factors lend structural clues into WRKY-domain function. Plant Molecular Biology 68:81-92

Collins NC, Tardieu F, Tuberosa R (2008) Quantitative trait loci and crop performance under abiotic stress: where do we stand? Plant Physiology 147:469-486
Condon AG, Richards RA, Rebetzke GJ, Farquhar GD (2002) Improving intrinsic water-use efficiency and crop yield. Crop Science 42:122-131
Condon AG, Richards RA, Rebetzke GJ, Farquhar GD (2004) Breeding for high water-use efficiency. Journal of Experimental Botany 55:2447-2460
Consortium IWGS (2014) A chromosome-based draft sequence of the hexaploid bread wheat (Triticum aestivum) genome. Science 345:1251788
Cui Y, Wang M, Zhou H, Li M, Huang L, Yin X, Zhao G, Lin F, Xia X, Xu G (2016) OsSGL, a Novel DUF1645 Domain-Containing Protein, Confers Enhanced Drought Tolerance in Transgenic Rice and Arabidopsis. Frontiers in Plant Science 7
Doležel J, Kubaláková M, Paux E, Bartoš J, Feuillet C (2007) Chromosome-based genomics in the cereals. Chromosome Research 15:51-66
Doležel J, Vrána J, Šafář J, Bartoš J, Kubaláková M, Šimková H (2012) Chromosomes in the flow to simplify genome analysis. Functional & Integrative Genomics 12:397-416
Dreher K, Callis J (2007) Ubiquitin, hormones and biotic stress in plants. Annals of botany 99:787-822
Duan Y-H, Guo J, Ding K, Wang S-J, Zhang H, Dai X-W, Chen Y-Y, Govers F, Huang L-L, Kang Z-S (2011) Characterization of a wheat HSP70 gene and its expression in response to stripe rust infection and abiotic stresses. Molecular Biology Reports 38:301-307
Edae EA, Bowden RL, Poland J (2015) Application of population sequencing (POPSEQ) for ordering and imputing genotyping-by-sequencing markers in hexaploid wheat. G3: Genes, Genomes, Genetics 5:2547-2553
Edwards J (2012) A genetic analysis of drought related traits in hexaploid wheat. The University of Adelaide, School of Agriculture. Food and Wine (PhD thesis)
Eulgem T, Rushton PJ, Robatzek S, Somssich IE (2000) The WRKY superfamily of plant transcription factors. Trends in Plant Science 5:199-206
Eversole K, Feuillet C, Mayer KFX, Rogers J (2014) Slicing the wheat genome. Science 345:285
Feuillet C, Eversole K (2007) Physical mapping of the wheat genome: a coordinated effort to lay the foundation for genome sequencing and develop tools for breeders. Israel Journal of Plant Sciences 55:307-313
Feuillet C, Stein N, Rossini L, Praud S, Mayer K, Schulman A, Eversole K, Appels R (2012) Integrating cereal genomics to support innovation in the Triticeae. Functional & integrative genomics 12:573-583
Finn RD, Bateman A, Clements J, Coggill P, Eberhardt RY, Eddy SR, Heger A, Hetherington K, Holm L, Mistry J (2013) Pfam: the protein families database. Nucleic Acids Research 42:D222-D230
Flaherty KM, DeLuca-Flaherty C, McKay DB (1990) Three-dimensional structure of the ATPase fragment of a 70K heat-shock cognate protein. Nature 346:623

Franklin KA, Quail PH (2009) Phytochrome functions in Arabidopsis development. Journal of Experimental Botany 61:11-24

Franklin KA, Whitelam GC (2007) Light-quality regulation of freezing tolerance in Arabidopsis thaliana. Nature Genetics 39:1410-1413

Gahlaut V, Jaiswal V, Tyagi BS, Singh G, Sareen S, Balyan HS, Gupta PK (2017) QTL mapping for nine drought-responsive agronomic traits in bread wheat under irrigated and rain-fed environments. PloS One 12:e0182857

Ghatak A, Chaturvedi P, Weckwerth W (2017) Cereal crop proteomics: Systemic analysis of crop drought stress responses towards marker-assisted selection breeding. Frontiers in Plant Science 8

Gill BS, Appels R, Botha-Oberholster A-M, Buell CR, Bennetzen JL, Chalhoub B, Chumley F, Dvořák J, Iwanaga M, Keller B (2004) A workshop report on wheat genome sequencing. Genetics 168:1087-1096

Goff SA, Ricke D, Lan T-H, Presting G, Wang R, Dunn M, Glazebrook J, Sessions A, Oeller P, Varma H (2002) A draft sequence of the rice genome (Oryza sativa L. ssp. japonica). Science 296:92-100

GonzáLez CV, Ibarra SE, Piccoli PN, Botto JF, Boccalandro HE (2012) Phytochrome B increases drought tolerance by enhancing ABA sensitivity in *Arabidopsis thaliana*. Plant, Cell and Environment 35:1958-1968

Gotter AL (2003) Tipin, a novel timeless-interacting protein, is developmentally co-expressed with timeless and disrupts its self-association. Journal of Molecular Biology 331:167-176

Grabowski PP, Evans J, Daum C, Deshpande S, Barry KW, Kennedy M, Ramstein G, Kaeppler SM, Buell CR, Jiang Y (2017) Genome- wide associations with flowering time in switchgrass using exome- capture sequencing data. New Phyiologist 213:154-169

Gupta P, Langridge P, Mir R (2010) Marker-assisted wheat breeding: present status and future possibilities. Molecular Breeding 26:145-161

Hoecker U, Xu Y, Quail PH (1998) SPA1: A new genetic locus involved in phytochrome A–specific signal transduction. Plant Cell 10:19-33

Hunter-Ensor M, Ousley A, Sehgal A (1996) Regulation of the Drosophila protein timeless suggests a mechanism for resetting the circadian clock by light. Cell 84:677-685

Jain M, Nijhawan A, Arora R, Agarwal P, Ray S, Sharma P, Kapoor S, Tyagi AK, Khurana JP (2007) F-box proteins in rice. Genome-wide analysis, classification, temporal and spatial gene expression during panicle and seed development, and regulation by light and abiotic stress. Plant Physiology 143:1467-1483

Jia J, Zhao S, Kong X, Li Y, Zhao G, He W, Appels R, Pfeifer M, Tao Y, Zhang X (2013) Aegilops tauschii draft genome sequence reveals a gene repertoire for wheat adaptation. Nature 496:91-95

Jiao Y, Lau OS, Deng XW (2007) Light-regulated transcriptional networks in higher plants. Nature Reviews Genetics 8:217-230

Kato N, Dubouzet E, Kokabu Y, Yoshida S, Taniguchi Y, Dubouzet JG, Yazaki K, Sato F (2007) Identification of a WRKY protein as a transcriptional regulator of benzylisoquinoline alkaloid biosynthesis in Coptis japonica. Plant and Cell Physiology 48:8-18

Klahr A, Zimmermann G, Wenzel G, Mohler V (2007) Effects of environment, disease progress, plant height and heading date on the detection of QTLs for resistance to Fusarium head blight in an European winter wheat cross. Euphytica 154:17-28

Kleine T (2012) Arabidopsis thaliana mTERF proteins: evolution and functional classification. Frontiers in Plant Science 3:233

Kong X, Zhou S, Yin S, Zhao Z, Han Y, Wang W (2016) Stress-inducible expression of an F-box gene TaFBA1 from wheat enhanced the drought tolerance in transgenic tobacco plants without impacting growth and development. Frontiers in Plant Science 7:1295

Lee B-h, Henderson DA, Zhu J-K (2005) The Arabidopsis cold-responsive transcriptome and its regulation by ICE1. The Plant Cell 17:3155-3175

Leroy P, Guilhot N, Sakai H, Bernard A, Choulet F, Theil S, Reboux S, Amano N, Flutre T, Pelegrin C (2012) TriAnnot: a versatile and high performance pipeline for the automated annotation of plant genomes. Frontiers in plant science 3:5

Li S, Fu Q, Chen L, Huang W, Yu D (2011) Arabidopsis thaliana WRKY25, WRKY26, and WRKY33 coordinate induction of plant thermotolerance. Planta 233:1237-1252

Lin B-L, Wang J-S, Liu H-C, Chen R-W, Meyer Y, Barakat A, Delseny M (2001) Genomic analysis of the Hsp70 superfamily in Arabidopsis thaliana. Cell Stress & Chaperones 6:201-208

Ling H-Q, Zhao S, Liu D, Wang J, Sun H, Zhang C, Fan H, Li D, Dong L, Tao Y (2013) Draft genome of the wheat A-genome progenitor Triticum urartu. Nature 496:87-90

Liu J, Zhang F, Zhou J, Chen F, Wang B, Xie X (2012) Phytochrome B control of total leaf area and stomatal density affects drought tolerance in rice. Plant Molecular Biology 78:289-300

Luo M-C, Thomas C, You FM, Hsiao J, Ouyang S, Buell CR, Malandro M, McGuire PE, Anderson OD, Dvorak J (2003) High-throughput fingerprinting of bacterial artificial chromosomes using the snapshot labeling kit and sizing of restriction fragments by capillary electrophoresis. Genomics 82:378-389

Luo M, Dennis ES, Berger F, Peacock WJ, Chaudhury A (2005) MINISEED3 (MINI3), a WRKY family gene, and HAIKU2 (IKU2), a leucine-rich repeat (LRR) KINASE gene, are regulators of seed size in Arabidopsis. Proceedings of the National Academy of Sciences of the United States of America 102:17531-17536

Mangelsen E, Kilian J, Berendzen KW, Kolukisaoglu ÜH, Harter K, Jansson C, Wanke D (2008) Phylogenetic and comparative gene expression analysis of barley (*Hordeum vulgar*e) WRKY transcription factor family reveals putatively retained functions between monocots and dicots. BMC Genomics 9:194

Mascher M, Muehlbauer GJ, Rokhsar DS, Chapman J, Schmutz J, Barry K, Muñoz- Amatriaín M, Close TJ, Wise RP, Schulman AH (2013) Anchoring and ordering NGS contig assemblies by population sequencing (POPSEQ). The Plant Journal 76:718-727

Mayer KF, Rogers J, Doležel J, Pozniak C, Eversole K, Feuillet C, Gill B, Friebe B, Lukaszewski AJ, Sourdille P (2014) A chromosome-based draft sequence of the hexaploid bread wheat (Triticum aestivum) genome. Science 345:1251788

Mewes H-W, Frishman D, Güldener U, Mannhaupt G, Mayer K, Mokrejs M, Morgenstern B, Münsterkötter M, Rudd S, Weil B (2002) MIPS: a database for genomes and protein sequences. Nucleic Acids Research 30:31-34

Mofatto LS, de Araújo Carneiro F, Vieira NG, Duarte KE, Vidal RO, Alekcevetch JC, Cotta MG, Verdeil J-L, Lapeyre-Montes F, Lartaud M (2016) Identification of candidate genes for drought tolerance in coffee by high-throughput sequencing in the shoot apex of different Coffea arabica cultivars. BMC Plant Biology 16:94

Morano KA (2007) New Tricks for an Old Dog. Annals of the New York Academy of Sciences 1113:1-14

Muthamilarasan M, Prasad M (2014) An overview of wheat genome sequencing and its implications for crop improvement. Journal of Genetics 93:619-622

Myers MP, Wager-Smith K, Wesley CS, Young MW, Sehgal A (1995) Positional cloning and sequence analysis of the Drosophila clock gene, timeless. Science-New York then Washognton:805-805

Niu CF, Wei W, Zhou QY, Tian AG, Hao YJ, Zhang WK, Ma B, Lin Q, Zhang ZB, Zhang JS (2012) Wheat WRKY genes TaWRKY2 and TaWRKY19 regulate abiotic stress tolerance in transgenic Arabidopsis plants. Plant, Cell & Environment 35:1156-1170

Osman KA, Tang B, Wang Y, Chen J, Yu F, Li L, Han X, Zhang Z, Yan J, Zheng Y (2013) Dynamic QTL analysis and candidate gene mapping for waterlogging tolerance at maize seedling stage. PLoS One 8:e79305

Parent B, Shahinnia F, Maphosa L, Berger B, Rabie H, Chalmers K, Kovalchuk A, Langridge P, Fleury D (2015) Combining field performance with controlled environment plant imaging to identify the genetic control of growth and transpiration underlying yield response to water-deficit stress in wheat. Journal of Experimental Botany 66:5481-5492

Paux E, Roger D, Badaeva E, Gay G, Bernard M, Sourdille P, Feuillet C (2006) Characterizing the composition and evolution of homoeologous genomes in hexaploid wheat through BAC- end sequencing on chromosome 3B. The Plant Journal 48:463-474

Petersen G, Seberg O, Yde M, Berthelsen K (2006) Phylogenetic relationships of *Triticum Aegilops* and evidence for the origin of the A, B, and D genomes of common wheat (*Triticum aestivum*). Molecular Phylogenetics and Evolution 39:70-82

Philippe R, Choulet F, Paux E, van Oeveren J, Tang J, Wittenberg AH, Janssen A, van Eijk MJ, Stormo K, Alberti A (2012) Whole Genome Profiling provides a robust framework for physical mapping and sequencing in the highly complex and repetitive wheat genome. BMC Genomics 13:47

Philippe R, Paux E, Bertin I, Sourdille P, Choulet F, Laugier C, Šimková H, Šafář J, Bellec A, Vautrin S (2013) A high density physical map of chromosome 1BL supports evolutionary studies, map-based cloning and sequencing in wheat. Genome Biology 14:R64

Qin Y, Wang M, Tian Y, He W, Han L, Xia G (2012) Over-expression of TaMYB33 encoding a novel wheat MYB transcription factor increases salt and drought tolerance in Arabidopsis. Molecular Biology Reports 39:7183-7192

Quesada V, Sarmiento- Mañús R, González- Bayón R, Hricová A, Pérez- Marcos R, Graciá-Martínez E, Medina- Ruiz L, Leyva- Díaz E, Ponce MR, Micol JL (2011) Arabidopsis RUGOSA2 encodes an mTERF family member required for mitochondrion, chloroplast and leaf development. The Plant Journal 68:738-753

Quevillon E, Silventoinen V, Pillai S, Harte N, Mulder N, Apweiler R, Lopez R (2005) InterProScan: protein domains identifier. Nucleic Acids Research 33:W116-W120

Raats D, Frenkel Z, Krugman T, Dodek I, Sela H, Šimková H, Magni F, Cattonaro F, Vautrin S, Bergès H (2013) The physical map of wheat chromosome 1BS provides insights into its gene space organization and evolution. Genome Biology 14:R138

Ramamoorthy R, Jiang S-Y, Kumar N, Venkatesh PN, Ramachandran S (2008) A comprehensive transcriptional profiling of the WRKY gene family in rice under various abiotic and phytohormone treatments. Plant and Cell Physiology 49:865-879

Ranjan A, Dickopf S, Ullrich KK, Rensing SA, Hoecker U (2014) Functional analysis of COP1 and SPA orthologs from Physcomitrella and rice during photomorphogenesis of transgenic Arabidopsis reveals distinct evolutionary conservation. BMC Plant Biology 14:178

Rebetzke GJ, Condon AG, Richards RA, Farquhar GD (2002) Selection for reduced carbon isotope discrimination increases aerial biomass and grain yield of rainfed bread wheat. Crop Sci 42:739-745

Rizhsky L, Liang H, Shuman J, Shulaev V, Davletova S, Mittler R (2004) When defense pathways collide. The response of Arabidopsis to a combination of drought and heat stress. Plant Physiology 134:1683-1696

Rousch JM, Bingham SE, Sommerfeld MR (2004) Protein expression during heat stress in thermo-intolerant and thermo-tolerant diatoms. Journal of Experimental Marine Biology and Ecology 306:231-243

Rushton DL, Tripathi P, Rabara RC, Lin J, Ringler P, Boken AK, Langum TJ, Smidt L, Boomsma DD, Emme NJ (2012) WRKY transcription factors: key components in abscisic acid signalling. Plant Biotechnology Journal 10:2-11

Rushton PJ, Somssich IE, Ringler P, Shen QJ (2010) WRKY transcription factors. Trends in Plant Science 15:247-258

Rustenholz C, Choulet F, Laugier C, Šafář J, Šimková H, Doležel J, Magni F, Scalabrin S, Cattonaro F, Vautrin S (2011) A 3,000-loci transcription map of chromosome 3B unravels the structural and functional features of gene islands in hexaploid wheat. Plant Physiology 157:1596-1608

Šafář J, Šimková H, Kubaláková M, Číhalíková J, Suchánková P, Bartoš J, Doležel J (2010) Development of chromosome-specific BAC resources for genomics of bread wheat. Cytogenetic and Genome Research 129:211-223

Sakuma Y, Maruyama K, Osakabe Y, Qin F, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2006) Functional analysis of an Arabidopsis transcription factor, DREB2A, involved in drought-responsive gene expression. The Plant Cell 18:1292-1309

Sarkar NK, Kundnani P, Grover A (2013) Functional analysis of Hsp70 superfamily proteins of rice (Oryza sativa). Cell Stress and Chaperones 18:427-437

Schnurbusch T, Paillard S, Schori A, Messmer M, Schachermayr G, Winzeler M, Keller B (2004) Dissection of quantitative and durable leaf rust resistance in Swiss winter wheat reveals a major resistance QTL in the Lr34 chromosomal region. Theoretical and Applied Genetics 108:477-484

Seki M, Narusaka M, Ishida J, Nanjo T, Fujita M, Oono Y, Kamiya A, Nakajima M, Enju A, Sakurai T (2002) Monitoring the expression profiles of 7000 Arabidopsis genes under drought, cold and high-salinity stresses using a full- length cDNA microarray. The Plant Journal 31:279-292

Smith H (2000) Phytochromes and light signal perception by plants—an emerging synthesis. Nature 407:585-591

Su P-H, Li H-m (2008) Arabidopsis stromal 70-kD heat shock proteins are essential for plant development and important for thermotolerance of germinating seeds. Plant Physiology 146:1231-1241

Suchecki R, Watson-Haigh NS, Baumann U (2017) POTAGE: A Visualisation Tool for Speeding up Gene Discovery in Wheat. Scientific Reports 7:14315

Swamy BPM, Vikram P, Dixit S, Ahmed HU, Kumar A (2011) Meta-analysis of grain yield QTL identified during agricultural drought in grasses showed consensus. BMC Genomics 12:319

Tang Y, Liu M, Gao S, Zhang Z, Zhao X, Zhao C, Zhang F, Chen X (2012) Molecular characterization of novel TaNAC genes in wheat and overexpression of TaNAC2a confers drought tolerance in tobacco. Physiologia Plantarum 144:210-224

Tavaria M, Gabriele T, Kola I, Anderson RL (1996) A hitchhiker's guide to the human Hsp70 family. Cell Stress & Chaperones 1:23

Trupkin SA, Legris M, Buchovsky AS, Rivero MBT, Casal JJ (2014) Phytochrome B nuclear bodies respond to the low red to far-red ratio and to the reduced irradiance of canopy shade in Arabidopsis. Plant Physiology 165:1698-1708

Tyagi S, Mir R, Kaur H, Chhuneja P, Ramesh B, Balyan H, Gupta P (2014) Marker-assisted pyramiding of eight QTLs/genes for seven different traits in common wheat (Triticum aestivum L.). Molecular Breeding 34:167-175

Ünsal-Kaçmaz K, Mullen TE, Kaufmann WK, Sancar A (2005) Coupling of human circadian and cell cycles by the timeless protein. Mol Cell Biology 25:3109-3116

Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, Yandell M, Evans CA, Holt RA (2001) The sequence of the human genome. Science 291:1304-1351

Wang A-y, Li Y, Zhang C-q (2012a) QTL mapping for stay-green in maize (Zea mays). Canadian Journal of Plant Science 92:249-256

Wang H-J, Wan A-R, Hsu C-M, Lee K-W, Yu S-M, Jauh G-Y (2007) Transcriptomic adaptations in rice suspension cells under sucrose starvation. Plant Molecular Biology 63:441-463

Wang H, Deng XW (2003) Dissecting the phytochrome A-dependent signaling network in higher plants. Trends Plant Science 8:172-178

Wang P, Xing Y, Li Z, Yu S (2012b) Improving rice yield and quality by QTL pyramiding. Molecular Breeding 29:903-913

Wen F, Wu X, Li T, Jia M, Liu X, Li P, Zhou X, Ji X, Yue X (2017) Genome-wide survey of heat shock factors and heat shock protein 70s and their regulatory network under abiotic stresses in Brachypodium distachyon. PloS One 12:e0180352

Wicker T, Buchmann JP, Keller B (2010) Patching gaps in plant genomes results in gene movement and erosion of colinearity. Genome Research 20:1229-1237

Wicker T, Schlagenhauf E, Graner A, Close TJ, Keller B, Stein N (2006) 454 sequencing put to the test using the complex genome of barley. BMC Genomics 7:275

Wong GK-S, Wang J, Tao L, Tan J, Zhang J, Passey DA, Yu J (2002) Compositional gradients in Gramineae genes. Genome Research 12:851-856

Wu X, Shiroto Y, Kishitani S, Ito Y, Toriyama K (2009) Enhanced heat and drought tolerance in transgenic rice seedlings overexpressing OsWRKY11 under the control of HSP101 promoter. Plant Cell Reports 28:21-30

Xiao B-Z, Chen X, Xiang C-B, Tang N, Zhang Q-F, Xiong L-Z (2009) Evaluation of seven function-known candidate genes for their effects on improving drought resistance of transgenic rice under field conditions. Molecular Plant 2:73-83

Xu X, Chen C, Fan B, Chen Z (2006) Physical and functional interactions between pathogen-induced Arabidopsis WRKY18, WRKY40, and WRKY60 transcription factors. The Plant Cell 18:1310-1326

Xue G-P, Sadat S, Drenth J, McIntyre CL (2013) The heat shock factor family from *Triticum aestivum* in response to heat and other major abiotic stresses and their role in regulation of heat shock protein genes. Journal of Experimental Botany 65:539-557

Yan Y-S, Chen X-Y, Yang K, Sun Z-X, Fu Y-P, Zhang Y-M, Fang R-X (2011) Overexpression of an F-box protein gene reduces abiotic stress tolerance and promotes root growth in rice. Molecular Plant 4:190-197

Yang M, Dong J, Zhao W, Gao X (2016) Characterization of proteins involved in early stage of wheat grain development by iTRAQ. Journal of Proteomics 136:157-166

Yoshida T, Mogami J, Yamaguchi-Shinozaki K (2014) ABA-dependent and ABA-independent signaling in response to osmotic stress in plants. Current Opinion in Plant Biology 21:133-139

Yoshimune K, Yoshimura T, Nakayama T, Nishino T, Esaki N (2002) Hsc62, Hsc56, and GrpE, the third Hsp70 chaperone system of Escherichia coli. Biochemical and Biophysical Research Communications 293:1389-1395

Yoshimura K, Masuda A, Kuwano M, Yokota A, Akashi K (2008) Programmed proteome response for drought avoidance/tolerance in the root of a C3 xerophyte (wild watermelon) under water deficits. Plant and Cell Physiology 49:226-241

Yoshizawa-Sugata N, Masai H (2007) Human Tim/Timeless-interacting protein, Tipin, is required for efficient progression of S phase and DNA replication checkpoint. Journal of Biological Chemistry 282:2729-2740

Yu J, Hu S, Wang J, Wong GK-S, Li S, Liu B, Deng Y, Dai L, Zhou Y, Zhang X (2002) A draft sequence of the rice genome (O*ryza sativa L. ssp. indica*). Science 296:79-92

Zhang J, Chiodini R, Badr A, Zhang G (2011) The impact of next-generation sequencing on genomics. Journal of Genetics and Genomics 38:95-109

Zhang Ye, Xu W, Li Z, Deng XW, Wu W, Xue Y (2008) F-box protein DOR functions as a novel inhibitory factor for abscisic acid-induced stomatal closure under drought stress in Arabidopsis. Plant Physiology 148:2121-2133

Zhao Y, Cai M, Zhang X, Li Y, Zhang J, Zhao H, Kong F, Zheng Y, Qiu F (2014) Genome-wide identification, evolution and expression analysis of mTERF gene family in maize. PLoS One 9:e94126

Zheng J, Yang Z, Madgwick PJ, Carmo-Silva E, Parry MA, Hu YG (2015) TaER Expression Is Associated with Transpiration Efficiency Traits and Yield in Bread Wheat. PLoS One 10:e0128415

Zhou S-M, Kong X-Z, Kang H-H, Sun X-D, Wang W (2015) The involvement of wheat F-box protein gene TaFBA1 in the oxidative stress tolerance of plants. Plos One 10:e0122117

Zhou S, Sun X, Yin S, Kong X, Zhou S, Xu Y, Luo Y, Wang W (2014) The role of the F-box gene TaFBA1 from wheat (*Triticum aestivum* L.) in drought tolerance. Plant Physiology and Biochemistry 84:213-223

Zimin AV, Puiu D, Hall R, Kingan S, Clavijo BJ, Salzberg SL (2017a) The first near-complete assembly of the hexaploid bread wheat genome, T*riticum aestivum*. GigaScience:6:11:pp1-7

Zimin AV, Puiu D, Luo M-C, Zhu T, Koren S, Marçais G, Yorke JA, Dvořák J, Salzberg SL (2017b) Hybrid assembly of the large and highly repetitive genome of Aegilops tauschii, a progenitor of bread wheat, with the MaSuRCA mega-reads algorithm. Genome Research 27:787-792

Ziyomo C, Bernardo R (2013) Drought tolerance in maize: Indirect selection through secondary traits versus genomewide selection. Crop Science 53:1269-1275

Tables and Figures

Table 1. QTL found on chromosome 1B in Drysdale/Gladius, Excalibur/Kukri and RAC875/Kukri populations positioned on the physical map. QTL peak position is from the respective genetic map.

Traits	QTL ID	Flanking markers	QTL peak position (cM)	LOD/ P-value	AE	Favourable allele	Population	Environments	Coordinates on 1B RefSeq v.1.0		Reference
									Start	End	
Yield	QYld.www-1B.1	BS00022093 - adw10	87.4	3	0.4	Drysdale	DG RIL	Polytunnel, severe drought and heat	531166669	619685924	Chapter 2
Seeds/spikelet	QSSp.www-1B	adw581 - adw582	89.7	4.2	0.2	Drysdale	DG RIL	Polytunnel, severe drought and heat	588628065	626724525	Chapter 2
Seeds/spike	QSS.www-1B	adw580 - adw582	89.7	3.7	0.3	Drysdale	DG RIL	Polytunnel, severe drought and heat	597555341	626724525	Chapter 2
Yield	QYld.www-1B.2	adw526 - adw608	210.4	2.8	0.4	Drysdale	DG RIL	Polytunnel, severe drought and heat	667965849	679226345	Chapter 2
Ave.growth rate	QGRO.atw-1B	Ex_c5296_9365847 CAP7_c4778_2155754	68.2	6.2	0 03	Drysdale	DG RIL	Glass house, well-watered	627946334	657177235	(Parent *et al.* , 2015)
Ave.leaf expansion rate	$QLER_{AVE.}$atw-1B.1	Ex_c5296_9365847 CAP7_c4778_2155754	68.8	4.4	242	Drysdale	DG RIL	Glass house, well-watered	627946334	657177235	(Parent *et al.* , 2015)
Ave.leaf expansion rate	$QLER_{AVE.}$atw-1B.2	Ex_c5296_9365847 CAP11_c1902_1022590	60.6	6.4	-304	Gladius	DG RIL	Glass house, well-watered	627946334	646167677	(Parent *et al.* , 2015)
Ave.relative leaf expansion rate	$QRER_{AVE}$ atw-1B	adw8 - adw582	91.9	4.5	0 03	Drysdale	DG RIL	Glass house, well-watered	603753308	626724525	Chapter 2
Ave. transpiration rate	QTR atw-1B	Ex_c5296_9365847 CAP7_c4778_2155754	72.8	4.9	6.2	Drysdale	DG RIL	Glass house, well-watered	627946334	657177235	(Parent *et al.* , 2015)
Spike number/plant	QSnp.apw11-1B	Ex_c5296_9365847 Ex_c29452_38489374	90.1	3.7	0.4	Drysdale	DG RIL	Ppolytunnel, well-waterd	657177175	678432373	(Parent *et al.* , 2015)
Yield	Qyld aww-1B.1	BS00004129 - adw1061145	150.1	0.001	78	Excalibur	EK DH	Field, drought, heat	643101588	645269286	Chapter 3, this chapter
Yield	QYld.aww-1B.2	ADW1005607 - adw555	171.4	0.01	63	Excalibur	EK DH	Field, drought, heat	654915464	673856220	(Edwards, 2012)
Yield	QYld.aww-1B.2	adw1218744 - BS00022342	170 8	0.001	312	Excalibur	EK NIL	Polytunnel, severe drought and heat	659985889	662154451	Chapter 3, this chapter
Yield	QYld.aww-1B.1	CAP8_c4595_252 CAP11_rep_c4760_280	125.1	5.8	28	RAC875	RK DH	Field, drought, heat	622218961	643246721	Chapter 2
Yield	QYld.aww-1B.2	Ra_c21994_996 Ku_c11537_539	178 9	5.2	11	RAC875	RK DH	Field, drought, heat	656852764	688769304	(Bennett *et al.* , 2012)

AE = additive effect, LOD = log of the odds ratio at = 0.05 from 1000 permutation test, DG = Drysdale/Gladius, EK = Excalibur/Kukri, RK = RAC875/Kukri.

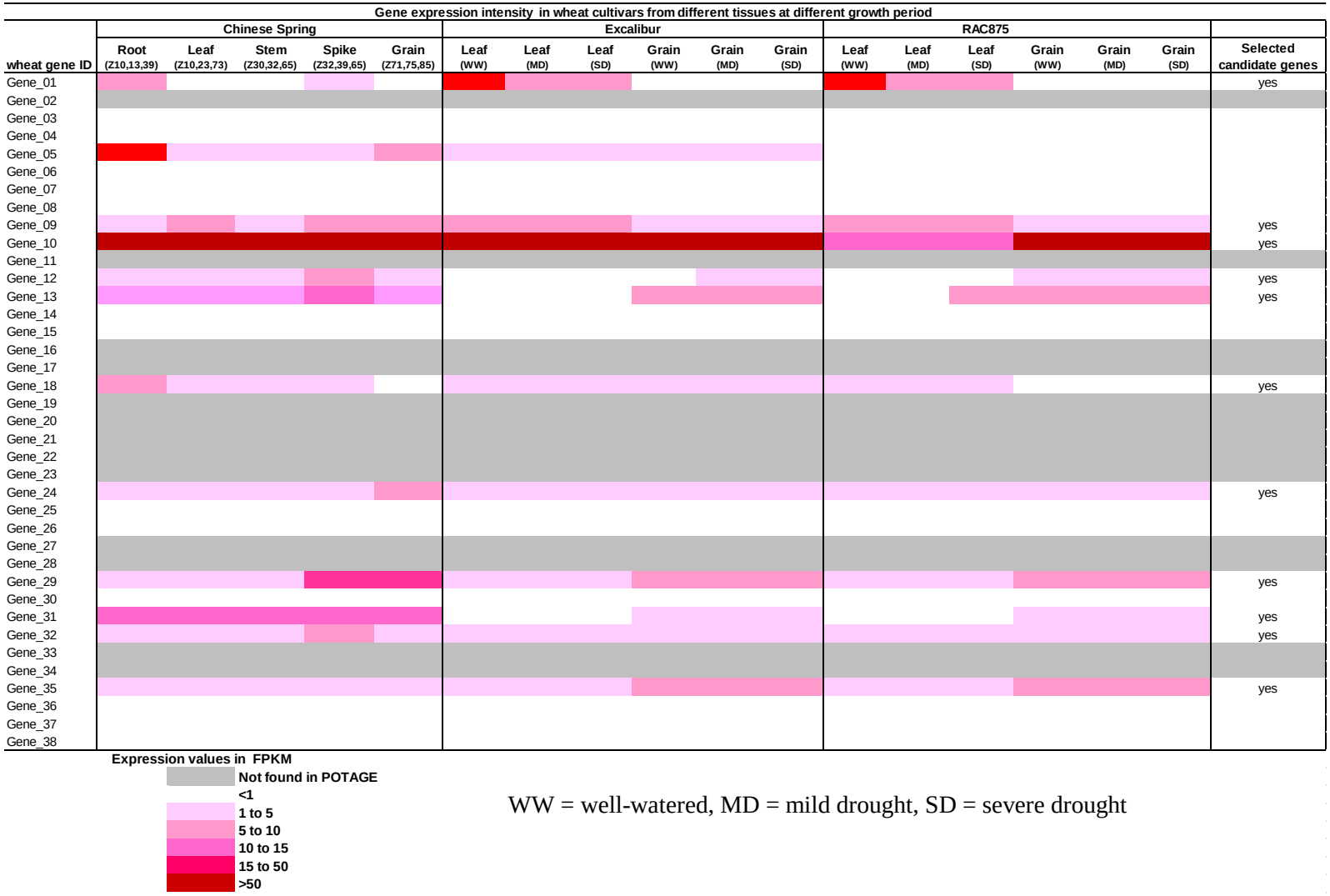
Table. 2. Putative function of 42 predicted genes under the Excalibur/Kukri yield QTL in Region III (2.17 Mb sequence interval) found by homology with rice and Brachypodium using Phytozome.

Gene ID	Rice annotation ID	Rice annotation description	score	e-value	Brachybodium ID	Brachybodium annotation description	score	e-value
Gene 01	LOC_Os05g49350.1	DUF1645 domain containing protein, putative, expressed	138.3	1.90E-38	Bradi2g45710.1	PF07816 - Protein of unknown function (DUF1645)	111.7	2.9E-28
Gene 02	LOC_Os05g49580.1	plastocyanin-like domain containing protein, putative, expressed	97.2	7.4E-62	Bradi2g15910 1	copper ion binding	216	9.80E-70
Gene 03	LOC_Os05g49380.2	OsDegp9 - Putative Deg protease homologue, expressed	632.1	0	Bradi2g15980.1	serine-type endopeptidase activity	651.7	0
Gene 04	LOC_Os07g35050.1	OsFBX237 - F-box domain containing protein, expressed	185.7	2.10E-52	Bradi2g37210.2	F-Box	317	9.40E-104
Gene 05	LOC_Os02g42810.1	oxidoreductase, short chain dehydrogenase/reductase family domain containing protein, expressed	314.7	2.9E-104	Bradi5g16660.1	K15095 - (+)-neomenthol dehydrogenase	334.3	4.1E-112
Gene 06	LOC_Os05g49580.1	plastocyanin-like domain containing protein, putative, expressed	195.3	1.60E-61	Bradi2g15920.1	copper ion binding	192.6	1.80E-60
Gene 07	LOC Os06g12100.1	mTERF family protein, expressed	162.9	1.90E-46	Bradi4g20547.1	PTHR13068//PTHR13068:SF37 - CGI-12 PROTEIN-RELATED	198.4	3E-60
Gene 08	LOC Os06g12100.1	mTERF family protein, expressed	162.9	1.90E-46	Bradi4g20547.1	PTHR13068//PTHR13068:SF37 - CGI-12 PROTEIN-RELATED	198.4	3.00E-60
Gene 09	LOC_Os05g49590.2	suppressor of phythchrome A, putative, expressed	1342.8	0	Bradi2g15900.1	Homologous to Arabidopsis SUPPRESSOR OF PHYA	1421.8	0
Gene 10	LOC_Os05g38530.1	DnaK family protein, putative, expressed	61.6	1.10E-10	Bradi1g66470.1	ATPase activity, coupled	59.3	7.90E-10
Gene 11	LOC_Os09g11140.1	expressed protein	42	3.70E-03		No homolog		
Gene 12	LOC_Os05g11980.1	timeless protein, expressed	114.8	1.60E-24	Bradi2g15960.1	K03155 - timeless	134.8	1.10E-30
Gene 13	LOC_Os05g11980.1	timeless protein, expressed	1192.2	0	Bradi2g15960.1	K03155 - timeless	1503.8	0
Gene 14	LOC_Os05g49350.1	DUF1645 domain containing protein, putative, expressed	119.8	1.3E-31	Bradi2g16010.1	PF07816 - Protein of unknown function (DUF1645)	96.7	8.9E-23
Gene 15		No homolog			Bradi2g51390.6	Single-stranded DNA binding	39.3	1.20E-02
Gene 16	LOC_Os07g35060.1	OsFBX238 - F-box domain containing protein, expressed	188.7	1.40E-53	Bradi2g37210.2	F-Box	228.8	1.30E-69
Gene 17	LOC_Os12g17480.1	MLA12, putative, expressed	144.8	1.60E-35	Bradi3g03597.1	PTHR23155//PTHR23155:SF605 - LEUCINE-RICH REPEAT-CONTAINING PROTEIN	129	2.50E-30
Gene 18	LOC_Os02g17304.1	resistance protein, putative, expressed	99	2.00E-23	Bradi4g04657.2	PTHR23155//PTHR23155:SF515 - LEUCINE-RICH REPEAT-CONTAINING PROTEIN	87	3.40E-19
Gene 19	LOC_Os11g16530.2	mal, putative, expressed	85.9	2.20E-17	Bradi4g04657.2	PTHR23155//PTHR23155:SF515 - LEUCINE-RICH REPEAT-CONTAINING PROTEIN	72	7.10E-13
Gene 20	LOC_Os07g18510.1	OsFBLD5 - F-box, LRR and FBD domain containing protein, expressed	122.9	6.70E-32	Bradi2g16190.2	PF08387 - FBD	140.6	2.10E-38
Gene 21	LOC_Os05g49650.2	expressed protein	212.6	1.40E-68	Bradi2g15870.1	PF12159 - Protein of unknown function (DUF3593)	204.9	1.20E-65
Gene 22	LOC_Os05g49610.1	ubiquitin carboxyl-terminal hydrolase, family 1, putative, expressed	565.1	0	Bradi2g15890.1	PTHR31476:SF4 - RNA RECOGNITION DOMAIN-CONTAINING PROTEIN WTF1	573.5	0
Gene 23	LOC_Os12g17140.1	mla1, putative, expressed	145.6	6.60E-38	Bradi3g00757.1	PTHR23155//PTHR23155:SF605 - LEUCINE-RICH REPEAT-CONTAINING PROTEIN	130.6	5.10E-31
Gene 24	LOC_Os07g18560.1	OsFBLD6 - F-box, LRR and FBD domain containing protein, expressed	192.2	2.8E-56	Bradi2g16200.1	F-Box	188.7	4E-55

Gene 25	LOC_Os02g17304.1	resistance protein, putative, expressed	64.3	9.60E-12	Bradi4g04662.3	PTHR23155//PTHR23155:SF544 - LEUCINE-RICH REPEAT-CONTAINING PROTEIN	47.8	3.70E-06
Gene 26	LOC_Os05g31730.1	transporter, monovalent cation:proton antiporter-2 family, putative, expressed	504.6	1.50E-174	Bradi2g26740.1	Potassium:hydrogen antiporter activity	559.3	0
Gene 27	LOC_Os03g24200.1	OsFBX85 - F-box domain containing protein, expressed	278.5	1.20E-86	Bradi4g42545.1	PF00646 - F-box domain	424.5	1.20E-143
Gene 28	LOC_Os07g35060.1	OsFBX238 - F-box domain containing protein, expressed	207.6	9.00E-61	Bradi2g37210.2	F-Box	290	1.50E-93
Gene 29	LOC_Os05g49430.1	aldose 1-epimerase, putative, expressed	526.6	0	Bradi2g15930.1	glucose-6-phosphate 1-epimerase activity	538.9	0
Gene 30	LOC_Os05g49620.1	WRKY19, expressed	52.4	3.00E-07	Bradi2g15872.1	PF03106 - WRKY DNA -binding domain	62.4	3.10E-11
Gene 31	LOC_Os05g49420.1	transcription factor, putative, expressed	364.4	3.10E-121	Bradi2g15940.1	bZIP transcription factor	416	1.60E-141
Gene 32	LOC_Os05g49620.1	WRKY19, expressed	179.9	6.20E-54	Bradi2g15877.1	F03106 - WRKY DNA -binding domain	180.3	1.70E-53
Gene 33	LOC_Os05g49410.1	1 expressed protein	184.9	3.60E-58	Bradi2g15950.1	PF11341 - Protein of unknown function (DUF3143)	187.6	4.50E-59
Gene 34	LOC_Os01g33520.1	ulp1 protease family, C-terminal catalytic domain containing protein, expressed	146.7	6.20E-36	Bradi1g72191.1	PTHR12606 - SENTRIN/SUMO-SPECIFIC PROTEASE	115.2	1.60E-27
Gene 35	LOC_Os06g12100.1	mTERF family protein, expressed	352.1	1.20E-115	Bradi4g20547.1	PTHR13068//PTHR13068:SF37 - CGI-12 PROTEIN-RELATED	422.2	1.70E-143
Gene 36	LOC_Os11g17330.1	stripe rust resistance protein Yr10, putative, expressed	78.6	6.50E-15	Bradi4g04655.5	PTHR23155//PTHR23155:SF515 - LEUCINE-RICH REPEAT-CONTAINING PROTEIN	78.2	1.00E-14
Gene 37		No homolog			Bradi1g51985.1	PF06839 - GRF zinc finger	41.6	3.60E-04
Gene 38	LOC_Os11g16530.2	mal, putative, expressed	142.5	1.80E-35	Bradi4g20527.4	PTHR23155//PTHR23155:SF578 - LEUCINE-RICH REPEAT-CONTAINING PROTEIN	137.1	1.80E-33
Gene 39		No homolog				No homolog		
Gene 40		No homolog				No homolog		
Gene 41		No homolog				No homolog		
Gene 42		No homolog				No homolog		

Not found in POTAGE/No homolog
Found in POTAGE
Selected genes

Table 3. Gene expression profile in different tissues of wheat cultivars including Excalibur and RAC875 using POTAGE platform.



WW = well-watered, MD = mild drought, SD = severe drought

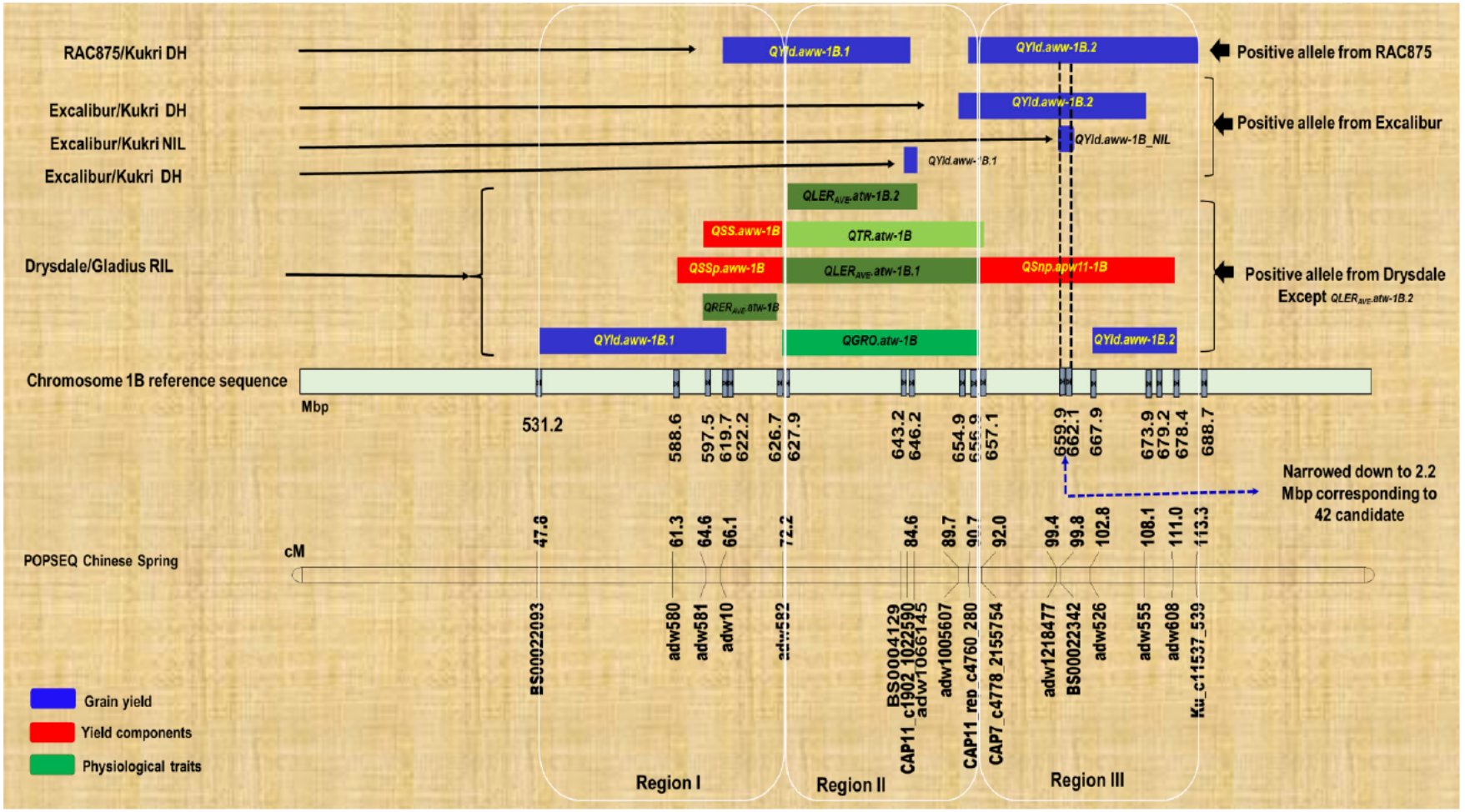


Figure 1. QTL for yield, yield components and physiological traits across three mapping populations and their relative position on IWGSC RefSeq v1.0. Yld = yield, SSp = Seeds/spikelet, SS = Seeds/spike, GRO = average growth rate, LER = average leaf expansion rate, QTR = average transpiration rate, Snp = spikes per plant. The yield QTL in Excalibur/Kukri DH that overlapped with yield and yield components QTL in RAC875/Kukri (Bennett et al., 2012) and Drysdale/Gladius, was fine mapped using NIL (chapter 3) and narrowed down to 2.2 Mbp which is corresponding to 42 candidate genes (blue line).

Table 4. Drysdale, Gladius, RAC875, Excalibur and Kukri haplotypes for the markers covering the three QTL hotspot regions on chromosome 1B Chinese Spring Ref Seq v1 map.

1B Ref Seq v1.0 map	QTL hotspot Region I					QTL hotspot Region II			QTL hotspot Region III						
Parental lines	BS00022093	adw580	adw581	adw10	adw582	CAP11_c1902_1022590	adw1005607	CAP11_rep_c4760_280	CAP7_c4778_2155754	adw1218477	BS00022342	adw526	adw555	adw608	CAP11_c11537_539
Drysdale	AA	AA	AA	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	AA
Gladius	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	AA	BB	AA	BB	BB
RAC875	NA	BB	BB	NA	AA	AA	NA	AA	AA	NA	NA	AA	BB	BB	BB
Excalibur	AA	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	AA	AA	AA
Kukri	AA	BB	AA	BB	AA	BB	BB	BB	AA	BB	BB	BB	BB	BB	BB

NA = marker data not avialable

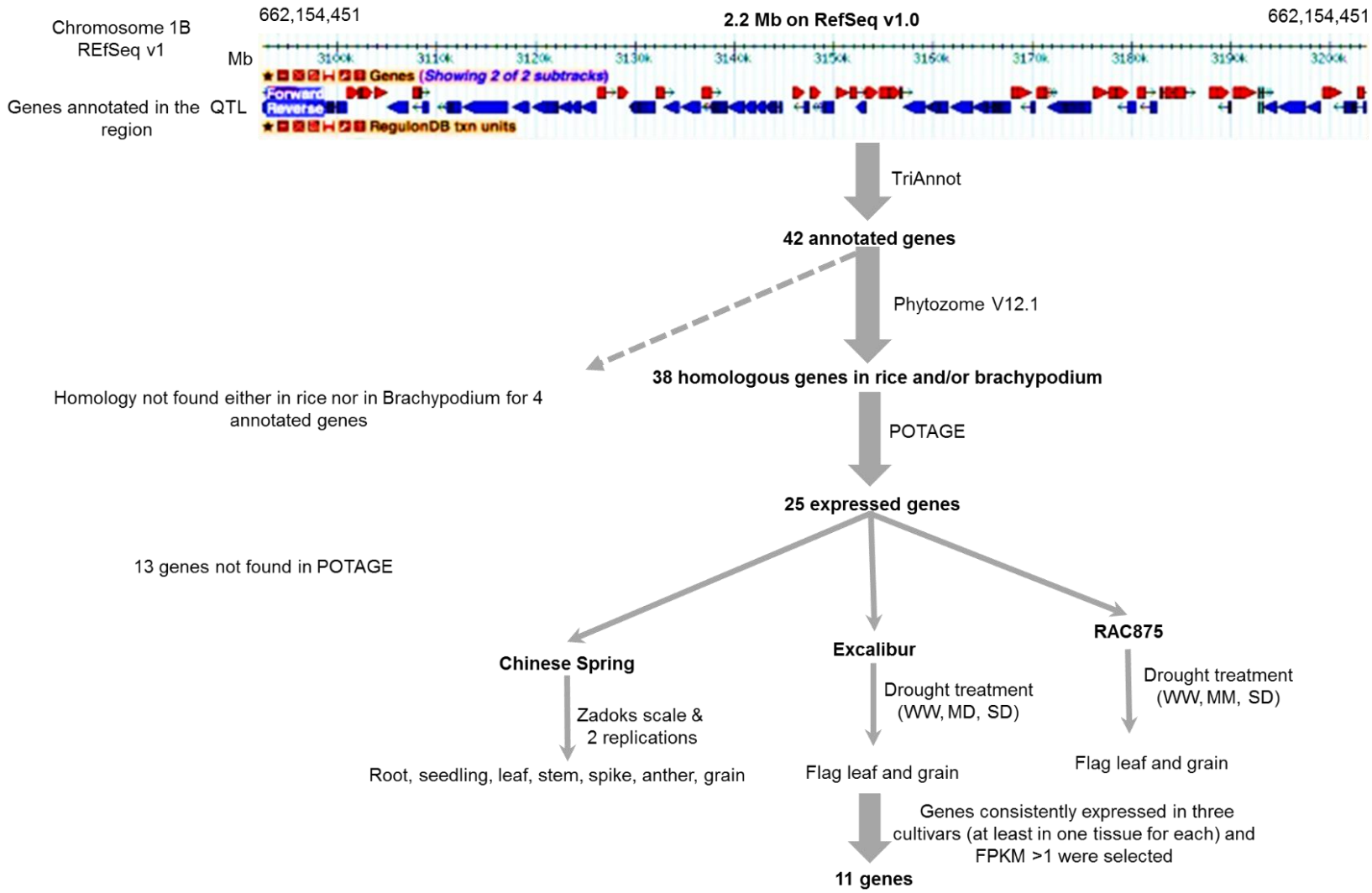


Figure 2. Stepwise method to select candidate genes from 42 genes for Excalibur/Kukri yield QTL (*QYld.aww-1B2*).

WW = well-watered, MD = mild drought, SD = severe drought, FPKM = Fragment per kilobase of exon per million fragments mapped

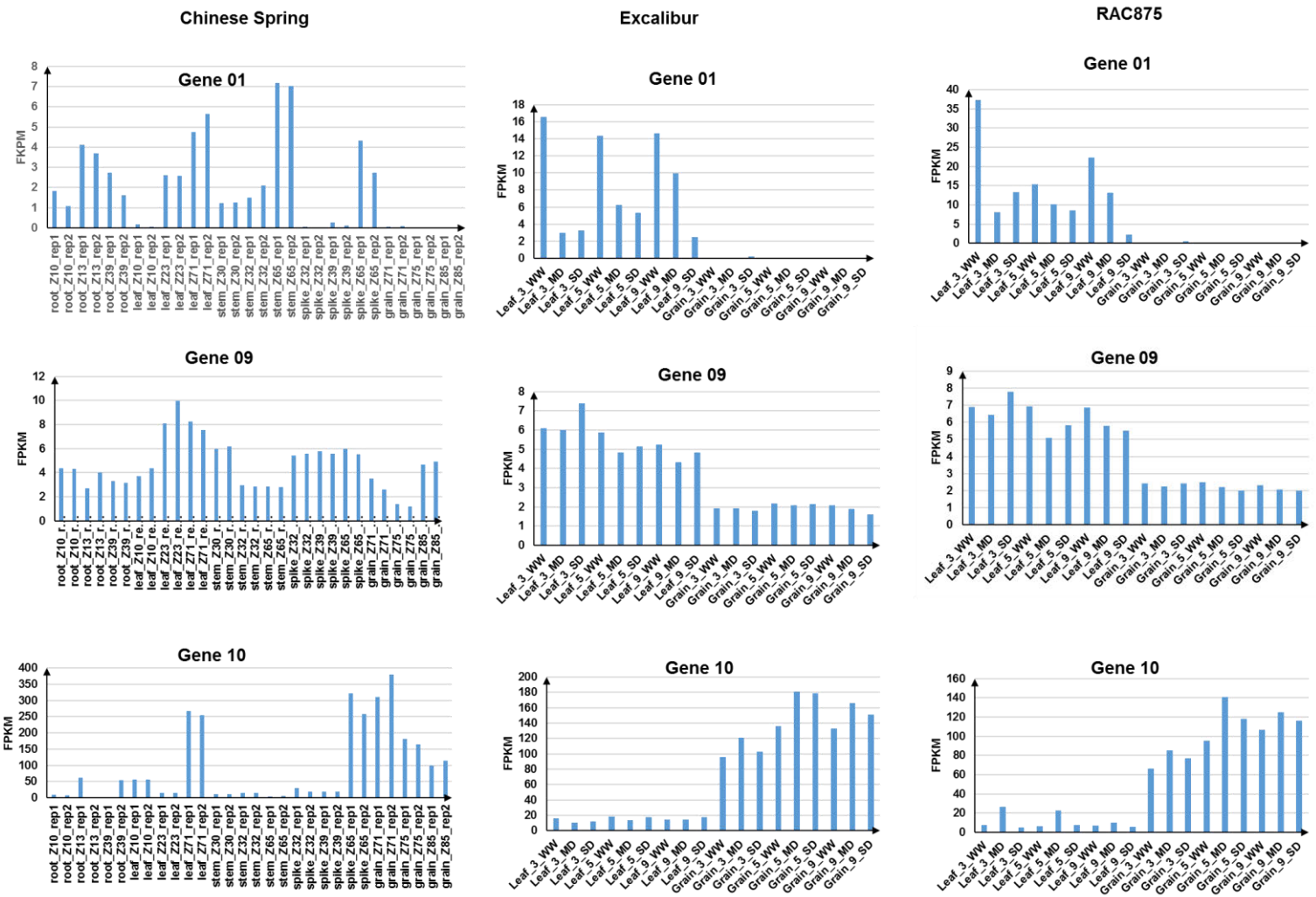


Figure 3. Gene expression profiles of 3 genes in three wheat cultivars (Chinese Spring, Excalibur and RAC875) across tissues, growth stage and drought treatment (Excalibur and RAC875). Z10 to Z85 = Zadoks' scale, 3, 5, 9 = days after treatment on which tissues sampled, WW = well-watered, MD = mild drought, SD = severe drought, FPKM = Fragment per kilobase of exon per million fragments mapped

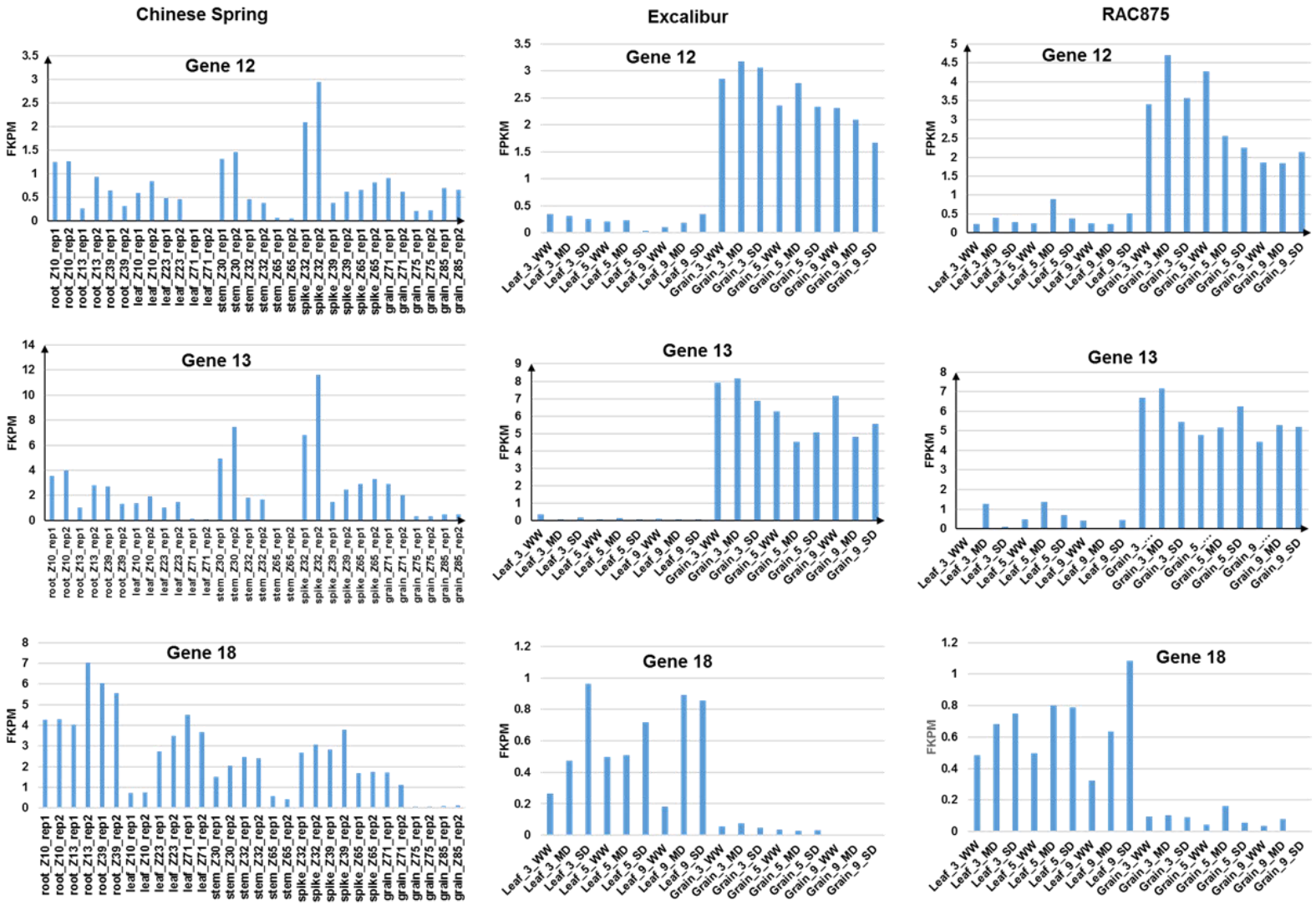


Figure 3. Continued

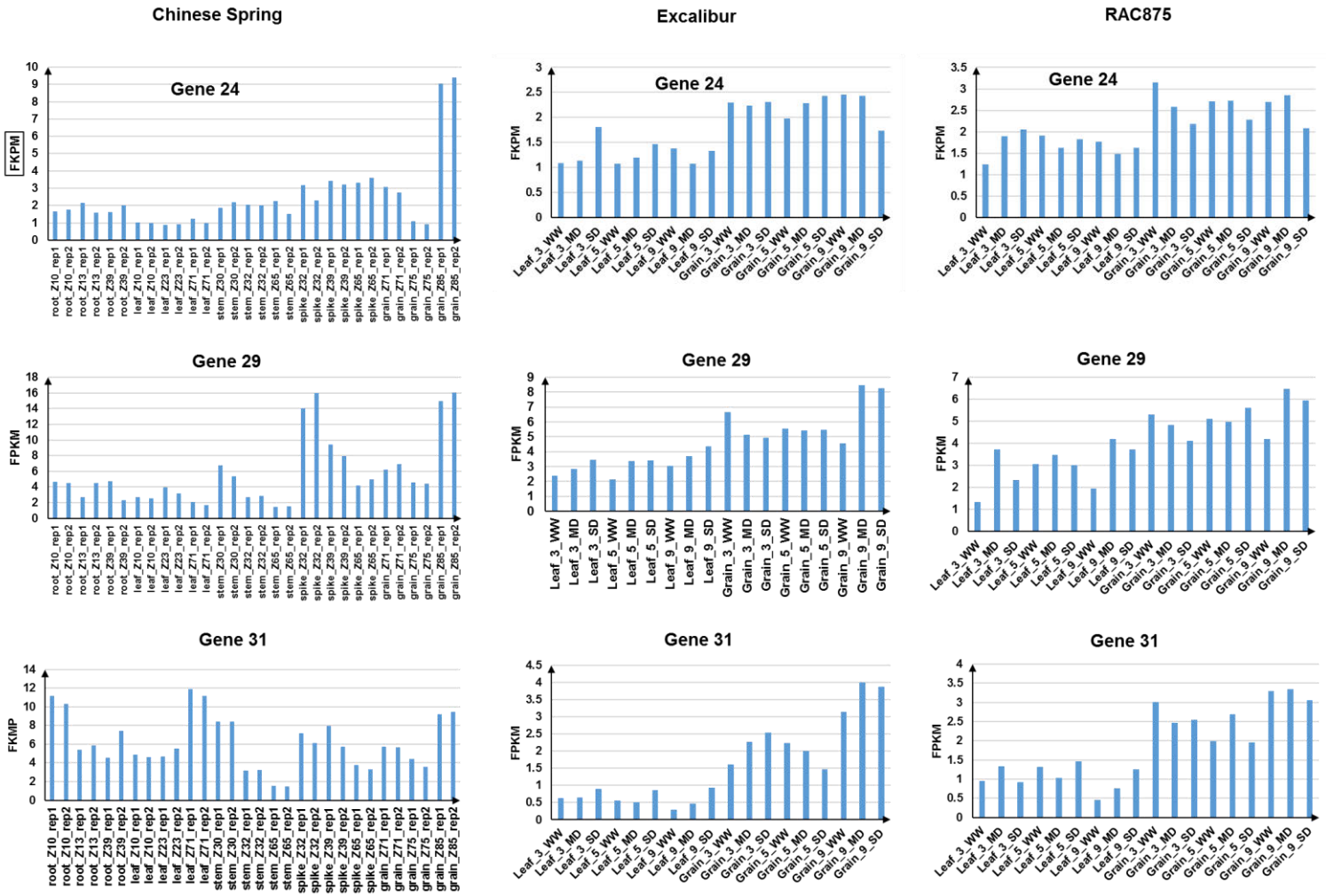


Figure 3. Continued

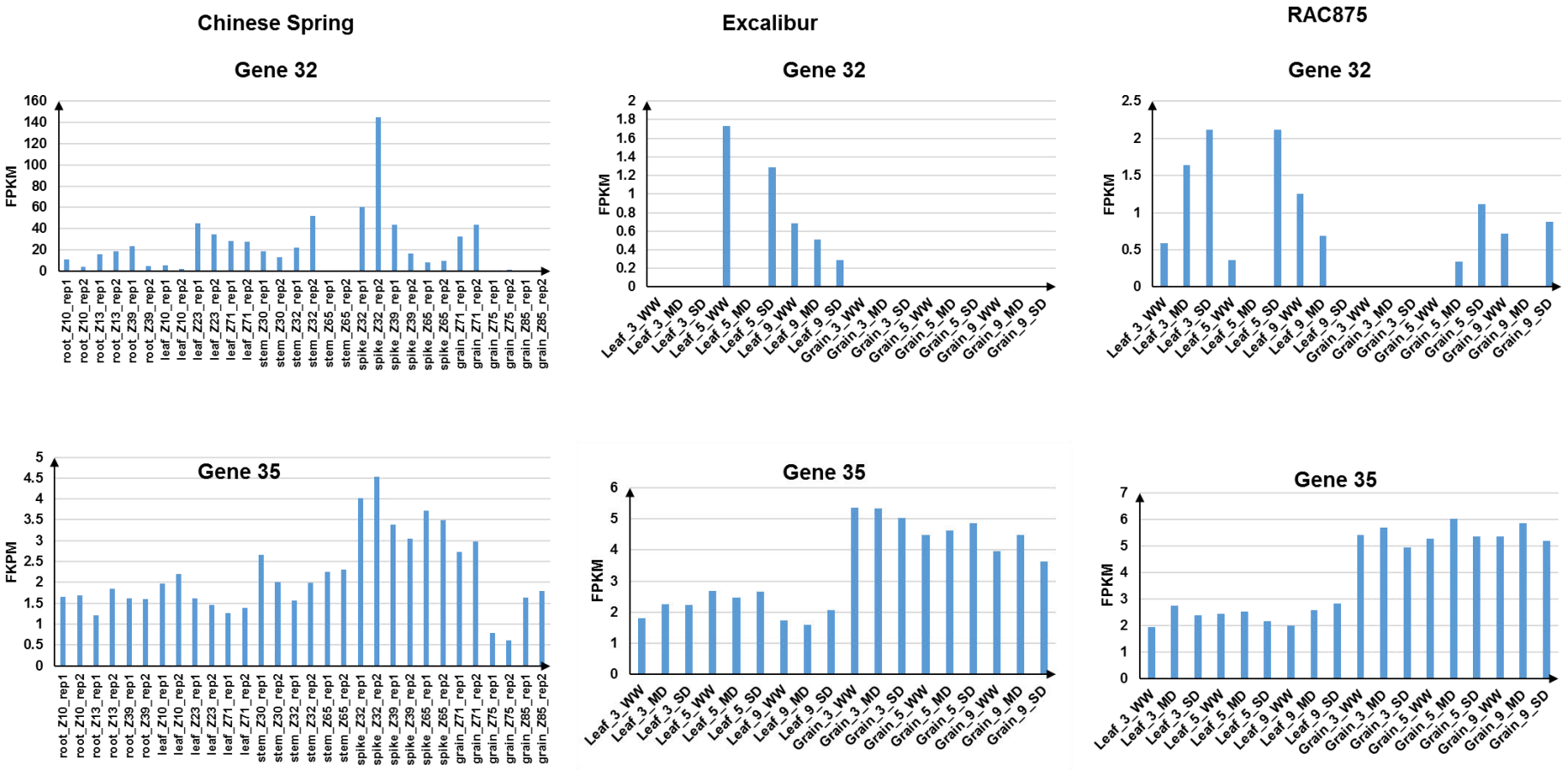


Figure 3. Continued

Chapter 6. General conclusion, contributions to scientific knowledge and future implications

6.1. Multiple QTL on chromosome 1B

Drought and heat stresses are threatening global wheat production and the challenge is worsening with the expected changes in climate (Bloem et al. 2009; Carnicer et al. 2011; Sohail et al. 2016). To increase wheat production under such conditions and feed the ever-increasing world population, new insights and knowledge on genomic loci controlling yield and related traits under dry and hot environments is essential. This information can lead to the development of diagnostic marker(s) for use in molecular marker assisted breeding to develop high yielding and stress tolerant cultivars. This study provided information on loci for grain yield, yield components and physiological traits on chromosome 1B in three wheat mapping populations, their position on the physical map, and candidate genes underlying wheat yield in dry and hot environments.

Another finding of this study was the positioning of 14 QTL that were identified in three mapping populations (Excalibur/Kukri, RAC875/Kukri DH and Drysdale/Gladius RIL) on the genomic region of chromosome 1B from 531.2 Mbp to 668.7 Mbp of the RefSeq v1 (Chapter 5, Fig 1). Excalibur, RAC875, Drysdale and Gladius are modern varieties adapted to Australian climate, with comparable average yield across sites (Fleury et al., 2010). They have different tolerance mechanisms to drought and heat (Fleury et al. 2010; Richards et al. 2002a) by contrast with Kukri which is susceptible. Excalibur and RAC875 are parental lines of Gladius, a cultivar released in the early 2000 and adapted to the southern region of Australia (Fleury et al. 2010). Drysdale was selected for its high transpiration efficiency and is adapted to the northern region (Condon et al. 2006). These lines were good sources to develop improved cultivars in dry and hot environments. As the three mapping populations have common pedigrees, we expected some common QTL among the three mapping populations. Indeed, our present study showed the alignement of chromosome 1B QTL from the three mapping populations and found clustered QTL for different traits in three major QTL hotspot regions. The co-location of yield and yield components QTL with QTL for physiological traits in similar genomic regions suggests pleiotropic effects of single or closely linked genes. These results could be further

used for fine mapping to identify genes underlying grain yield using physiological traits as proxy traits.

These results can also be deployed in marker assisted selection to pyramid positive alleles by using linked molecular markers flanking the QTL. Even though, successful QTL pyramiding in different crops including wheat has been mainly for major genes such as disease resistance, grain quality, sprouting tolerance, etc (Kumar et al. 2010; Soriano and Royo 2015; Tyagi et al. 2014), a promising MAS pyramiding approach for yield QTL under drought stress is recently reported in wheat (Gahlaut et al. 2017) and in rice (Ashikari and Matsuoka 2006; Wang et al. 2012c). In this study, the co-located QTL found in Region II (627.9 Mbp to 657.1 Mbp) explained a range of phenotypic variation (15% to 46%) for the traits and could be used in marker assisted QTL pyramiding to accumulate the minor QTL into a single genotype to increase the overall gains. Interestingly, the yield QTL in RAC875/Kukri (*QYld.aww-1B.2*) co-located with yield QTL in Excalibur/Kukri NIL (*QYld.aww.2*) and spikes number/plant in Drysdale/Gladius in Region III (657.1 Mbp to 688.7 Mbp) could also be considered for MAS pyramiding using the already fine mapped yield QTL in Excalibur/Kukri NIL as a background.

6.2. Yield QTL in Drysdale/Gladius RIL and its features

The first research component of this study was to discover whether the genetic variation on chromosome 1B for growth and transpiration rate at vegetative stage in Drysdale/Gladius RIL under glasshouse conditions has an effect on yield. Drysdale is known for its high transpiration efficiency and high yield in New South Wales region where sufficient underground soil moisture available. Gladius is widely adapted to southern region under the prevailing cyclic drought and episodes of high temperatures at grain filling period. The main reason why we focused on the Drysdale/Gladius mapping population in the current study is to combine the favourable features of the two varieties and thus, identify QTL for yield and physiological traits under dry and hot environment of overall Australia. We confirmed the presence of QTL for yield and seeds/spikelet on chromosome 1B and found that these QTL were expressed specifically under severe terminal drought and high temperature conditions (Chapter 3, Table 4). We also found that these QTL co-located with QTL for biomass and relative rate of leaf area expansion assessed from the imaging platform data (Parent *et al.*, 2015). This result was supported by significant positive correlations observed between grain yield, seeds/spike and

biomass (Tables 2 and 3). The Drysdale allele increased leaf expansion rate at early stage, which in turn increased biomass accumulation before the onset of drought stress, leading to improvement in floral fertility (seeds/spikelet) and increased grain yield. This result suggests that:

- Positive selection for the leaf expansion rate in areas where terminal soil moisture stress and sporadic high temperature prevail, such as in South Australia, may help plants maximize the use of available moisture during the favourable growing conditions of the autumn and winter period to increase early vigour/biomass production. This biomass could be remobilized and used for grain filling to maintain economic yield during the cyclic drought and heat stress in the spring and summer. Current developments in field-based high-throughput phenotyping equipment (Araus and Cairns 2014) could be used to measure vegetative plant growth rate to predict final grain yield responses under drought and heat stressed field conditions.
- As the Drysdale and Excalibur allele increased all the QTL in the entire QTL hotspot region of this study (Fig 5.1), breeders could target this haplotype for selection in crossing programs to improve most of the traits co-located in this linkage block (Table 5.4). Moreover, these markers could be used in genomic selection program to facilitate rapid selection of superior genotypes from large germplasms and accelerate the breeding cycle to improve wheat yield under stressed climate.
- The co-location between yield QTL and QTL for growth, leaf expansion and transpiration rate shows that automated high throughput imaging platform TPA could provide a rapid means of phenotyping large mapping populations and support the positional cloning of the 1B yield QTL.

6.3. Fine map of Excalibur/Kukri 1B yield QTL

In this study, we confirmed the yield QTL in Excalibur/Kukri DH reported by Edwards (2012c) and fine mapped this locus using NIL grown under severe terminal drought and heat stress (Chapter 4). The Excalibur parent had an allelic effect of 123 kg/ha in DH and 285 kg/ha in NIL. Grain yield co-segregated with fertile tillers, seeds number per plot, NDVI and biomass in four NIL pairs (Chapter 4, Fig 3 and Supp. Table 7) in which the Excalibur allele increased grain yield by 54.5%, biomass by 43%, and fertile tillers by 32.8%. We hypothesized that the

number of fertile tillers per plot determined the number of seeds/plot, which eventually turned to grain yield. This matches our knowledge of the Excalibur parental line which is able to adjust tiller number to the available soil moisture (Izanloo et al. 2008).

Eleven candidate genes showed high expression in reproductive and seedling tissues of Chinese Spring, Excalibur and RAC875 cultivars under drought treatment and were selected for further study (Chapter 5, Table 2). These candidate genes could be involved in crop developmental, physiological and reproductive process and supporting early vegetative stage biomass accumulation, source translocation, maintaining fertile tillers and spike fertility and grain development under terminal drought and heat stress. However, this preliminary list of candidate genes needs to be annotated manually as it may not represent the full gene content of the region. Further gene expression analysis needs to be done in different tissues and different developmental stages from Excalibur/Kukri NIL under drought and heat stress. Gene complementation would be needed to validate the biological function of the genes in the three mapping populations (Excalibur/Kukri, Drysdale/Gladius and RAC875/Kukri) using over expression or silencing with alternative alleles from Excalibur, RAC875 and Drysdale versus Kukri and Gladius. Transgenic performance can be compared to the original parental lines for early growth traits, fertile spikes, floret fertility and grain yield (Langridge and Reynolds 2015). TILLING (Targeting Induced Local Lesions in Genomes) approach (Comai et al. 2004; Till et al. 2006) may also be useful as a source of alternative haplotypes for gene complementation study.

6.4. Conclusions

In this study we used new wheat genomic resources such as genome sequencing and high-density linkage maps to finely map yield and yield components QTL on chromosome 1B in three interlinked mapping populations across locations, and identify candidate genes that underly grain yield under dry and hot climate of southern Australia. The information on QTL for yield and physiological traits and candidate genes described in this thesis are significant resources that could be used by breeders in wheat improvement programs through various breeding approaches, including:

- developing diagnostic markers for MAS. We developed KASP assay that are tightly linked to yield QTL in the region III, and overlapped QTL for yield and physiological traits in the region II of chromosome 1B. Those will help introgressing QTL avoiding linkage drag. These markers might be added to the suites of markers for genomic selection (GS) program for quick selection of elite lines from large populations to develop drought and heat tolerant wheat cultivars.
- using Drysdale and Excalibur haplotypes in the three QTL hotspot of chromosome 1B (Table 5.4) in crossing program to create new desirable allelic combinations and select transgressive segregants leading to superior high yielding genotypes for southern Australian climate.
- using proxy traits such as growth rate/leaf expansion rate in high throughput imaging phenotyping platform for further fine mapping RAC875/Kukri yield QTL (*QYld.aww-1B-1*) at QTL hotspot region II. Hyperspectral imaging technologies could also be used to select those physiological QTL colocated to grain yield QTL.

Ultimately, we would clone the gene(s) responsible of the 1B yield QTL and understand its function. This would provide the opportunity to create novel alleles by genome editing or TILLING mutations that could increase drought and heat tolerance. Eleven candidate genes have been proposed here for further studies. We need to look at more genomic resources and further molecular techniques such as manual annotations, structural variants and validate them (Sanger or KASP) and QPCR to investigate these genes and identify possibly new molecular mechanisms controlling drought and heat tolerance in wheat.

6.5. References

Abdolshahi R, Nazari M, Safarian A, Sadathossini TS, Salarpour M, Amiri H (2015) Integrated selection criteria for drought tolerance in wheat (*Triticum aestivum* L.) breeding programs using discriminant analysis. Field Crops Res 174:20-29

Acreche MM, Slafer GA (2006) Grain weight response to increases in number of grains in wheat in a Mediterranean area. Field Crops Res 98:52-59

Adams MD, Celniker SE, Holt RA, Evans CA, Gocayne JD, Amanatides PG, Scherer SE, Li PW, Hoskins RA, Galle RF (2000) The genome sequence of Drosophila melanogaster. Science 287:2185-2195

Adjemian M, Janzen JP (2014) USDA Economic Research Service-Wheat Prices Driven By Supply and Demand, Not Speculators

Agarwal PK, Agarwal P, Reddy M, Sopory SK (2006) Role of DREB transcription factors in abiotic and biotic stress tolerance in plants. Plant cell reports 25:1263-1274

Ahmadi A, Baker D (2001) The effect of water stress on grain filling processes in wheat. The Journal of Agricultural Science 136:257-269

Ahmed HU, Henry A, Mauleon R, Dixit S, Vikram P, Tilatto R, Verulkar SB, Perraju P, Mandal NP, Variar M (2013) Genetic, physiological, and gene expression analyses reveal that multiple QTL enhance yield of rice mega-variety IR64 under drought. PLoS One 8:e62795

Akhtar M, Jaiswal A, Taj G, Jaiswal J, Qureshi M, Singh N (2012) DREB1/CBF transcription factors: their structure, function and role in abiotic stress tolerance in plants. Journal of genetics 91:385-395

Al-Whaibi MH (2011) Plant heat-shock proteins: a mini review. Journal of King Saud University-Science 23:139-150

Alaux M, Letellier T, Alfama-Depauw F, Jamilloux V, Rogers J, Choulet F, Guerche C, Loaec M, Flores R-G, Michotey C (2016) IWGSC Sequence Repository: Moving towards tools to facilitate data integration for the reference sequence of wheat. PAG XXIV-Plant and Animal Genome Conference, p np

Alexandratos N, Bruinsma J (2012) World agriculture towards 2030/2050: the 2012 revision. ESA Working paper Rome, FAO

Allard W (1960) Principles of Plant Breeding (John Willey and Sons. Inc. London)

Allen AM, Barker GLA, Wilkinson P, Burridge A, Winfield M, Coghill J, Uauy C, Griffiths S, Jack P, Berry S (2013) Discovery and development of exome-based, co-dominant single nucleotide polymorphism markers in hexaploid wheat (Triticum aestivum L.). Plant Biotechnol J 11:279-295

Almeida GD, Nair S, Borém A, Cairns J, Trachsel S, Ribaut J-M, Bänziger M, Prasanna BM, Crossa J, Babu R (2014) Molecular mapping across three populations reveals a QTL hotspot region on chromosome 3 for secondary traits associated with drought tolerance in tropical maize. Mol Breed 34:701-715

Alonso-Blanco C, Koornneef M (2000) Naturally occurring variation in< i> Arabidopsis</i>: an underexploited resource for plant genetics. Trends in plant science 5:22-29

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389-3402

Alvarez S, Marsh EL, Schroeder SG, Schachtman DP (2008) Metabolomic and proteomic changes in the xylem sap of maize under drought. Plant, Cell & Environment 31:325-340

Amano N, Tanaka T, Numa H, Sakai H, Itoh T (2010) Efficient plant gene identification based on interspecies mapping of full-length cDNAs. DNA Res 17:271-279

Araus J, Slafer G, Reynolds M, Royo C (2002) Plant breeding and drought in C3 cereals: what should we breed for? Annals of botany 89:925-940

Araus J, Villegas D, Aparicio N, Del Moral L, El Hani S, Rharrabti Y, Ferrio J, Royo C (2003) Environmental factors determining carbon isotope discrimination and yield in durum wheat under Mediterranean conditions. Crop Science 43:170-180

Araus JL, Slafer GA, Royo C, Serret MD (2008) Breeding for yield potential and stress adaptation in cereals. Critical Reviews in Plant Science 27:377-412

Arrigo A (1998) Small stress proteins: chaperones that act as regulators of intracellular redox state and programmed cell death. Biological chemistry 379:19-26

Asada K (2006) Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol 141:391-396
Ashikari M, Matsuoka M (2006) Identification, isolation and pyramiding of quantitative trait loci for rice breeding. Trends in Plant Science 11:344-350
Asseng S, Van Herwaarden AF (2003) Analysis of the benefits to wheat yield from assimilates stored prior to grain filling in a range of environments. Plant Soil 256:217-229
Atkinson NJ, Jain R, Urwin PE (2015) The response of plants to simultaneous biotic and abiotic stress. Combined stresses in plants. Springer, pp 181-201
Atkinson NJ, Lilley CJ, Urwin PE (2013) Identification of genes involved in the response of Arabidopsis thaliana to simultaneous biotic and abiotic stresses. Plant physiology:pp. 113.222372
Austin R, Jones H (1975) The physiology of wheat. Part III. Plant Breeding Institute Annual Report 1974:20-71
Austin RB, Morgan CL, Ford MA, Blackwell RD (1980) Contributions to grain yield from pre-anthesis assimilation in tall and dwarf barley phenotypes in two contrasting seasons. Ann Bot 45:309-319
Babiychuk E, Vandepoele K, Wissing J, Garcia-Diaz M, De Rycke R, Akbari H, Joubès J, Beeckman T, Jänsch L, Frentzen M (2011) Plastid gene expression and plant development require a plastidic protein of the mitochondrial transcription termination factor family. Proceedings of the National Academy of Sciences 108:6674-6679
Babu RC, Nguyen BD, Chamarerk V, Shanmugasundaram P, Chezhian P, Jeyaprakash P, Ganesh S, Palchamy A, Sadasivam S, Sarkarung S (2003) Genetic analysis of drought resistance in rice by molecular markers. Crop Science 43:1457-1469
Bahieldin A, Mahfouz HT, Eissa HF, Saleh OM, Ramadan AM, Ahmed IA, Dyer WE, El-Itriby HA, Madkour MA (2005) Field evaluation of transgenic wheat plants stably expressing the HVA1 gene for drought tolerance. Physiologia Plantarum 123:421-427
Baker R (1969) Genotype-environment interactions in yield of wheat. Canadian Journal of Plant Science 49:743-751
Barker T, Campos H, Cooper M, Dolan D, Edmeades G, Habben J, Schussler J, Wright D, Zinselmeier C (2005) Improving drought tolerance in maize. Plant Breed Rev 25:173-253
Beales J, Turner A, Griffiths S, Snape JW, Laurie DA (2007) A pseudo-response regulator is misexpressed in the photoperiod insensitive Ppd-D1a mutant of wheat (Triticum aestivum L.). Theoretical and Applied Genetics 115:721-733
Belkhadir Y, Subramaniam R, Dangl JL (2004) Plant disease resistance protein signaling: NBS–LRR proteins and their partners. Current opinion in plant biology 7:391-399
Bennett D, Izanloo A, Reynolds M, Kuchel H, Langridge P, Schnurbusch T (2012a) Genetic dissection of grain yield and physical grain quality in bread wheat (Triticum aestivum L.) under water-limited environments. Theor Appl Genet 125:255-271
Bennett D, Reynolds M, Mullan D, Izanloo A, Kuchel H, Langridge P, Schnurbusch T (2012b) Detection of two major grain yield QTL in bread wheat (Triticum aestivum L.) under heat, drought and high yield potential environments. Theoretical and Applied Genetics 125:1473-1485
Bita C, Gerats T (2013) Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. Frontiers in plant science 4:273
Bloem MW, Semba RD, Kraemer K (2009) Castel Gandolfo Workshop: An Introduction to the Impact of Climate Change, the Economic Crisis, and the Increase in the Food Prices on Malnutrition, 2. The Journal of Nutrition 140:132S-135S
Blum A (1997) Improving wheat grain filling under stress by stem reserve mobilisation. Wheat: Prospects for Global Improvement. Springer, pp 135-141
Blum A (2005) Drought resistance, water-use efficiency, and yield potential—are they compatible, dissonant, or mutually exclusive? Crop and Pasture Science 56:1159-1168
Blum A (2011) Drought resistance–is it really a complex trait? Funct Plant Biol 38:753-757
Blum A, Klueva N, Nguyen H (2001) Wheat cellular thermotolerance is related to yield under heat stress. Euphytica 117:117-123

Blum A, Mayer J, Gozlan G (1982) Infrared thermal sensing of plant canopies as a screening technique for dehydration avoidance in wheat. Field Crops Research 5:137-146
Blum A, Zhang J, Nguyen H (1999) Consistent differences among wheat cultivars in osmotic adjustment and their relationship to plant production. Field Crops Research 64:287-291
Blum U (1996) Allelopathic interactions involving phenolic acids. Journal of Nematology 28:259
Bogale A, Tesfaye K, Geleto T (2011) Morphological and physiological attributes associated to drought tolerance of Ethiopian durum wheat genotypes under water deficit condition. Journal of Biodiversity and Environmental Sciences 1:22-36
Boggs JZ, Loewy K, Bibee K, Heschel MS (2010) Phytochromes influence stomatal conductance plasticity in Arabidopsis thaliana. Plant growth regulation 60:77-81
Bonneau J, Taylor J, Parent B, Bennett D, Reynolds M, Feuillet C, Langridge P, Mather D (2013a) Multi-environment analysis and improved mapping of a yield-related QTL on chromosome 3B of wheat. Theoretical and Applied Genetics 126:747-761
Bonneau J, Taylor J, Parent B, Bennett D, Reynolds M, Feuillet C, Langridge P, Mather D (2013b) Multi-environment analysis and improved mapping of a yield-related QTL on chromosome 3B of wheat. Theor Appl Genet 126:747-761
Boopathi NM (2013) Mapping Population Development. Genetic Mapping and Marker Assisted Selection. Springer, pp 23-37
Borrás L, Slafer GA, Otegui MaE (2004) Seed dry weight response to source–sink manipulations in wheat, maize and soybean: a quantitative reappraisal. Field Crops Res 86:131-146
Bovill W, Horne M, Herde D, Davis M, Wildermuth G, Sutherland M (2010) Pyramiding QTL increases seedling resistance to crown rot (Fusarium pseudograminearum) of wheat (Triticum aestivum). Theoretical and Applied Genetics 121:127-136
Bray EA, Bailey-Serres, J., and Weretilnyk, E. (2000) Responses to abiotic stresses. In: B.B. Buchanan WG, and R.L. Jones (ed) Biochemistry and Molecular Biology of Plants. American Society of Plant Physiologists, Rockville, MD, pp 1158–1203
Brenchley R, Spannagl M, Pfeifer M, Barker GL, D'Amore R, Allen AM, McKenzie N, Kramer M, Kerhornou A, Bolser D (2012) Analysis of the bread wheat genome using whole-genome shotgun sequencing. Nature 491:705-710
Budak H, Kantar M, Yucebilgili Kurtoglu K (2013) Drought tolerance in modern and wild wheat. The Scientific World Journal 2013
Cai H, Morishima H (2002) QTL clusters reflect character associations in wild and cultivated rice. Theor Appl Genet 104:1217-1228
Calderini D, Quintero A, Hasan A, Wingen L, Griffiths S, Girousse C, Mouzeyar S, Le Gouis J (2013) Improving grain filling and potential grain size. International Workshop of the Wheat Yield Consortium, 3 Proceedings; CENEB, CIMMYT Cd Obregon, Sonora (Mexico); 5-7 Mar 2013. CIMMYT, p 39
Campbell KG, Bergman CJ, Gualberto DG, Anderson JA, Giroux MJ, Hareland G, Fulcher RG, Sorrells ME, Finney PL (1999) Quantitative trait loci associated with kernel traits in a soft× hard wheat cross. Crop Sci 39:1184-1195
Carnicer J, Coll M, Ninyerola M, Pons X, Sanchez G, Penuelas J (2011) Widespread crown condition decline, food web disruption, and amplified tree mortality with increased climate change-type drought. Proceedings of the National Academy of Sciences 108:1474-1478
Cattivelli L, Rizza F, Badeck F-W, Mazzucotelli E, Mastrangelo AM, Francia E, Marè C, Tondelli A, Stanca AM (2008) Drought tolerance improvement in crop plants: an integrated view from breeding to genomics. Field Crops Res 105:1-14
Cavanagh CR, Chao S, Wang S, Huang BE, Stephen S, Kiani S, Forrest K, Saintenac C, Brown-Guedira GL, Akhunova A (2013) Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars. Proceedings of the national academy of sciences 110:8057-8062

Chandrasekar V, K Sairam R, Srivastava G (2000) Physiological and biochemical responses of hexaploid and tetraploid wheat to drought stress. Journal of Agronomy and Crop Science 185:219-227
Chaves MM, Maroco JP, Pereira JS (2003) Understanding plant responses to drought—from genes to the whole plant. Functional plant biology 30:239-264
Chen L, Song Y, Li S, Zhang L, Zou C, Yu D (2012) The role of WRKY transcription factors in plant abiotic stresses. Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms 1819:120-128
Chini A, Grant JJ, Seki M, Shinozaki K, Loake GJ (2004) Drought tolerance established by enhanced expression of the CC–NBS–LRR gene, ADR1, requires salicylic acid, EDS1 and ABI1. The Plant Journal 38:810-822
Choulet F, Alberti A, Theil S, Glover N, Barbe V, Daron J, Pingault L, Sourdille P, Couloux A, Paux E (2014) Structural and functional partitioning of bread wheat chromosome 3B. Science 345:1249721
Choulet F, Wicker T, Rustenholz C, Paux E, Salse J, Leroy P, Schlub S, Le Paslier M-C, Magdelenat G, Gonthier C (2010) Megabase level sequencing reveals contrasted organization and evolution patterns of the wheat gene and transposable element spaces. The Plant Cell Online 22:1686-1701
Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963-971
Clavijo BJ, Venturini L, Schudoma C, Accinelli GG, Kaithakottil G, Wright J, Borrill P, Kettleborough G, Heavens D, Chapman H (2017) An improved assembly and annotation of the allohexaploid wheat genome identifies complete families of agronomic genes and provides genomic evidence for chromosomal translocations. Genome Res 27:885-896
Colasuonno P, Gadaleta A, Giancaspro A, Nigro D, Giove S, Incerti O, Mangini G, Signorile A, Simeone R, Blanco A (2014) Development of a high-density SNP-based linkage map and detection of yellow pigment content QTLs in durum wheat. Mol Breed 34:1563-1578
Collins NC, Tardieu F, Tuberosa R (2008) Quantitative trait loci and crop performance under abiotic stress: where do we stand? Plant Physiol 147:469-486
Comai L, Young K, Till BJ, Reynolds SH, Greene EA, Codomo CA, Enns LC, Johnson JE, Burtner C, Odden AR (2004) Efficient discovery of DNA polymorphisms in natural populations by Ecotilling. The Plant Journal 37:778-786
Condon A, Richards R, Farquhar G (1993a) Relationships between carbon isotope discrimination, water use efficiency and transpiration efficiency for dryland wheat. Australian Journal of Agricultural Research 44:1693-1711
Condon A, Richards R, Farquhar G (1993b) Relationships between carbon isotope discrimination, water use efficiency and transpiration efficiency for dryland wheat. Crop and Pasture Science 44:1693-1711
Condon A, Richards R, Rebetzke G, Farquhar G (2002a) Improving intrinsic water-use efficiency and crop yield. Crop Science 42:122-131
Condon A, Richards R, Rebetzke G, Farquhar G (2004a) Breeding for high water-use efficiency. Journal of Experimental Botany 55:2447-2460
Condon AG, Farquar G, Rebetzke GJ, Richards RA (2006) The application of carbon isotope discrimination in cereal improvement for water-limited environments. Drought adaptation in cereals:171-219
Condon AG, Richards RA, Rebetzke GJ, Farquhar GD (2002b) Improving intrinsic water-use efficiency and crop yield. Crop Sci 42:122-131
Condon AG, Richards RA, Rebetzke GJ, Farquhar GD (2004b) Breeding for high water-use efficiency. Journal of experimental botany 55:2447-2460
Consortium IWGS (2014) A chromosome-based draft sequence of the hexaploid bread wheat (Triticum aestivum) genome. Science 345:1251788
Cui Y, Wang M, Zhou H, Li M, Huang L, Yin X, Zhao G, Lin F, Xia X, Xu G (2016) OsSGL, a Novel DUF1645 Domain-Containing Protein, Confers Enhanced Drought Tolerance in Transgenic Rice and Arabidopsis. Frontiers in plant science 7

de Oliveira ED, Bramley H, Siddique KHM, Henty S, Berger J, Palta JA (2013) Can elevated CO2 combined with high temperature ameliorate the effect of terminal drought in wheat? Funct Plant Biol 40:160-171
Dhungana P, Eskridge KM, Baenziger PS, Campbell BT, Gill KS, Dweikat I (2007) Analysis of genotype-by-environment interaction in wheat using a structural equation model and chromosome substitution lines. Crop Sci 47:477-484
Diab AA, Kantety R, Ozturk N, Benscher D, Nachit M, Sorrells M (2008) Drought-inducible genes and differentially expressed sequence tags associated with components of drought tolerance in durum wheat
Diamant S, Eliahu N, Rosenthal D, Goloubinoff P (2001) Chemical chaperones regulate molecular chaperones in vitro and in cells under combined salt and heat stresses. Journal of Biological Chemistry 276:39586-39591
Díaz A, Zikhali M, Turner AS, Isaac P, Laurie DA (2012) Copy number variation affecting the Photoperiod-B1 and Vernalization-A1 genes is associated with altered flowering time in wheat (Triticum aestivum). PLoS One 7:e33234
Dietrich CR, Cui F, Packila ML, Li J, Ashlock DA, Nikolau BJ, Schnable PS (2002) Maize Mu transposons are targeted to the 5′ untranslated region of the gl8 gene and sequences flanking Mu target-site duplications exhibit nonrandom nucleotide composition throughout the genome. Genetics 160:697-716
Doležel J, Kubaláková M, Paux E, Bartoš J, Feuillet C (2007) Chromosome-based genomics in the cereals. Chromosome Research 15:51-66
Doležel J, Vrána J, Šafář J, Bartoš J, Kubaláková M, Šimková H (2012) Chromosomes in the flow to simplify genome analysis. Functional & integrative genomics 12:397-416
Dolferus R, Powell N, Xuemei J, Ravash R, Edlington J, Oliver S, Van Dongen J, Shiran B (2013) The physiology of reproductive-stage abiotic stress tolerance in cereals. Molecular Stress Physiology of Plants. Springer, pp 193-216
Dorion S, Lalonde S, Saini HS (1996) Induction of male sterility in wheat by meiotic-stage water deficit is preceded by a decline in invertase activity and changes in carbohydrate metabolism in anthers. Plant Physiol 111:137-145
Dreher K, Callis J (2007) Ubiquitin, hormones and biotic stress in plants. Annals of botany 99:787-822
Duan Y-H, Guo J, Ding K, Wang S-J, Zhang H, Dai X-W, Chen Y-Y, Govers F, Huang L-L, Kang Z-S (2011) Characterization of a wheat HSP70 gene and its expression in response to stripe rust infection and abiotic stresses. Molecular biology reports 38:301-307
Duveiller E, Singh RP, Nicol JM (2007) The challenges of maintaining wheat productivity: pests, diseases, and potential epidemics. Euphytica 157:417-430
Eagles HA, Cane K, Vallance N (2009) The flow of alleles of important photoperiod and vernalisation genes through Australian wheat. Crop and Pasture Science 60:646-657
Edae EA, Bowden RL, Poland J (2015) Application of population sequencing (POPSEQ) for ordering and imputing genotyping-by-sequencing markers in hexaploid wheat. G3: Genes, Genomes, Genetics 5:2547-2553
Edwards D, Wilcox S, Barrero RA, Fleury D, Cavanagh CR, Forrest KL, Hayden MJ, Moolhuijzen P, Keeble-Gagnere G, Bellgard MI, Lorenc MT, Shang CA, Baumann U, Taylor JM, Morell MK, Langridge P, Appels R, Fitzgerald A (2012) Bread matters: a national initiative to profile the genetic diversity of Australian wheat. Plant biotechnology journal 10:703-708
Edwards J (2012a) A genetic analysis of drought related traits in hexaploid wheat. PhD thesis. University of Adelaide, Adelaide, Australia (PhD thesis)
Ehdaie B, Alloush G, Madore M, Waines J (2006a) Genotypic variation for stem reserves and mobilization in wheat. Crop science 46:2093-2103
Ehdaie B, Alloush G, Waines J (2008) Genotypic variation in linear rate of grain growth and contribution of stem reserves to grain yield in wheat. Field Crops Research 106:34-43

Ehdaie B, Alloush GA, Madore MA, Waines JG (2006b) Genotypic variation for stem reserves and mobilization in wheat. Crop Science 46:2093-2103
Estrada-Campuzano G, Slafer GA, Miralles DJ (2012) Differences in yield, biomass and their components between triticale and wheat grown under contrasting water and nitrogen environments. Field Crops Res 128:167-179
Evans L (1993) Crop evolution, adaptation, and yield. Cambridge Univ. Press, Cambridge, UK. Crop evolution, adaptation, and yield Cambridge Univ Press, Cambridge, UK:-
Eversole K, Feuillet C, Mayer KFX, Rogers J (2014) Slicing the wheat genome. Science 345:285
FAOSTAT D (2013a) Food and agriculture organization of the United Nations. Statistical database.
FAOSTAT F (2013b) Agriculture Database. Available: htt p. faostat3 fao org/faostat-gateway/go/to/download/Q/*/E Accessed
FAOSTAT F (2015) Food and Agriculture Organization of the United Nations Statistics Division. Rome: FAO
Farooq M, Bramley H, Palta JA, Siddique KHM (2011) Heat stress in wheat during reproductive and grain-filling phases. Critical Reviews in Plant Sciences 30:491-507
Farooq M, Hussain M, Siddique KH (2014) Drought stress in wheat during flowering and grain-filling periods. Crit Rev Plant Sci 33:331-349
Farooq M, Wahid A, Kobayashi N, Fujita D, Basra S (2009) Plant drought stress: effects, mechanisms and management. Agronomy for sustainable development 29:185-212
Feuillet C, Eversole K (2007) Physical mapping of the wheat genome: a coordinated effort to lay the foundation for genome sequencing and develop tools for breeders. Israel Journal of Plant Sciences 55:307-313
Feuillet C, Langridge P, Waugh R (2008) Cereal breeding takes a walk on the wild side. Trends Genet 24:24-32
Feuillet C, Stein N, Rossini L, Praud S, Mayer K, Schulman A, Eversole K, Appels R (2012) Integrating cereal genomics to support innovation in the Triticeae. Functional & integrative genomics 12:573-583
Figlan S, Baloyi T, Hlongoane T, Terefe T, Shimelis H, Tsilo T (2017) Adult plant resistance of selected Kenyan wheat cultivars to leaf rust and stem rust diseases. Cereal Research Communications 45:68-82
Finn RD, Bateman A, Clements J, Coggill P, Eberhardt RY, Eddy SR, Heger A, Hetherington K, Holm L, Mistry J (2013) Pfam: the protein families database. Nucleic Acids Res 42:D222-D230
Fischer R (2007) Understanding the physiological basis of yield potential in wheat. The Journal of Agricultural Science 145:99
Fischer R, Rees D, Sayre K, Lu Z-M, Condon A, Saavedra AL (1998) Wheat yield progress associated with higher stomatal conductance and photosynthetic rate, and cooler canopies. Crop science 38:1467-1475
Fleury D, Jefferies S, Kuchel H, Langridge P (2010) Genetic and genomic tools to improve drought tolerance in wheat. Journal of experimental botany 61:3211-3222
Fleury D, Langridge P (2014) QTL and association mapping for plant abiotic stress tolerance. Plant Abiotic Stress, Second Edition:257-287
Flor HH (1971) Current status of the gene-for-gene concept. Annual review of phytopathology 9:275-296
Foulkes MJ, Scott RK, Sylvester-Bradley R (2002) The ability of wheat cultivars to withstand drought in UK conditions: formation of grain yield. The Journal of Agricultural Science 138:153-169
Francia E, Tacconi G, Crosatti C, Barabaschi D, Bulgarelli D, Dall'Aglio E, Valè G (2005) Marker assisted selection in crop plants. Plant Cell, Tissue and Organ Culture 82:317-342
Gahlaut V, Jaiswal V, Tyagi BS, Singh G, Sareen S, Balyan HS, Gupta PK (2017) QTL mapping for nine drought-responsive agronomic traits in bread wheat under irrigated and rain-fed environments. PloS One 12:e0182857

Garbus I, Romero JR, Valarik M, Vanžurová H, Karafiátová M, Cáccamo M, Doležel J, Tranquilli G, Helguera M, Echenique V (2015) Characterization of repetitive DNA landscape in wheat homeologous group 4 chromosomes. BMC Genomics 16:375
Gavran M (2012) Australian plantation statistics 2012 update. ABARES
Ghatak A, Chaturvedi P, Weckwerth W (2017) Cereal crop proteomics: Systemic analysis of crop drought stress responses towards marker-assisted selection breeding. Frontiers in Plant Science 8
Gill BS, Appels R, Botha-Oberholster A-M, Buell CR, Bennetzen JL, Chalhoub B, Chumley F, Dvořák J, Iwanaga M, Keller B (2004) A workshop report on wheat genome sequencing. Genetics 168:1087-1096
Giunta F, Motzo R, Pruneddu G (2003) Comparison of temperate cereals and grain legumes in a Mediterranean environment. Agricoltura mediterranea 133:234-248
Goff SA, Ricke D, Lan T-H, Presting G, Wang R, Dunn M, Glazebrook J, Sessions A, Oeller P, Varma H (2002) A draft sequence of the rice genome (Oryza sativa L. ssp. japonica). Science 296:92-100
GonzáLez CV, Ibarra SE, Piccoli PN, Botto JF, Boccalandro HE (2012) Phytochrome B increases drought tolerance by enhancing ABA sensitivity in Arabidopsis thaliana. Plant, Cell Environ 35:1958-1968
Grabowski PP, Evans J, Daum C, Deshpande S, Barry KW, Kennedy M, Ramstein G, Kaeppler SM, Buell CR, Jiang Y (2017) Genome-wide associations with flowering time in switchgrass using exome-capture sequencing data. New Phytol 213:154-169
Graziani M, Maccaferri M, Royo C, Salvatorelli F, Tuberosa R (2014) QTL dissection of yield components and morpho-physiological traits in a durum wheat elite population tested in contrasting thermo-pluviometric conditions. Crop and Pasture Science 65:80-95
Griffiths S, Sharp R, Foote TN, Bertin I, Wanous M, Reader S, Colas I, Moore G (2006) Molecular characterization of Ph1 as a major chromosome pairing locus in polyploid wheat. Nature 439:749-752
Griffiths S, Wingen L, Pietragalla J, Garcia G, Hasan A, Miralles D, Calderini DF, Ankleshwaria JB, Waite ML, Simmonds J (2015) Genetic dissection of grain size and grain number trade-offs in CIMMYT wheat germplasm. PloS one 10:e0118847
Gupta P, Langridge P, Mir R (2010) Marker-assisted wheat breeding: present status and future possibilities. Molecular Breeding 26:145-161
Gutiérrez-Rodríguez M, Reynolds MP, Escalante-Estrada JA, Rodríguez-González MT (2004) Association between canopy reflectance indices and yield and physiological traits in bread wheat under drought and well-irrigated conditions. Aust J Agric Res 55:1139-1147
Hayden MJ, Nguyen TM, Waterman A, McMichael GL, Chalmers KJ (2008) Application of multiplex-ready PCR for fluorescence-based SSR genotyping in barley and wheat. Molecular Breeding 21:271-281
He C, Holme J, Anthony J (2014) SNP genotyping: the KASP assay. Crop Breeding: Methods and Protocols:75-86
Hill CB, Taylor JD, Edwards J, Mather D, Bacic A, Langridge P, Roessner U (2013) Whole-Genome Mapping of Agronomic and Metabolic Traits to Identify Novel Quantitative Trait Loci in Bread Wheat Grown in a Water-Limited Environment1 [W][OA]
Hill CB, Taylor JD, Edwards J, Mather D, Langridge P, Bacic A, Roessner U (2015) Detection of QTL for metabolic and agronomic traits in wheat with adjustments for variation at genetic loci that affect plant phenology. Plant Science 233:143-154
Hoecker U, Xu Y, Quail PH (1998) SPA1: A new genetic locus involved in phytochrome A–specific signal transduction. The Plant Cell Online 10:19-33
Houshyar E, Grundmann P (2017) Environmental impacts of energy use in wheat tillage systems: A comparative life cycle assessment (LCA) study in Iran. Energy 122:11-24
Hui Z, ZhengBin Z, HongBo S, Ping X, Foulkes M (2008) Genetic correlation and path analysis of transpiration efficiency for wheat flag leaves. Environmental and experimental botany 64:128-134
Hunter-Ensor M, Ousley A, Sehgal A (1996) Regulation of the Drosophila protein timeless suggests a mechanism for resetting the circadian clock by light. Cell 84:677-685
Ingram J, Bartels D (1996) The molecular basis of dehydration tolerance in plants. Annu Rev Plant Biol 47:377-403

Innes P, Blackwell RD, Austin RB, Ford MA (1981) The effects of selection for number of ears on the yield and water economy of winter wheat. The Journal of Agricultural Science 97:523-532
International VSN (2013) GenStat for Windows, 16th Edn. VSN International Hemel Hempstead, UK
International Wheat Genome Sequencing C (2014) A chromosome-based draft sequence of the hexaploid bread wheat (Triticum aestivum) genome. Science 345:1251788
Izanloo A, Condon AG, Langridge P, Tester M, Schnurbusch T (2008) Different mechanisms of adaptation to cyclic water stress in two South Australian bread wheat cultivars. Journal of Experimental Botany 59:3327-3346
Ji X, Shiran B, Wan J, Lewis DC, Jenkins CL, Condon AG, Richards RA, Dolferus R (2010) Importance of pre-anthesis anther sink strength for maintenance of grain number during reproductive stage water stress in wheat. Plant, Cell & Environment 33:926-942
Jia J, Zhao S, Kong X, Li Y, Zhao G, He W, Appels R, Pfeifer M, Tao Y, Zhang X (2013) Aegilops tauschii draft genome sequence reveals a gene repertoire for wheat adaptation. Nature 496:91-95
Jiafeng J, Xin H, Ling L, Jiangang L, Hanliang S, Qilai X, Renhong Y, Yuanhua D (2014) Effect of cold plasma treatment on seed germination and growth of wheat. Plasma Science and Technology 16:54
Jones PD, New M, Parker DE, Martin S, Rigor IG (1999) Surface air temperature and its changes over the past 150 years. Reviews of Geophysics 37:173-199
Khan MM, Khan N (2017) Effect of Water Logging and Salinity Stress on Physiological and Biochemical Changes in Tolerant and Susceptible Varieties of Triticum aestivum L. Int J Curr Microbiol App Sci 6:975-981
Kimber Gv, Sears E (1987) Evolution in the genus Triticum and the origin of cultivated wheat. Wheat and wheat improvement:154-164
Kirby SH (1983) Rheology of the lithosphere. Reviews of Geophysics 21:1458-1487
Klahr A, Zimmermann G, Wenzel G, Mohler V (2007) Effects of environment, disease progress, plant height and heading date on the detection of QTLs for resistance to Fusarium head blight in an European winter wheat cross. Euphytica 154:17-28
Kleine T (2012) Arabidopsis thaliana mTERF proteins: evolution and functional classification. Frontiers in plant science 3
Kong X, Zhou S, Yin S, Zhao Z, Han Y, Wang W (2016) Stress-inducible expression of an F-box gene TaFBA1 from wheat enhanced the drought tolerance in transgenic tobacco plants without impacting growth and development. Frontiers in plant science 7
Kosina P, Reynolds M, Dixon J, Joshi A (2007) Stakeholder perception of wheat production constraints, capacity building needs, and research partnerships in developing countries. Euphytica 157:475-483
Kumar A, Singh ON, Mandal NP, Dwivedi JL, Variar M, Kikuchi S, Satoh K, Mauleon R, Dixit S, Leung H (2013) Genetic, physiological, and gene expression analyses reveal that multiple QTL enhance yield of rice mega-variety IR64 under drought
Kumar J, Mir R, Kumar N, Kumar A, Mohan A, Prabhu K, Balyan H, Gupta P (2010) Marker-assisted selection for pre-harvest sprouting tolerance and leaf rust resistance in bread wheat. Plant Breeding 129:617-621
Lagi M, Bar-Yam Y, Bertrand KZ, Bar-Yam Y (2011) The food crises: A quantitative model of food prices including speculators and ethanol conversion
Lagudah ES, Dubcovsky J, Powell W (2001) Wheat genomics. Plant Physiology and Biochemistry 39:335-344
Lanceras JC, Pantuwan G, Jongdee B, Toojinda T (2004) Quantitative trait loci associated with drought tolerance at reproductive stage in rice. Plant Physiology 135:384-399
Langridge P, Reynolds MP (2015) Genomic tools to assist breeding for drought tolerance. Current Opinion in Biotechnol 32:130-135
Lawes R, Huth N, Hochman Z (2016) Commercially available wheat cultivars are broadly adapted to location and time of sowing in Australia's grain zone. European Journal of Agronomy 77:38-46
Lee T, Hwang S, Kim CY, Shim H, Kim H, Ronald PC, Marcotte EM, Lee I (2017) WheatNet: A genome-scale functional network for hexaploid bread wheat, Triticum aestivum. Molecular Plant

Leroy P, Guilhot N, Sakai H, Bernard A, Choulet F, Theil S, Reboux S, Amano N, Flutre T, Pelegrin C (2012) TriAnnot: a versatile and high performance pipeline for the automated annotation of plant genomes. Frontiers in plant science 3
Levitt J (972) Responses of plants to encironmental stresses. cademic Press, New York, N.Y
Liang J, Zhang J, Cao X (2001) Grain sink strength may be related to the poor grain filling of indica-japonica rice (Oryza sativa) hybrids. Physiol Plant 112:470-477
Ling H-Q, Zhao S, Liu D, Wang J, Sun H, Zhang C, Fan H, Li D, Dong L, Tao Y (2013) Draft genome of the wheat A-genome progenitor Triticum urartu. Nature 496:87-90
Liu J, Zhang F, Zhou J, Chen F, Wang B, Xie X (2012) Phytochrome B control of total leaf area and stomatal density affects drought tolerance in rice. Plant Mol Biol 78:289-300
Lopes M, Reynolds M, Manes Y, Singh R, Crossa J, Braun H (2012) Genetic yield gains and changes in associated traits of CIMMYT spring bread wheat in a "historic" set representing 30 years of breeding. Crop Science 52:1123-1131
Lopes MS, Rebetzke GJ, Reynolds M (2014) Integration of phenotyping and genetic platforms for a better understanding of wheat performance under drought. J Exp Bot 65:6167-6177
Lopes MS, Reynolds MP (2011) Drought adaptive traits and wide adaptation in elite lines derived from resynthesized hexaploid wheat. Crop Science 51:1617-1626
Loss SP, Siddique KHM (1994) Morphological and physiological traits associated with wheat yield increases in Mediterranean environments. Advances in agronomy 52:229-276
Ludlow M, Muchow R (1990) A critical evaluation of traits for improving crop yields in water-limited environments. Advances in agronomy 43:107-153
Lukina EV, Stone ML, Raun WR (1999) Estimating vegetation coverage in wheat using digital images. J Plant Nutr 22:341-350
Luo M-C, Thomas C, You FM, Hsiao J, Ouyang S, Buell CR, Malandro M, McGuire PE, Anderson OD, Dvorak J (2003) High-throughput fingerprinting of bacterial artificial chromosomes using the snapshot labeling kit and sizing of restriction fragments by capillary electrophoresis. Genomics 82:378-389
Lutts S, Almansouri M, Kinet J-M (2004) Salinity and water stress have contrasting effects on the relationship between growth and cell viability during and after stress exposure in durum wheat callus. Plant Science 167:9-18
Maccaferri M, Ricci A, Salvi S, Milner SG, Noli E, Martelli PL, Casadio R, Akhunov E, Scalabrin S, Vendramin V (2015) A high-density, SNP-based consensus map of tetraploid wheat as a bridge to integrate durum and bread wheat genomics and breeding. Plant Biotechnol J 13:648-663
Maccaferri M, Sanguineti MC, Corneti S, Ortega JLA, Salem MB, Bort J, DeAmbrogio E, del Moral LFG, Demontis A, El-Ahmed A (2008) Quantitative trait loci for grain yield and adaptation of durum wheat (Triticum durum Desf.) across a wide range of water availability. Genetics 178:489-511
Maccaferri M, Sanguineti MC, Demontis A, El-Ahmed A, Garcia del Moral L, Maalouf F, Nachit M, Nserallah N, Ouabbou H, Rhouma S (2010) Association mapping in durum wheat grown across a broad range of water regimes. J Exp Bot 62:409-438
Maldonado CA, Zuñiga GE, Corcuera LJ, Alberdi M (1997) Effect of water stress on frost resistance of oat leaves. Environ Exp Bot 38:99-107
Mammadov J, Aggarwal R, Buyyarapu R, Kumpatla S (2012) SNP markers and their impact on plant breeding. International journal of plant genomics 2012
Maphosa L, Langridge P, Taylor H, Emebiri LC, Mather DE (2015) Genetic control of grain protein, dough rheology traits and loaf traits in a bread wheat population grown in three environments. Journal of Cereal Science 64:147-152
Maphosa L, Langridge P, Taylor H, Parent B, Emebiri LC, Kuchel H, Reynolds MP, Chalmers KJ, Okada A, Edwards J (2014) Genetic control of grain yield and grain physical characteristics in a bread wheat population grown under a range of environmental conditions. Theoretical and applied genetics 127:1607-1624
Marcotuli I, Gadaleta A, Mangini G, Signorile AM, Zacheo SA, Blanco A, Simeone R, Colasuonno P (2017) Development of a High-Density SNP-Based Linkage Map and Detection of QTL for β-Glucans,

Protein Content, Grain Yield per Spike and Heading Time in Durum Wheat. International journal of molecular sciences 18:1329
Mardis ER (2008) Next-generation DNA sequencing methods. Annu Rev Genomics Hum Genet 9:387-402
Martínez-Pérez E, Shaw P, Reader S, Aragón-Alcaide L, Miller T, Moore G (1999) Homologous chromosome pairing in wheat. Journal of cell science 112:1761-1769
Mascher M, Muehlbauer GJ, Rokhsar DS, Chapman J, Schmutz J, Barry K, Muñoz-Amatriaín M, Close TJ, Wise RP, Schulman AH (2013) Anchoring and ordering NGS contig assemblies by population sequencing (POPSEQ). The Plant Journal 76:718-727
Mason RE, Hays DB, Mondal S, Ibrahim AM, Basnet BR (2013) QTL for yield, yield components and canopy temperature depression in wheat under late sown field conditions. Euphytica 194:243-259
Mayer KF, Rogers J, Doležel J, Pozniak C, Eversole K, Feuillet C, Gill B, Friebe B, Lukaszewski AJ, Sourdille P (2014) A chromosome-based draft sequence of the hexaploid bread wheat (Triticum aestivum) genome. Science 345:1251788
Mayer KFX, Taudien S, Martis M, Šimková H, Suchánková P, Gundlach H, Wicker T, Petzold A, Felder M, Steuernagel B (2009) Gene content and virtual gene order of barley chromosome 1H. Plant Physiol 151:496-505
McIntyre CL, Mathews KL, Rattey A, Chapman SC, Drenth J, Ghaderi M, Reynolds M, Shorter R (2010) Molecular detection of genomic regions associated with grain yield and yield-related components in an elite bread wheat cross evaluated under irrigated and rainfed conditions. Theor Appl Genet 120:527-541
Meng L, Li H, Zhang L, Wang J (2015) QTL IciMapping: Integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. The Crop Journal 3:269-283
Merchuk-Ovnat L, Barak V, Fahima T, Ordon F, Lidzbarsky GA, Krugman T, Saranga Y (2016) Ancestral QTL alleles from wild emmer wheat improve drought resistance and productivity in modern wheat cultivars. Frontiers in plant science 7
Metzker ML (2010) Sequencing technologies—the next generation. Nature reviews genetics 11:31-46
Mewes H-W, Frishman D, Güldener U, Mannhaupt G, Mayer K, Mokrejs M, Morgenstern B, Münsterkötter M, Rudd S, Weil B (2002) MIPS: a database for genomes and protein sequences. Nucleic Acids Res 30:31-34
Millet EJ, Welcker C, Kruijer W, Negro S, Coupel-Ledru A, Nicolas SD, Laborde J, Bauland C, Praud S, Ranc N (2016) Genome-wide analysis of yield in Europe: allelic effects vary with drought and heat scenarios. Plant physiology 172:749-764
Mir R, Kumar N, Jaiswal V, Girdharwal N, Prasad M, Balyan H, Gupta P (2012a) Genetic dissection of grain weight in bread wheat through quantitative trait locus interval and association mapping. Mol Breed 29:963-972
Mir RR, Zaman-Allah M, Sreenivasulu N, Trethowan R, Varshney RK (2012b) Integrated genomics, physiology and breeding approaches for improving drought tolerance in crops. Theoretical and Applied Genetics 125:625-645
Miralles DJ, Slafer GA (1995) Yield, biomass and yield components in dwarf, semi-dwarf and tall isogenic lines of spring wheat under recommended and late sowing dates. Plant Breeding 114:392-396
Miralles DJ, Slafer GA (2007) Sink limitations to yield in wheat: how could it be reduced? Journal of Agricultural Science, 2007, vol 145, núm 2, p 139-149
Mitchell JH, Chapman SC, Rebetzke GJ, Bonnett DG, Fukai S (2012) Evaluation of a reduced-tillering (tin) gene in wheat lines grown across different production environments. Crop and Pasture Science 63:128-141
Moeller C, Evers JB, Rebetzke G (2014) Canopy architectural and physiological characterization of near-isogenic wheat lines differing in the tiller inhibition gene tin. Frontiers in plant science 5

Mofatto LS, de Araújo Carneiro F, Vieira NG, Duarte KE, Vidal RO, Alekcevetch JC, Cotta MG, Verdeil J-L, Lapeyre-Montes F, Lartaud M (2016) Identification of candidate genes for drought tolerance in coffee by high-throughput sequencing in the shoot apex of different Coffea arabica cultivars. BMC plant biology 16:94
Moffatt J, Sears R, Paulsen G (1990) Wheat high temperature tolerance during reproductive growth. I. Evaluation by chlorophyll fluorescence. Crop science 30:881-885
Monneveux P, Jing R, Misra SC (2012) Phenotyping for drought adaptation in wheat using physiological traits. Frontiers in physiology 3
Morgan JM (2000) Increases in grain yield of wheat by breeding for an osmoregulation gene: relationship to water supply and evaporative demand. Australian Journal Of Agricultural Research 51:971-978
Morran S, Eini O, Pyvovarenko T, Parent B, Singh R, Ismagul A, Eliby S, Shirley N, Langridge P, Lopato S (2011) Improvement of stress tolerance of wheat and barley by modulation of expression of DREB/CBF factors. Plant biotechnology journal 9:230-249
Mphande W, Nicolas ME, Seneweera S, Bahrami H (2016a) Dynamics and contribution of stem water-soluble carbohydrates to grain yield in two wheat lines contrasted under Drought and elevated CO2 conditions. J Plant Physi 214:1037-1058
Mphande W, Nicolas ME, Seneweera S, Bahrami H, Bbebe N (2016b) Dynamics and contribution of stem water-soluble carbohydrates to grain yield in two wheat lines contrasted under Drought and elevated CO2 conditions. J Plant Physi 214:1037-1058
Murray GM, Brennan JP (2009) Estimating disease losses to the Australian wheat industry. Australasian Plant Pathology 38:558-570
Muthamilarasan M, Prasad M (2014) An overview of wheat genome sequencing and its implications for crop improvement. Journal of genetics 93:619-622
Myers MP, Wager-Smith K, Wesley CS, Young MW, Sehgal A (1995) Positional cloning and sequence analysis of the Drosophila clock gene, timeless. SCIENCE-NEW YORK THEN WASHINGTON-:805-805
Nakhforoosh A, Bodewein T, Fiorani F, Bodner G (2016) Identification of water use strategies at early growth stages in durum wheat from shoot phenotyping and physiological measurements. Frontiers in plant science 7
Naruoka Y, Talbert L, Lanning S, Blake N, Martin J, Sherman J (2011) Identification of quantitative trait loci for productive tiller number and its relationship to agronomic traits in spring wheat. Theoretical and Applied Genetics 123:1043
Nawaz F, Ashraf MY, Ahmad R, Waraich EA (2013) Selenium (Se) seed priming induced growth and biochemical changes in wheat under water deficit conditions. Biol Trace Elem Res 151:284-293
Nevo E, Chen G (2010) Drought and salt tolerances in wild relatives for wheat and barley improvement. Plant, cell & environment 33:670-685
Nezhad KZ, Weber W, Röder M, Sharma S, Lohwasser U, Meyer R, Saal B, Börner A (2012) QTL analysis for thousand-grain weight under terminal drought stress in bread wheat (Triticum aestivum L.). Euphytica 186:127-138
Nie Z, Miller S, Moore G, Hackney B, Boschma S, Reed K, Mitchell M, Albertsen T, Clark S, Craig A (2008) Field evaluation of perennial grasses and herbs in southern Australia. 2. Persistence, root characteristics and summer activity. Australian Journal of Experimental Agriculture 48:424-435
NIU CF, Wei W, ZHOU QY, TIAN AG, HAO YJ, ZHANG WK, Ma B, Lin Q, ZHANG ZB, ZHANG JS (2012a) Wheat WRKY genes TaWRKY2 and TaWRKY19 regulate abiotic stress tolerance in transgenic Arabidopsis plants. Plant, cell & environment 35:1156-1170
Niu CF, Wei W, Zhou QY, Tian AG, Hao YJ, Zhang WK, Ma B, Lin Q, Zhang ZB, Zhang JS, Chen SY (2012b) Wheat WRKY genes TaWRKY2 and TaWRKY19 regulate abiotic stress tolerance in transgenic Arabidopsis plants. Plant Cell Environ 35:1156-1170
Nollen EA, Morimoto RI (2002) Chaperoning signaling pathways: molecular chaperones as stress-sensingheat shock'proteins. Journal of cell science 115:2809-2816

Noutoshi Y, Ito T, Seki M, Nakashita H, Yoshida S, Marco Y, Shirasu K, Shinozaki K (2005) A single amino acid insertion in the WRKY domain of the Arabidopsis TIR–NBS–LRR–WRKY-type disease resistance protein SLH1 (sensitive to low humidity 1) causes activation of defense responses and hypersensitive cell death. The Plant Journal 43:873-888
O'Leary B, O'Leary BM, Lee CP, Atkin OK, Cheng R, Brown TB, Harvey A (2017) Plant Physiology Preview. Published on June 14, 2017, as DOI: 10.1104/pp. 17.00610. synthesis 20:21
Ohki K (1984) Manganese deficiency and toxicity effects on growth, development, and nutrient composition in wheat. Agronomy Journal 76:213-218
Olivares-Villegas JJ, Reynolds MP, McDonald GK (2007) Drought-adaptive attributes in the Seri/Babax hexaploid wheat population. Functional Plant Biology 34:189-203
Onyemaobi I, Liu H, Siddique KH, Yan G (2016a) Both male and female malfunction contributes to yield reduction under water stress during meiosis in bread wheat. Frontiers in Plant Science 7:2071
Onyemaobi I, Liu H, Siddique KH, Yan G (2016b) Both male and female malfunction contributes to yield reduction under water stress during meiosis in bread wheat. Frontiers in plant science 7
Osman KA, Tang B, Wang Y, Chen J, Yu F, Li L, Han X, Zhang Z, Yan J, Zheng Y (2013) Dynamic QTL analysis and candidate gene mapping for waterlogging tolerance at maize seedling stage. PLoS One 8:e79305
Ovenden B, Milgate A, Lisle C, Wade LJ, Rebetzke GJ, Holland JB (2017) Selection for water-soluble carbohydrate accumulation and investigation of genetic× environment interactions in an elite wheat breeding population. Theor Appl Genet:1-17
Pallotta M, Graham R, Langridge P, Sparrow D, Barker S (2000) RFLP mapping of manganese efficiency in barley. Theor Appl Genet 101:1100-1108
Pallotta M, Schnurbusch T, Hayes J, Hay A, Baumann U, Paull J, Langridge P, Sutton T (2014) Molecular basis of adaptation to high soil boron in wheat landraces and elite cultivars. Nature
Panchuk II, Volkov RA, Schöffl F (2002) Heat stress-and heat shock transcription factor-dependent expression and activity of ascorbate peroxidase in Arabidopsis. Plant Physiology 129:838-853
Parent B, Bonneau J, Maphosa L, Kovalchuk A, Langridge P, Fleury D (2017) Quantifying wheat sensitivities to environmental constraints to dissect G x E in the field. Plant physiology:pp-00372
Parent B, Shahinnia F, Maphosa L, Berger B, Rabie H, Chalmers K, Kovalchuk A, Langridge P, Fleury D (2015) Combining field performance with controlled environment plant imaging to identify the genetic control of growth and transpiration underlying yield response to water-deficit stress in wheat. Journal of experimental botany 66:5481-5492
Parent B, Tardieu F (2012) Temperature responses of developmental processes have not been affected by breeding in different ecological areas for 17 crop species. New Phytologist 194:760-774
Páscoa P, Gouveia C, Russo A, Trigo R (2017) The role of drought on wheat yield interannual variability in the Iberian Peninsula from 1929 to 2012. International journal of biometeorology 61:439-451
Passioura J (1997) Drought and drought tolerance. Drought tolerance in higher plants: genetical, physiological and molecular biological analysis. Springer, pp 1-5
Passioura J (2002) Soil conditions and plant growth. Plant, cell & environment 25:311-318
Passioura J (2007) The drought environment: physical, biological and agricultural perspectives. Journal of experimental Botany 58:113-117
Passioura J (2012) Phenotyping for drought tolerance in grain crops: when is it useful to breeders? Functional Plant Biology 39:851-859
Patrick J (1972) Distribution of assimilate during stem elongation in wheat. Australian Journal of Biological Sciences 25:455-468
Paux E, Faure S, Choulet F, Roger D, Gauthier V, Martinant JP, Sourdille P, Balfourier F, Le Paslier MC, Chauveau A, Cakir M, Gandon B, Feuillet C (2010) Insertion site-based polymorphism markers open new perspectives for genome saturation and marker-assisted selection in wheat. Plant biotechnology journal 8:196-210

Paux E, Roger D, Badaeva E, Gay G, Bernard M, Sourdille P, Feuillet C (2006) Characterizing the composition and evolution of homoeologous genomes in hexaploid wheat through BAC-end sequencing on chromosome 3B. The Plant Journal 48:463-474
Peltonen-Sainio P, Kangas A, Salo Y, Jauhiainen L (2007) Grain number dominates grain weight in temperate cereal yield determination: Evidence based on 30 years of multi-location trials. Field crops research 100:179-188
Pepler S, Gooding M, Ford K, Ellis R (2005) A temporal limit to the association between flag leaf life extension by fungicides and wheat yields. European journal of agronomy 22:363-373
Perdomo JA, Capó-Bauçà S, Carmo-Silva E, Galmés J (2017) Rubisco and rubisco activase play an important role in the biochemical limitations of photosynthesis in rice, wheat, and maize under high temperature and water deficit. Frontiers in plant science 8:490
Petersen G, Seberg O, Yde M, Berthelsen K (2006) Phylogenetic relationships of< i> Triticum</i> and< i> Aegilops</i> and evidence for the origin of the A, B, and D genomes of common wheat (< i> Triticum aestivum</i>). Molecular phylogenetics and evolution 39:70-82
Philippe R, Choulet F, Paux E, van Oeveren J, Tang J, Wittenberg AH, Janssen A, van Eijk MJ, Stormo K, Alberti A (2012) Whole Genome Profiling provides a robust framework for physical mapping and sequencing in the highly complex and repetitive wheat genome. BMC genomics 13:47
Philippe R, Paux E, Bertin I, Sourdille P, Choulet F, Laugier C, Šimková H, Šafář J, Bellec A, Vautrin S (2013a) A high density physical map of chromosome 1BL supports evolutionary studies, map-based cloning and sequencing in wheat. Genome Biol 14:R64
Philippe R, Paux E, Bertin I, Sourdille P, Choulet F, Laugier C, Šimková H, Šafář J, Bellec A, Vautrin S (2013b) A high density physical map of chromosome 1BL supports evolutionary studies, map-based cloning and sequencing in wheat. Genome biology 14:R64
Pinto RS, Reynolds MP, Mathews KL, McIntyre CL, Olivares-Villegas J-J, Chapman SC (2010) Heat and drought adaptive QTL in a wheat population designed to minimize confounding agronomic effects. Theor Appl Genet 121:1001-1021
Pnueli L, Hallak-Herr E, Rozenberg M, Cohen M, Goloubinoff P, Kaplan A, Mittler R (2002) Molecular and biochemical mechanisms associated with dormancy and drought tolerance in the desert legume Retama raetam. The Plant Journal 31:319-330
Powell N, Ji X, Ravash R, Edlington J, Dolferus R (2012) Yield stability for cereals in a changing climate. Functional Plant Biology 39:539-552
Pradhan GP, Prasad PV, Fritz AK, Kirkham MB, Gill BS (2012) Effects of drought and high temperature stress on synthetic hexaploid wheat. Functional Plant Biology 39:190-198
Pradhan GP, Prasad PVV (2015) Evaluation of wheat chromosome translocation lines for high temperature stress tolerance at grain filling stage. PloS one 10:e0116620
Prasad PVV, Djanaguiraman M (2014) Response of floret fertility and individual grain weight of wheat to high temperature stress: sensitive stages and thresholds for temperature and duration. Functional Plant Biology 41:1261-1269
Qin Y, Wang M, Tian Y, He W, Han L, Xia G (2012) Over-expression of TaMYB33 encoding a novel wheat MYB transcription factor increases salt and drought tolerance in Arabidopsis. Molecular biology reports 39:7183-7192
Qiu Y, Yu D (2009) Over-expression of the stress-induced OsWRKY45 enhances disease resistance and drought tolerance in Arabidopsis. Environmental and experimental botany 65:35-47
Quarrie S, Pekic Quarrie S, Radosevic R, Rancic D, Kaminska A, Barnes J, Leverington M, Ceoloni C, Dodig D (2006a) Dissecting a wheat QTL for yield present in a range of environments: from the QTL to candidate genes. Journal of Experimental Botany 57:2627-2637
Quarrie S, Quarrie SP, Radosevic R, Rancic D, Kaminska A, Barnes J, Leverington M, Ceoloni C, Dodig D (2006b) Dissecting a wheat QTL for yield present in a range of environments: from the QTL to candidate genes. Journal of Experimental Botany 57:2627-2637
Quarrie S, Steed A, Calestani C, Semikhodskii A, Lebreton C, Chinoy C, Steele N, Pljevljakusić D, Waterman E, Weyen J (2005a) A high-density genetic map of hexaploid wheat (Triticum aestivum L.)

from the cross Chinese Spring× SQ1 and its use to compare QTLs for grain yield across a range of environments. Theor Appl Genet 110:865-880
Quarrie SA, Pekic Quarrie S, Radosevic R, Rancic D, Kaminska A, Barnes JD, Leverington M, Ceoloni C, Dodig D (2006c) Dissecting a wheat QTL for yield present in a range of environments: from the QTL to candidate genes. Journal of Experimental Botany 57:2627-2637
Quarrie SA, Steed A, Calestani C, Semikhodskii A, Lebreton C, Chinoy C, Steele N, Pljevljakusic D, Waterman E, Weyen J, Schondelmaier J, Habash DZ, Farmer P, Saker L, Clarkson DT, Abugalieva A, Yessimbekova M, Turuspekov Y, Abugalieva S, Tuberosa R, Sanguineti MC, Hollington PA, Aragues R, Royo A, Dodig D (2005b) A high-density genetic map of hexaploid wheat (*Triticum aestivum* L.) from the cross Chinese Spring X SQ1 and its use to compare QTLs for grain yield across a range of environments. Theor Appl Genet 110:865-880
Quarrie SA, Stojanović J, Pekić S (1999) Improving drought resistance in small-grained cereals: A case study, progress and prospects. Plant growth regulation 29:1-21
Quesada V, Sarmiento-Mañús R, González-Bayón R, Hricová A, Pérez-Marcos R, Graciá-Martínez E, Medina-Ruiz L, Leyva-Díaz E, Ponce MR, Micol JL (2011) Arabidopsis RUGOSA2 encodes an mTERF family member required for mitochondrion, chloroplast and leaf development. The Plant Journal 68:738-753
Quevillon E, Silventoinen V, Pillai S, Harte N, Mulder N, Apweiler R, Lopez R (2005) InterProScan: protein domains identifier. Nucleic Acids Res 33:W116-W120
Raats D, Frenkel Z, Krugman T, Dodek I, Sela H, Šimková H, Magni F, Cattonaro F, Vautrin S, Bergès H (2013) The physical map of wheat chromosome 1BS provides insights into its gene space organization and evolution. Genome biology 14:R138
Rahaie M, Xue G-P, Naghavi MR, Alizadeh H, Schenk PM (2010) A MYB gene from wheat (Triticum aestivum L.) is up-regulated during salt and drought stresses and differentially regulated between salt-tolerant and sensitive genotypes. Plant cell reports 29:835-844
Rasheed A, Wen W, Gao F, Zhai S, Jin H, Liu J, Guo Q, Zhang Y, Dreisigacker S, Xia X (2016) Development and validation of KASP assays for genes underpinning key economic traits in bread wheat. Theoretical and applied genetics 129:1843-1860
Raun WR, Solie JB, Johnson GV, Stone ML, Lukina EV, Thomason WE, Schepers JS (2001) In-season prediction of potential grain yield in winter wheat using canopy reflectance. Agron J 93:131-138
Rawson HM, Macpherson HG (2000) Irrigated wheat: managing your crop. Food and Agriculture Organization of the United Nations
Ray DK, Mueller ND, West PC, Foley JA (2013) Yield trends are insufficient to double global crop production by 2050. PLoS One 8:e66428
Rebetzke G, Condon A, Farquhar G, Appels R, Richards R (2008a) Quantitative trait loci for carbon isotope discrimination are repeatable across environments and wheat mapping populations. Theoretical and Applied Genetics 118:123-137
Rebetzke G, Condon A, Richards R, Farquhar G (2002a) Selection for reduced carbon isotope discrimination increases aerial biomass and grain yield of rainfed bread wheat. Crop Science 42:739-745
Rebetzke G, Condon AG, Richards R, Farquhar G (2002b) Selection for reduced carbon isotope discrimination increases aerial biomass and grain yield of rainfed bread wheat. Crop Science 42:739-745
Rebetzke G, Richards R, Condon A, Farquhar G (2006) Inheritance of carbon isotope discrimination in bread wheat (Triticum aestivum L.). Euphytica 150:97-106
Rebetzke GJ, Condon AG, Richards RA, Farquhar GD (2002c) Selection for reduced carbon isotope discrimination increases aerial biomass and grain yield of rainfed bread wheat. Crop Sci 42:739-745
Rebetzke GJ, Van Herwaarden AF, Jenkins C, Weiss M, Lewis D, Ruuska S, Tabe L, Fettell NA, Richards RA (2008b) Quantitative trait loci for water-soluble carbohydrates and associations with agronomic traits in wheat. Aust J Agric Res 59:891-905

Regan KL, Siddique KHM, Turner NC, Whan BR (1992) Potential for increasing early vigour and total biomass in spring wheat. II. Characteristics associated with early vigour. Aust J Agric Res 43:541-553
Reynolds JF, Bugmann H, Pitelka LF (2001) How much physiology is needed in forest gap models for simulating long-term vegetation response to global change? Challenges, limitations, and potentials. Climatic Change 51:541-557
Reynolds M, Dreccer F, Trethowan R (2007) Drought-adaptive traits derived from wheat wild relatives and landraces. Journal of experimental botany 58:177-186
Reynolds M, MUJEEB-KAZI A, Sawkins M (2005) Prospects for utilising plant-adaptive mechanisms to improve wheat and other crops in drought-and salinity-prone environments. Annals of Applied Biology 146:239-259
Reynolds M, Skovmand B, Trethowan R, Pfeiffer W (2000) Evaluating a conceptual model for drought tolerance. Molecular approaches for genetic improvement of cereals for stable production in water-limited environments:49-53
Reynolds M, Tuberosa R (2008) Translational research impacting on crop productivity in drought-prone environments. Current opinion in plant biology 11:171-179
Reynolds MP, Pask AJ, Hoppitt WJ, Sonder K, Sukumaran S, Molero G, Saint Pierre C, Payne T, Singh RP, Braun HJ (2017) Strategic crossing of biomass and harvest index—source and sink—achieves genetic gains in wheat. Euphytica 213:257
Reynolds MP, Singh RP, Ibrahim A, Ageeb OAA, Larque-Saavedra A, Quick JS (1998) Evaluating physiological traits to complement empirical selection for wheat in warm environments (Reprinted from Wheat: Prospects for global improvement, 1998). Euphytica 100:85-94
Ribaut J-M, Ragot M (2007) Marker-assisted selection to improve drought adaptation in maize: the backcross approach, perspectives, limitations, and alternatives. Journal of Experimental Botany 58:351-360
Richards G (1996a) Cultural tourism in Europe. Greg Richards
Richards JC (1992) Longman dictionary of language teaching and applied linguistics. Longman
Richards R (2000) Selectable traits to increase crop photosynthesis and yield of grain crops. Journal of Experimental Botany 51:447-458
Richards R, Passioura J (1989) A breeding program to reduce the diameter of the major xylem vessel in the seminal roots of wheat and its effect on grain yield in rain-fed environments. Crop and Pasture Science 40:943-950
Richards R, Rebetzke G, Condon A, Van Herwaarden A (2002a) Breeding opportunities for increasing the efficiency of water use and crop yield in temperate cereals. Crop science 42:111-121
Richards RA (1996b) Increasing the yield potential of wheat: manipulating sources and sinks. Increasing yield potential in wheat: breaking the barriers:134-149
Richards RA (2006) Physiological traits used in the breeding of new cultivars for water-scarce environments. Agricultural Water Management 80:197-211
Richards RA, Rebetzke GJ, Condon AG, Van Herwaarden AF (2002b) Breeding opportunities for increasing the efficiency of water use and crop yield in temperate cereals. Crop Sci 42:111-121
Rizhsky L, Liang H, Shuman J, Shulaev V, Davletova S, Mittler R (2004) When defense pathways collide. The response of Arabidopsis to a combination of drought and heat stress. Plant Physiol 134:1683-1696
Rosegrant M, Agcaoili M (2010) Global food demand, supply, and price prospects to 2010. International Food Policy Research Institute, Washington, DC USA
Rosegrant MW, Paisner MS, Meijer S, Witcover J (2001) 2020 Global food outlook: Trends, alternatives, and choices. Intl Food Policy Res Inst
Rousch JM, Bingham SE, Sommerfeld MR (2004) Protein expression during heat stress in thermo-intolerant and thermo-tolerant diatoms. J Exp Mar Biol Ecol 306:231-243
Rushton DL, Tripathi P, Rabara RC, Lin J, Ringler P, Boken AK, Langum TJ, Smidt L, Boomsma DD, Emme NJ (2012) WRKY transcription factors: key components in abscisic acid signalling. Plant biotechnology journal 10:2-11

Rushton PJ, Somssich IE, Ringler P, Shen QJ (2010) WRKY transcription factors. Trends in plant science 15:247-258
Rustenholz C, Choulet F, Laugier C, Šafář J, Šimková H, Doležel J, Magni F, Scalabrin S, Cattonaro F, Vautrin S (2011) A 3,000-loci transcription map of chromosome 3B unravels the structural and functional features of gene islands in hexaploid wheat. Plant physiology 157:1596-1608
Rustgi S, Shafqat MN, Kumar N, Baenziger PS, Ali ML, Dweikat I, Campbell BT, Gill KS (2013) Genetic dissection of yield and its component traits using high-density composite map of wheat chromosome 3A: bridging gaps between QTLs and underlying genes. PLoS One 8:e70526
Ruuska SA, Rebetzke GJ, van Herwaarden AF, Richards RA, Fettell NA, Tabe L, Jenkins CLD (2006) Genotypic variation in water-soluble carbohydrate accumulation in wheat. Funct Plant Biol 33:799-809
Šafář J, Šimková H, Kubaláková M, Číhalíková J, Suchánková P, Bartoš J, Doležel J (2010) Development of chromosome-specific BAC resources for genomics of bread wheat. Cytogenetic and genome research 129:211-223
Saint Pierre C, Crossa J, Manes Y, Reynolds MP (2010a) Gene action of canopy temperature in bread wheat under diverse environments. Theoretical and applied genetics 120:1107-1117
Saint Pierre C, Trethowan R, Reynolds M (2010b) Stem solidness and its relationship to water-soluble carbohydrates: association with wheat yield under water deficit. Functional Plant Biology 37:166-174
Sakuma Y, Maruyama K, Osakabe Y, Qin F, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2006) Functional analysis of an Arabidopsis transcription factor, DREB2A, involved in drought-responsive gene expression. The Plant Cell 18:1292-1309
Salvi S, Tuberosa R (2005a) How many genes underlie a primary QTL? Trends in Plant Science 10:297-304
Salvi S, Tuberosa R (2005b) How many genes underlie a primary QTL? Trends in Plant Science 6:297-304
Salvi S, Tuberosa R (2005c) To clone or not to clone plant QTLs: present and future challenges. Trends in Plant Science 10:297-304
Sarkar NK, Kundnani P, Grover A (2013) Functional analysis of Hsp70 superfamily proteins of rice (Oryza sativa). Cell Stress and Chaperones 18:427-437
Savin R, Slafer GA (1991) Shading effects on the yield of an Argentinian wheat cultivar. The Journal of Agricultural Science 116:1-7
Schnurbusch T, Paillard S, Schori A, Messmer M, Schachermayr G, Winzeler M, Keller B (2004) Dissection of quantitative and durable leaf rust resistance in Swiss winter wheat reveals a major resistance QTL in the Lr34 chromosomal region. Theoretical and Applied Genetics 108:477-484
Semagn K, Babu R, Hearne S, Olsen M (2014) Single nucleotide polymorphism genotyping using Kompetitive Allele Specific PCR (KASP): overview of the technology and its application in crop improvement. Mol Breed 33:1-14
Semenov MA, Shewry PR (2011) Modelling predicts that heat stress, not drought, will increase vulnerability of wheat in Europe. Scientific Reports 1
Shahbaz M, Muhammad S, Ahmad F, Anwar M, Sajjad M, Riaz M, Nabi G, Waqar MQ (2017) Efficacy of different herbicides for controlling narrow leave weeds in wheat crop at different locations under adaptive research zone Sheikhupura. Pure and Applied Biology.
Shahinnia F, Le Roy J, Laborde B, Sznajder B, Kalambettu P, Mahjourimajd S, Tilbrook J, Fleury D (2016) Genetic association of stomatal traits and yield in wheat grown in low rainfall environments. BMC Plant Biol 16:150
Shakiba MR, Ehdaie B, Madore MA, Waines JG (1996) Contribution of internode reserves to grain yield in a tall and semidwarf spring wheat [Triticum aestivum]. Journal of Genetics & Breeding (Italy)
Shamsudin NAA, Swamy BPM, Ratnam W, Cruz MTS, Raman A, Kumar A (2016) Marker assisted pyramiding of drought yield QTLs into a popular Malaysian rice cultivar, MR219. BMC genetics 17:30
Shao M, Tang X, Zhang Y, Li W (2006) City clusters in China: air and surface water pollution. Frontiers in Ecology and the Environment 4:353-361

Shavrukov Y, Baho M, Lopato S, Langridge P (2016) The TaDREB3 transgene transferred by conventional crossings to different genetic backgrounds of bread wheat improves drought tolerance. Plant biotechnology journal 14:313-322
Shewry P (2009) Wheat. Journal of experimental Botany 60:1537-1553
Shiferaw B, Smale M, Braun H-J, Duveiller E, Reynolds M, Muricho G (2013) Crops that feed the world 10. Past successes and future challenges to the role played by wheat in global food security. Food Security 5:291-317
Shirdelmoghanloo H, Taylor JD, Lohraseb I, Rabie H, Brien C, Timmins A, Martin P, Mather DE, Emebiri L, Collins NC (2016) A QTL on the short arm of wheat (Triticum aestivum L.) chromosome 3B affects the stability of grain weight in plants exposed to a brief heat shock early in grain filling. BMC plant biology 16:1
Shukla S, Singh K, Patil RV, Kadam S, Bharti S, Prasad P, Singh NK, Khanna-Chopra R (2015) Genomic regions associated with grain yield under drought stress in wheat (Triticum aestivum L.). Euphytica 203:449-467
Siddique K, Tennant D, Perry M, Belford R (1990) Water use and water use efficiency of old and modern wheat cultivars in a Mediterranean-type environment. Crop and Pasture Science 41:431-447
Simmons SR (1987) Growth, development, and physiology. Wheat and wheat improvement:77-113
Singh B, Singh A (2015) Fingerprinting and Gene Cloning. Marker-Assisted Plant Breeding: Principles and Practices. Springer, pp 341-365
Slafer GA (2003) Genetic basis of yield as viewed from a crop physiologist's perspective. Ann Appl Biol 142:117-128
Slafer GA, Andrade FH (1993) Physiological attributes related to the generation of grain yield in bread wheat cultivars released at different eras. Field Crops Res 31:351-367
Slafer GA, Araus JL, Richards RA (1999a) Physiological traits that increase the yield potential of wheat. Wheat: ecology and physiology of yield determination:379-415
Slafer GA, Araus JL, Richards RA (1999b) Physiological traits that increase the yield potential of wheat. In 'Wheat: ecology and physiology of yield determination'.(Eds EH Satorre, GA Slafer) pp. 379–415. Food Products Press: New York
Slafer GA, ARAUS JL, ROYO C, MORAL LFG (2005) Promising eco-physiological traits for genetic improvement of cereal yields in Mediterranean environments. Ann Appl Biol 146:61-70
Slafer GA, Calderini DF, Miralles DJ (1996) Yield components and compensation in wheat: opportunities for further increasing yield potential. Increasing yield potential in wheat: Breaking the Barriers:101-133
Slafer GA, Miralles DJ (1992) Green area duration during the grain filling period of an Argentine wheat cultivar as influenced by sowing date, temperature and sink strength. Journal of Agronomy and Crop Science 168:191-200
Slafer GA, Savin R (1994) Source—sink relationships and grain mass at different positions within the spike in wheat. Field Crops Res 37:39-49
Sloane D (1999) Early vigour: its role in enhancing the productivity of wheat grown in South Australia
Smiley RW, Nicol JM (2009) Nematodes which challenge global wheat production. Wheat Science and Trade BF Carver, ed Wiley-Blackwell, Ames, IA 10:171-187
Smith H (2000) Phytochromes and light signal perception by plants—an emerging synthesis. Nature 407:585-591
Snape J, Butterworth K, Whitechurch E, Worland A (2001) Waiting for fine times: genetics of flowering time in wheat. Euphytica 119:185-190
Sohail Q, Naheed H, Mohammadi R (2016) Breeding and Genetic Enhancement of Dryland Crops. Innovations in Dryland Agriculture. Springer, pp 257-296
Soriano JM, Malosetti M, Roselló M, Sorrells ME, Royo C (2017) Dissecting the old Mediterranean durum wheat genetic architecture for phenology, biomass and yield formation by association mapping and QTL meta-analysis. PloS one 12:e0178290

Soriano JM, Royo C (2015) Dissecting the genetic architecture of leaf rust resistance in wheat by QTL meta-analysis. Phytopathology 105:1585-1593
Specht RL (1973) Structure and functional response of ecosystems in the Mediterranean climate of Australia. Mediterranean Type Ecosystems. Springer, pp 113-120
Staňková H, Hastie AR, Chan S, Vrána J, Tulpová Z, Kubaláková M, Visendi P, Hayashi S, Luo M, Batley J (2016) BioNano genome mapping of individual chromosomes supports physical mapping and sequence assembly in complex plant genomes. Plant Biotechnol J 14:1523-1531
Su P-H, Li H-m (2008) Arabidopsis stromal 70-kD heat shock proteins are essential for plant development and important for thermotolerance of germinating seeds. Plant Physiology 146:1231-1241
Suchecki R, Watson-Haigh NS, Baumann U (2017) POTAGE: A Visualisation Tool for Speeding up Gene Discovery in Wheat. Scientific Reports 7:14315
Swamy BPM, Kumar A (2013) Genomics-based precision breeding approaches to improve drought tolerance in rice. Biotechnology advances 31:1308-1318
Swamy BPM, Vikram P, Dixit S, Ahmed HU, Kumar A (2011) Meta-analysis of grain yield QTL identified during agricultural drought in grasses showed consensus. BMC Genomics 12:319
Swindale L, Bidinger F (1981) The human consequences of drought and crop research priorities for their alleviation. The physiology and biochemistry of drought resistance in plants New York, Academic Press USA:1-13
Tahmasebi S, Heidari B, Pakniyat H, McIntyre CL (2016) Mapping QTLs associated with agronomic and physiological traits under terminal drought and heat stress conditions in wheat (Triticum aestivum L.). Genome 60:26-45
Taiz L, Zeiger E (2006) Stress physiology. Plant physiology 4
Tambussi EA, Nogués S, Araus JL (2005) Ear of durum wheat under water stress: water relations and photosynthetic metabolism. Planta 221:446-458
Tang Y, Liu M, Gao S, Zhang Z, Zhao X, Zhao C, Zhang F, Chen X (2012) Molecular characterization of novel TaNAC genes in wheat and overexpression of TaNAC2a confers drought tolerance in tobacco. Physiologia plantarum 144:210-224
Tardieu F (2011a) Any trait or trait-related allele can confer drought tolerance: just design the right drought scenario. Journal of Experimental Botany:err269
Tardieu F (2011b) Any trait or trait-related allele can confer drought tolerance: just design the right drought scenario. J Exp Bot 63:25-31
Teixeira EI, Fischer G, van Velthuizen H, Walter C, Ewert F (2013) Global hot-spots of heat stress on agricultural crops due to climate change. Agricultural and Forest Meteorology 170:206-215
Till BJ, Zerr T, Comai L, Henikoff S (2006) A protocol for TILLING and Ecotilling in plants and animals. Nature protocols 1:2465-2477
Tiwari C, Wallwork H, Kumar U, Dhari R, Arun B, Mishra V, Reynolds M, Joshi AK (2013) Molecular mapping of high temperature tolerance in bread wheat adapted to the Eastern Gangetic Plain region of India. Field Crops Research 154:201-210
Trethowan R, Reynolds M, Skovmand B, van Ginkel M (1998) The effect of glume pubescence on floret temperature in wheat. Agronomy Abstracts, p 161
Tricker PJ, ElHabti A, Schmidt J, Fleury D (2018) The physiological and genetic basis of combined drought and heat tolerance in wheat. Journal of experimental botany 69:3195-3210
Tuberosa R, Salvi S (2006) Genomics-based approaches to improve drought tolerance of crops. Trends in plant science 11:405-412
Tucker CJ (1979) Red and photographic infrared linear combinations for monitoring vegetation. Remote Sens Environ 8:127-150
Tuinstra MR, Ejeta G, Goldsbrough PB (1997) Heterogeneous inbred family (HIF) analysis: a method for developing near-isogenic lines that differ at quantitative trait loci. TAG Theoretical and Applied Genetics 95:1005-1011

Turner NC (1979) Drought resistance and adaptation to water deficits in crop plants. Stress Physiology in Crop Plants Eds H Mussel and RC Staples Wiley, New York:344-372
Tyagi S, Mir R, Kaur H, Chhuneja P, Ramesh B, Balyan H, Gupta P (2014) Marker-assisted pyramiding of eight QTLs/genes for seven different traits in common wheat (Triticum aestivum L.). Molecular Breeding 34:167-175
Uauy C (2017) Wheat genomics comes of age. Curr Opin Plant Biol 36:142-148
Ünsal-Kaçmaz K, Mullen TE, Kaufmann WK, Sancar A (2005) Coupling of human circadian and cell cycles by the timeless protein. Mol Cell Biol 25:3109-3116
Venkateswarlu B, Shanker AK, Shanker C, Maheswari M (2011) Crop stress and its management: perspectives and strategies. Springer Science & Business Media
Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, Yandell M, Evans CA, Holt RA (2001) The sequence of the human genome. science 291:1304-1351
Verma V, Foulkes M, Worland A, Sylvester-Bradley R, Caligari P, Snape J (2004) Mapping quantitative trait loci for flag leaf senescence as a yield determinant in winter wheat under optimal and drought-stressed environments. Euphytica 135:255-263
Wang A-y, Li Y, Zhang C-q (2012a) QTL mapping for stay-green in maize (Zea mays). Canadian Journal of Plant Science 92:249-256
Wang H-J, Wan A-R, Hsu C-M, Lee K-W, Yu S-M, Jauh G-Y (2007) Transcriptomic adaptations in rice suspension cells under sucrose starvation. Plant molecular biology 63:441-463
Wang L-N, Liu Y-F, Zhang Y-M, Fang R-X, Liu Q-L (2012b) The expression level of Rosa Terminal Flower 1 (RTFL1) is related with recurrent flowering in roses. Molecular biology reports 39:3737-3746
Wang P, Xing Y, Li Z, Yu S (2012c) Improving rice yield and quality by QTL pyramiding. Molecular Breeding 29:903-913
Wang S, Basten C, Zeng Z (2012d) Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC, 2010.
Wang S, Wong D, Forrest K, Allen A, Chao S, Huang BE, Maccaferri M, Salvi S, Milner SG, Cattivelli L (2014) Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array. Plant Biotechnology Journal 12:787-796
Wardlaw I, Wrigley C (1994) Heat tolerance in temperate cereals: an overview. Functional Plant Biology 21:695-703
Wehmeyer N, Vierling E (2000) The expression of small heat shock proteins in seeds responds to discrete developmental signals and suggests a general protective role in desiccation tolerance. Plant Physiol 122:1099-1108
Wellmer F, Alves-Ferreira M, Dubois A, Riechmann JL, Meyerowitz EM (2006) Genome-wide analysis of gene expression during early Arabidopsis flower development. PLoS genetics 2:e117
Wen F, Wu X, Li T, Jia M, Liu X, Li P, Zhou X, Ji X, Yue X (2017) Genome-wide survey of heat shock factors and heat shock protein 70s and their regulatory network under abiotic stresses in Brachypodium distachyon. PloS one 12:e0180352
Wicker T, Buchmann JP, Keller B (2010) Patching gaps in plant genomes results in gene movement and erosion of colinearity. Genome research 20:1229-1237
Wicker T, Schlagenhauf E, Graner A, Close TJ, Keller B, Stein N (2006) 454 sequencing put to the test using the complex genome of barley. BMC Genomics 7:275
Wilkinson PA, Winfield MO, Barker GL, Allen AM, Burridge A, Coghill JA, Edwards KJ (2012) CerealsDB 2.0: an integrated resource for plant breeders and scientists. BMC bioinformatics 13:219
Wong GK-S, Wang J, Tao L, Tan J, Zhang J, Passey DA, Yu J (2002) Compositional gradients in Gramineae genes. Genome Res 12:851-856
Worland A, Appendino M, Sayers E (1994) The distribution, in European winter wheats, of genes that influence ecoclimatic adaptability whilst determining photoperiodic insensitivity and plant height. euphytica 80:219-228

Worland A, Korzun V, Röder M, Ganal M, Law C (1998) Genetic analysis of the dwarfing gene Rht8 in wheat. Part II. The distribution and adaptive significance of allelic variants at the Rht8 locus of wheat as revealed by microsatellite screening. Theoretical and Applied Genetics 96:1110-1120
Worland AJ (1996) The influence of flowering time genes on environmental adaptability in European wheats. Euphytica 89:49-57
Wu Q-H, Chen Y-X, Zhou S-H, Fu L, Chen J-J, Xiao Y, Zhang D, Ouyang S-H, Zhao X-J, Cui Y (2015) High-density genetic linkage map construction and QTL mapping of grain shape and size in the wheat population Yanda1817× Beinong6. PloS one 10:e0118144
Wu X, Cheng R, Xue S, Kong Z, Wan H, Li G, Huang Y, Jia H, Jia J, Zhang L (2014) Precise mapping of a quantitative trait locus interval for spike length and grain weight in bread wheat (Triticum aestivum L.). Molecular breeding 33:129-138
Xiao B-Z, Chen X, Xiang C-B, Tang N, Zhang Q-F, Xiong L-Z (2009) Evaluation of seven function-known candidate genes for their effects on improving drought resistance of transgenic rice under field conditions. Molecular Plant 2:73-83
Xing Y, Zhang Q (2010) Genetic and molecular bases of rice yield. Annu Rev Plant Biol 61:421-442
Xue G-P, Sadat S, Drenth J, McIntyre CL (2013) The heat shock factor family from Triticum aestivum in response to heat and other major abiotic stresses and their role in regulation of heat shock protein genes. Journal of experimental botany 65:539-557
YAN HP, Kang MZ, De Reffye P, Dingkuhn M (2004) A dynamic, architectural plant model simulating resource-dependent growth. Annals of botany 93:591-602
Yan Y-S, Chen X-Y, Yang K, Sun Z-X, Fu Y-P, Zhang Y-M, Fang R-X (2011) Overexpression of an F-box protein gene reduces abiotic stress tolerance and promotes root growth in rice. Molecular plant 4:190-197
Yáñez A, Tapia G, Guerra F, del Pozo A (2017) Stem carbohydrate dynamics and expression of genes involved in fructan accumulation and remobilization during grain growth in wheat (Triticum aestivum L.) genotypes with contrasting tolerance to water stress. PloS one 12:e0177667
Yang J, Zhang J (2006) Grain filling of cereals under soil drying. New phytologist 169:223-236
Yang J, Zhang J, Wang Z, Zhu Q, Liu L (2001) Water deficit–induced senescence and its relationship to the remobilization of pre-stored carbon in wheat during grain filling. Agron J 93:196-206
Yang J, Zhang J, Wang Z, Zhu Q, Liu L (2003) Involvement of abscisic acid and cytokinins in the senescence and remobilization of carbon reserves in wheat subjected to water stress during grain filling. Plant, Cell Environ 26:1621-1631
Yang M, Dong J, Zhao W, Gao X (2016) Characterization of proteins involved in early stage of wheat grain development by iTRAQ. Journal of proteomics 136:157-166
Yang X, Tian Z, Sun L, Chen B, Tubiello FN, Xu Y (2017) The impacts of increased heat stress events on wheat yield under climate change in China. Climatic Change:1-16
Yoshida T, Mogami J, Yamaguchi-Shinozaki K (2014) ABA-dependent and ABA-independent signaling in response to osmotic stress in plants. Current opinion in plant biology 21:133-139
Yoshizawa-Sugata N, Masai H (2007) Human Tim/Timeless-interacting protein, Tipin, is required for efficient progression of S phase and DNA replication checkpoint. J Biol Chem 282:2729-2740
Yu J, Hu S, Wang J, Wong GK-S, Li S, Liu B, Deng Y, Dai L, Zhou Y, Zhang X (2002) A draft sequence of the rice genome (Oryza sativa L. ssp. indica). science 296:79-92
Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. Weed research 14:415-421
Zamani MM, Nabipour M, Meskarbashee M (2014) Stem water soluble carbohydrate remobilization in wheat under heat stress during the grain filling. International Journal of Agriculture and Biology 16:401-405
Zhang J, Chiodini R, Badr A, Zhang G (2011) The impact of next-generation sequencing on genomics. Journal of genetics and genomics 38:95-109

Zhang Ye, Xu W, Li Z, Deng XW, Wu W, Xue Y (2008) F-box protein DOR functions as a novel inhibitory factor for abscisic acid-induced stomatal closure under drought stress in Arabidopsis. Plant Physiology 148:2121-2133
Zhou S-M, Kong X-Z, Kang H-H, Sun X-D, Wang W (2015) The involvement of wheat F-box protein gene TaFBA1 in the oxidative stress tolerance of plants. Plos one 10:e0122117
Zhou S, Sun X, Yin S, Kong X, Zhou S, Xu Y, Luo Y, Wang W (2014) The role of the F-box gene TaFBA1 from wheat (Triticum aestivum L.) in drought tolerance. Plant physiology and biochemistry 84:213-223
Zhu J, Vos J, van der Werf W, van der Putten PE, Evers JB (2013) Early competition shapes maize whole-plant development in mixed stands. Journal of experimental botany 65:641-653
Zikhali M, Wingen LU, Leverington-Waite M, Specel S, Griffiths S (2017) The identification of new candidate genes Triticum aestivum FLOWERING LOCUS T3-B1 (TaFT3-B1) and TARGET OF EAT1 (TaTOE1-B1) controlling the short day photoperiod response in bread wheat. Plant, Cell & Environment
Zimin AV, Puiu D, Hall R, Kingan S, Clavijo BJ, Salzberg SL (2017a) The first near-complete assembly of the hexaploid bread wheat genome, Triticum aestivum. GigaScience
Zimin AV, Puiu D, Luo M-C, Zhu T, Koren S, Marçais G, Yorke JA, Dvořák J, Salzberg SL (2017b) Hybrid assembly of the large and highly repetitive genome of Aegilops tauschii, a progenitor of bread wheat, with the MaSuRCA mega-reads algorithm. Genome Res 27:787-792
Zivy M (1987) Genetic variability for heat shock proteins in common wheat. Theoretical and applied genetics 74:209-213
Ziyomo C, Bernardo R (2013) Drought tolerance in maize: Indirect selection through secondary traits versus genomewide selection. Crop Sci 53:1269-1275