GENETIC ANALYSIS OF A REGION ASSOCIATED WITH HEAT AND DROUGHT TOLERANCE ON CHROMOSOME 3B OF HEXAPLOID WHEAT (*Triticum aestivum*)

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Abbreviations

ABA	Abscisic acid
BAC	Bacterial artificial chromosome
BIC	Bayesian information criterion
Bp	Base pair
CIM	Composite interval mapping
cM	Centimorgan
CT	Canopy temperature
ctg	Contig
DArT	Diversity arrays technology
DH	Doubled haploid
FA	Factor analytic
GC	Growth chamber
ISBP	Insertion site-based polymorphism
Kb	Kilo base pair
LR	Leaf rolling
MARS	Marker assisted recurrent selection
MAS	Marker assisted selection
Mb	Mega base pair
MEIM	Multi-environment inferred marker
MET	Multi-environment trial
NDVI	Normalized difference vegetative index
NIL	Near isogenic line
PAV	Presence absence variation
PCR	Polymerase chain reaction
QTL	Quantitative trait locus
RIL	Recombinant inbred line
RING	Really interesting new gene
SIM	Single interval mapping
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeat
TGW	Thousand grain weight
TILLING	Targeting induced local lesions in genomes
WGAIM	Whole genome average interval mapping

Abstract

Drought and heat can occur during the growth cycle of crops and severely reduce yield. A QTL associated with yield and yield-related component was found in four wheat populations (*Triticum aestivum* L.) on the long arm of chromosome 3B "*qYDH.3BL*". The four populations were grown under various climatic conditions including drought, heat and combinations of both in a number of different areas (Australia and Mexico). Linear mixed models that partition and account for genetic and non-genetic or extraneous variation were used to detect loci in single-environment and/or multi-environment QTL analysis using ASReml-R. The alleles carried by RAC875, Excalibur or Drysdale improved grain yield by between 5% and 12.5%. Two doubled haploid populations (RAC875/Kukri and Excalibur/Kukri) and two recombinant inbred line populations (RAC875/Kukri and Gladius/Drysdale) were used to fine map *qYDH.3BL* and identify candidate gene(s). A total of thirty-seven molecular markers were mapped on one or both genetic maps of chromosome 3B enabling development of a consensus genetic map of the *qYDH.3BL* region. The markers were selected based on comparisons with a published "neighbour map" of chromosome 3B or designed using either BAC-end, contig or gene sequences from the chromosome 3B sequencing project; 3BSEQ http://urgi.versailles.inra.fr/ (cv. Chinese Spring).

A positional cloning approach was used to identify candidate genes for *qYDH.3BL*. Molecular markers from the targeted region were assigned to physical contigs by screening the chromosome 3B BAC library experimentally using PCR or *in silico* by sequence comparison. A total of eight physical contigs containing 85 genes, were anchored to the *qYDH.3BL* region. Public and in-house resources of wheat transcript sequences were used to restrict the gene list to 65 expressed genes.

Based on comparison of the 65 gene sequences to gene probes in a drought transcriptomic database, three genes were found to be differentially expressed between RAC875 and Kukri under drought conditions. Short genomic sequence reads (10× coverage) from each of the five parental lines (RAC875, Kukri, Excalibur, Gladius and Drysdale) were mapped against the 65 genes for polymorphism discovery. One gene exhibited sequence polymorphism between the drought tolerant parents (RAC875, Excalibur and Drysdale)

and the drought-sensitive parents (Gladius and Kukri). In addition, presence/absence polymorphisms were consistently detected throughout a region containing 12 genes, indicating that the drought tolerant parents may have a deletion (or alien introgression) in this region. Thus, in this work, we confirmed the genetic effect of *qYDH.3BL* in multiple environments and multiple populations, saturated the target region with new molecular markers and defined a preliminary list of genes located in the *qYDH.3BL* region and selected candidate genes for further investigations.

Résumé

Des épisodes climatiques de sècheresse et/ou de hautes températures peuvent engendrer de fortes pertes de rendement pour les cultures de céréales au champ. Un QTL associé au rendement et à ses composantes a été détecté dans quatre populations de blé (*Triticum aestivum* L.) sur le bras long du chromosome 3B « *qYDH.3BL* ».

Deux populations d'haploïdes doublés (RAC875/Kukri et Excalibur/Kukri) et deux populations de lignées recombinantes (RAC875/Kukri et Gladius/Drysdale) ont été utilisées pour cartographier finement le QTL, au même titre que l'identification de gènes candidats. Ces quatre populations ont été testées sous des conditions environnementales variées, incluant des périodes de sécheresse et/ou hautes températures en Australie et au Mexique. Des modèles statistiques mixtes et linéaires décomposant les variations génétiques et non-génétiques ont été utilisés pour la détection de QTL en considérant dans un premier temps chaque environnement unique, puis en considérant les environnements multiples dans une analyse commune. Les allèles de RAC875, Drysdale et Excalibur à ce locus ont montré une hausse du rendement de 5 à 12.5 % comparées à celles de Gladius ou Kukri.

Un total de trente-sept marqueurs moléculaires a été cartographié dans la région du QTL. Les marqueurs moléculaires ont été sélectionnés (i) par comparaison avec une carte génétique publiée du chromosome 3B, ou (ii) en désignant de nouveaux marqueurs moléculaires sur les séquences de BAC-end, de contig ou de gènes provenant du projet de séquençage du chromosome 3B (3BSEQ, http://urgi.versailles.inra.fr/, cv. Chinese Spring). Ceci a permis la construction d'une carte génétique consensus du locus *qYDH.3BL* .

A ce jour, aucun QTL associé au rendement ou ses composantes en condition de sécheresse et/ou de hautes températures n'a encore été cloné positionellement chez le blé tendre. Les marqueurs moléculaires de la région d'intérêt ont été utilisés pour cartographier physiquement des contigs, soit par PCR, soit par comparaison de séquences *in silico*. La région du QTL inclus un total de huit contigs physiques comprenant 85 gènes annotés.

L’utilisation de base de données de transcris biologiques publiques ou internes ont été utilisées pour détecter la présence de ces gènes, réduisant la liste à soixante-cinq gènes. Sur les contigs ayant une confiance élevée, aucun des vingt gènes n’a été exprimé différentiellement entre RAC875 et Kukri. Cependant, un gène présentant du polymorphisme dans sa séquence ainsi qu’une délétion/insertion d’un segment portant 12 gènes ont été découvert permettant ainsi de continuer à affiner la liste de gènes candidats. Les trois lignées parentales (RAC875, Drysdale et Excalibur) qui ont l’allèle liée au haut rendement ont le même haplotype pour ce gène, et la même délétion/insertion en opposition au deux autres lignées parentales Gladius et Kukri.

Ainsi, dans ce travail de thèse nous avons pu confirmer la présence d’un QTL répondant aux stresses environnementaux sur le chromosome 3BL dans différentes populations et différents environnements, identifier des gènes candidats sous le QTL, et proposer une liste restreinte pour de futures analyses sur la base de données d’expression et de polymorphismes entre les parents des populations de cartographie.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Julien Bonneau 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.

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Julien Bonneau Date: 19 of March 2013

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Chapter 1

Chapter 1: Introduction and literature review

I. Introduction

Cereal production will need to increase by 37% to meet the food security challenge by 2050 (Tester and Langridge 2010). Bread wheat (*Triticum aestivum* L.) is the staple food for 1/3 of the world population and is one of the most produced (tonnes) cereals in the world in third position after maize (*Zea mays* L.) and rice (*Oryza sativa* L.) (www.fao.org). World population is predicted to increase to 9 billion people in 2044 (http://www.census.gov/), challenges related to the decreased availability of agricultural land (Tilman et al. 2011), the decreased availability of water and vulnerability of many areas of the world to drought (Cosgrove and Cosgrove 2012), as well as predicted global warming (Battisti and Naylor 2009) all coincide to make it very challenging to address the requirements for 2050.

Increasing crop yield is one of the many challenges of modern agriculture. To be sustainable, crop production has to increase, through a combination of greater yield and yield stability no matter the environmental conditions, improved disease resistance and improve efficiencies of water use and nutrient uptake (Foley et al. 2011).

Advancements in plant science to improve yield in any of the three major crops hold the potential to contribute significantly to finding solutions to the food security challenges. The 'green revolution' pioneered by Norman Borlaug in wheat at CIMMYT was also based on scientific knowledge. He and his team produced rust resistant cultivars and identified semi-dwarf wheat (*Rht*) genes (Ellis et al. 2002) that had a tremendous impact on wheat yield improvement across the world. A second "green revolution" (Pingali 2012) is needed now to break the yield barrier and improve grain yield across the globe. With the advancement of genomics and genome sequencing, additional resources are becoming available to support this endeavour as illustrated in rice where the genome sequence (IRGSP 2005) is being used extensively for gene discovery to improve rice cultivars (Jiang et al. 2012). Sequencing the wheat genome is a scientific challenge that was launched 7 years ago by the International Wheat Genome Sequencing Consortium project (www.wheatgenome.org) with the idea of delivering high quality information to the breeders to accelerate mapping and gene discovery to improve wheat.

II. Breeding technology: one way to combat climate change

1. Environmental impact on crop productivity

Climate trends in the past 30 years have affected crop production. Global warming is in part involved in the observed worldwide stagnation of crop production and genetic progress has not compensated for that effect over the past 15 years (Lobell et al. 2011). According to recent reviews, heat has dramatically affected wheat production in India (Lobell et al. 2012) and heat events are predicted to rise in Europe, affecting wheat production (Semenov and Shewry 2011). Brisson et al. (2010) detailed climatic changes being responsible for year to year variability which was masked overall when considering total production in France. In 2012, drought caused a "natural disaster" in the USA, with maize yield reduced by 1.3 to 3.7 tonnes per hectare (Gilbert 2012). In Australia, heat is predicted to have an important negative impact on wheat production (Zheng et al. 2012). Thus, there is an urgent need to better understand the genetic and molecular mechanisms underlying heat and drought tolerance and implement breeding methodologies that will rapidly lead to new varieties improved for these traits while maintaining quality and resistance to diseases.

2. Opportunities and challenges in breeding

The challenge that farmers, breeders and scientists face is to increase crop yield across the world to meet the food demand for 2050 (Tester and Langridge 2010). New breeding technologies can contribute to wheat yield improvement and be applied to many traits such as tolerance to biotic or abiotic stresses, increase nutrient uptake and end-use quality. Several technologies have been developed to improve and accelerate the development of new cultivars for better crop production. One of them relies on the possibility to introduce new and more efficient genes or alleles into genetically modified plants. Although well established in many laboratories, this technology is encountering problems of popular acceptance in many countries which is reducing its application in plant breeding (Clive 2011). In allogamous plants such as maize and sunflower (*Helianthus annuus* L.), heterosis (hybrid vigour) has been exploited through the development of large hybrid programs. This technology would also be highly relevant for wheat but it has not been deployed at a large scale so far because of production cost and stability problems (Longin et al. 2012). In the 1990's the advent of molecular markers made it possible to apply marker assisted

selection (MAS) in breeding programs (Ribaut and Hoisington 1998), particularly for gene pyramiding and backcrossing and in marker-assisted recurrent selection (MARS). More recently, genomic selection (GS), which was initially developed and applied in animal breeding (Meuwissen 2007) has been explored as a new direction in plant breeding. Genomic selection is a form of marker-assisted selection in which genetic markers covering the whole genome are used to predict the breeding value of an individual. It relies on statistical analysis of a training population that is genotyped and phenotyped (Nakaya and Isobe 2012).

3. QTL analysis, a widely used method to detect genomic regions affecting traits of interest

The identification of polygenic effects (QTLs) is the first step in identifying genetic regions contributing to a specific trait. The general process consists of (1) identifying a phenotype (agronomic, stress response, metabolite expression level etc…) that is related to the target trait, e.g. the response of a plant to unfavourable conditions (biotic or abiotic stresses), (2) selecting lines from germplasm collections that differ in the expression of the phenotype, (3) generating populations such as F_2, backcross derived, doubled haploid, recombinant inbred lines from crosses between the selected lines, (4) phenotyping these progenies for the selected traits and genotyping them using molecular markers to generate genetic maps, (5) combining data from the genotype and phenotype to conduct QTL analysis. QTL analysis can be performed using different computing software such as MultiQTL (http://www.multiqtl.com/), MapManager QTX (Manly et al. 2001), Cartographer (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm), GeneStat (http://www.vsni.co.uk). It is essential that the genetic maps are effectively curated to increase the accuracy of QTL detection (Lehmensiek et al. 2005). After QTL analysis, the final step is to validate the association between the genetic and phenotypic information to provide a solid foundation for the application of molecular markers in breeding and for future map base cloning projects.

4. Forward genetics to identify candidate genes

Forward genetics identifies genes responsible for a specific phenotype whereas reverse genetics identifies phenotypic responses to a known gene. QTL analysis (part of forward genetics) allows the detection of a genomic region which contains the gene(s) of interest.

Once the region is clearly defined, different methods are available for more detailed gene identification and characterization. In the absence of a physical map and/or genome sequence information, the conservation between genomes (synteny) of related species can be used to identify regions of sequenced genomes carrying markers close to the QTL identified by genetic mapping in the target species. In grasses, synteny analyses started in the 1990's (Gale and Devos 1998; Moore et al. 1995) with RFLP markers and showed a relatively good level of conservation between the genomes of rice, wheat and other cereals. With the sequencing of grass genomes including rice (IRGSP 2005), *Brachypodium* (IBI 2010); maize (Schnable et al. 2009), sorghum (*Sorghum bicolor* L.) (Paterson et al. 2009), and foxtail millet (*Setaria italica* L.) (Zhang et al. 2012), it became easier to directly access the sequence of the syntenic regions and develop markers or identify candidate genes. In wheat, positional cloning combined with synteny has been used to identify a number of genes. However until recently, this was extremely challenging due to the lack of a reference genome sequence and physical maps therefore requiring long and laborious chromosome walking. So far, fewer than a dozen genes are now positionally cloned in wheat (Krattinger et al. 2009). Most have a clear phenotype, i.e. a trait relatively easy to measure. These include *VRN1*, *VRN2* and *VRN3* (phenology associated with vernalization); *Lr1*, *Lr10*, *Lr21*, *Pm3* (fungal disease resistance), *Ph1* (control of chromosome pairing); *Gpc-B1* (nutrient content and senescence) and *Q* (threshing and spike morphology). Other traits such as yield or responses to abiotic stresses or a combination of both are far more complex and involve multiple genes each having a small effect on the phenotype.

5. Grain yield: a complex but valuable trait

Grain yield is the final result of a cereal life cycle. During the growth cycle, plants are confronted with biotic and abiotic stresses such as pathogens, water deficit, and extremes temperature (frost and heat) that affect yield. Farmers, breeders and scientists have over decades selected wheat varieties more tolerant, more resistant and with high yield and quality. Several genes are known to contribute to yield increase; they are mainly related to plant growth and development (phenology) (*Vrn* Zhang et al. 2008), plant height (*Rht*, Ellis et al. 2007; Ellis et al. 2002) and photoperiod sensitivity (*Ppd*, Diaz et al. 2012; Wilhelm et al. 2009; Beales et al. 2007). These genes are deployed and used across the world to benefit wheat production. However, many other genes could contribute to yield improvement. QTL detection and positional cloning of unrevealed genes can still

contribute to improve grain yield in wheat. Grain yield is the most important agronomic trait but also genetically complex (Cooper et al. 2009; Holland 2007) controlled by many genes that interact with each other but also with the environment in so-called "genotype by environment" interactions. Since 2007, at least eighteen published studies shown QTL associated with yield and yield-related components in wheat (*Triticum aestivum* and *Triticum turgidum* subsp. *durum*) under heat and/or drought environmental conditions (Appendix 1.1). However, none of these QTL have been positionally cloned and the molecular basis of their action remains unknown.

6. Progress in methods for genotyping and phenotyping

Because of the size (17 Gb), complexity (hexaploid, 2n = 6x = 42) and repetitive nature (> 80% transposable elements) of the wheat genome, progress in sequencing and genotyping has been slow. However, in the past decade, tremendous achievements have been made through large international initiatives that take advantage of developments in sequencing technologies. The development of new high throughput genotyping platforms such as the wheat 9K iSelect SNP assay http://wheat.pw.usda.gov/ggpages/9K_assay_available.html) has dramatically improved the construction of dense genetic maps that can be used for QTL analysis. Similarly, phenotyping is also difficult to achieve at large scales. New phenotyping platforms are now available for high throughput phenotyping in controlled conditions to partially tackle the issue, generating large amounts of phenotypic data (Berger et al. 2012). Progress is also underway to achieve more efficient and precise phenotyping .

7. Adapted statistical tools

To perform QTL analysis using high throughput platforms, good statistical models that embrace large data sets and also perform appropriate analysis are required. Van Eeuwijk et al. (2010a) have described a list of statistical models and analytic procedures to analyse large genotypic datasets. To analyse data from a population tested in multiple environments multi-environment trial (MET) analysis is a powerful and appropriate method for estimating the genetic effects of the genotypes at each site (Smith et al. 2001; Smith et al. 2005). MET uses a linear mixed model framework that parsimoniously models the variance-covariance structure for the genotype by environment interaction, taking into account extraneous design or spatial variation that might be present in each environment.

These models have been adapted in various ways to incorporate QTL detection and estimation (Mathews et al. 2008; Boer et al. 2007; Malosetti et al. 2011; van Eeuwijk et al. 2010b; Malosetti et al. 2007; Malosetti et al. 2008). One common multi-environment QTL analysis approach involves a two-step MET procedure (Mathews et al. 2008). In the first step, each trial is analysed independently using a single site linear mixed model (Glimour et al. 1995) where extraneous variation is captured using appropriate random effects and correlation structures. At this stage plant genotypes are fitted as fixed effects. In the second step, the best linear unbiased estimates (BLUEs) of the genotypes across environments are then combined and used as a response (usually with an appropriate weight for each genotype) in a MET analysis. QTL are then determined by incorporating genetic marker information, usually one marker or interval at a time, into the MET as a simple fixed effect and performing Single Interval Mapping (SIM) or Composite Interval Mapping (CIM). Many software packages can perform multi-environment QTL analysis (QTLNetwork (Yang et al. 2008), Multi-QTL software available at http://www.multiqtl.com (Haifa, Israel) or GenStat software available at http://www.vsni.co.uk/software/genstat). Unfortunately, most are limited in their flexibility. Multi-QTL and QTLNetwork can only perform the second stage of the two-stage procedure, thus requiring the BLUEs of the genotypes in advance. In addition, they do not allow complex structures for the variance-covariance matrix of the genotype by environment interaction and do not have the ability to incorporate weights for a more appropriate analysis. In contrast, GenStat can perform both stages of the analysis with the correct use of weights into the second stage as well as attempt to provide the best modelling strategy for the genotype by environment interaction. Unfortunately, all packages use a piecemeal CIM approach to QTL analysis in the second stage that neglects background variation of all the other markers on the same or other linkage groups and inevitably requires the calculation of a threshold for the induced multiple testing problem.

III. Physiology, yield improvement under heat and drought

Understanding the physiology of plants under abiotic stresses can lead to a better understanding of the complex genetic control of grain yield. Grain yield is optimal when the environmental conditions including soil moisture, light, temperature, wind, UV, humidity and so on are favourable for the plant. The optimal temperature for wheat was reported to be between 18 to 24°C (Stone and Nicolas 1994). Any parameters that differ

from the optimal conditions for growth will affect physiological and biochemical processes of the plant and thereby the genetic responses. From a plant physiology perspective there are two relevant mechanisms for wheat improvement under water deficit and/or high temperature. These are tolerance defined as the ability of the plant to withstand the stress and avoidance defined as the ability to prevent exposure to the stress (Barnabas et al. 2008). Plants are sessile and have therefore developed biochemical and physiological mechanisms to respond to environmental changes either at the morphological level, through photosynthetic / stomatal conductance efficiency or by molecular signal suppression/activation turning on leaf senescence, seed abortion and early desiccation.

Avoidance of high temperature stress is explained by a cascade of physiological phenomena. Under higher temperatures, the plant will increase water uptake and opens its stomata increasing transpiration rate. Canopy temperature is a good empirical measure for genotype selection in warm environments (Reynolds et al. 1998). Tolerance of high temperature is variable between genotypes (Kumar et al. 2012) where tolerance could be explained as maintenance of functional metabolism and continuity of growth.

Avoidance mechanism in water-limited environment is characterized by limiting water loss during the crop cycle including stomata closure, and a reduction of leaf area development, adaptive processes to reduce transpiration and senescence. The plant can also avoid drought by maximizing water uptake which would include developing a deeper root system and/or a more efficient water uptake. Tolerance of water deficit may include efficient osmotic adjustment with thicker cell walls or small cells (Barnabas et al. 2008). Drought tolerance might also be associated with an efficient Reactive Oxygen Species (ROS) scavenging system.

Drought is often accompanied by heat, and heat often generates soil water evaporation which triggers drought. Antagonistically elevated temperatures enhance stomata aperture whereas drought induces stomatal closure. In the US, the damage from the combination of both stresses was studied by Mittler (2006). During the period from 1980 to 2004 drought stress cost on its own (no heat events) was evaluated at $20 billion, whereas the cost in damages reached $120 billion when both stresses were considered simultaneously.

1. Transpiration is affected by abiotic stresses

Grain yield is the result of carbon assimilation through photosynthesis and transpiration and subsequent accumulation of matter in the grain. These mechanisms are complex and involve a large number of biochemical pathways. Efficient transpiration and photosynthesis enable optimal biomass production therefore grain yield. When the plant is under unfavourable conditions such as high temperature many enzyme function within biochemical pathways will be affected due to temperatures above their optimum. Under water deficit the transpiration mechanism will be disrupted and this will trigger dysfunction of metabolic pathways affecting grain yield quantity and quality.

Transpiration corresponds to the mechanical flux of water involving specific cells (mesophyll cells, stomata) regulated by biophysical and biochemical pathways and controlled by a large number of genes. The water status of the plant is a good indicator of the stress level that the plant encounters. Measurement of water status can and measured directly or indirectly to understand the intrinsic responses in the plant. Stomatal conductance to water vapour, canopy temperature (CT) and leaf water potential (an essential measurement to identify the stress level applied to the plant especially in the field) are the most common measurements. A recent publication by Rebetzke (2012) identified QTL related to canopy temperature and genetic association with stomatal conductance under well-watered conditions. It is believed that CT and stomatal conductance are both integrated within a plant-environment system. However in the literature stomatal conductance was reported to be correlated with CT or CTD (canopy temperature depression) (Amani et al. 1996; Pinter et al. 1990) or uncorrelated (Giunta et al. 2008).

2. Physiological responses and genetics

Canopy temperature is reported to be a reliable measurement for yield prediction under different environmental conditions such as heat and/or drought with different water regimes. Fluctuation in canopy temperature as a response to heat and drought indicates that the plant can maintain its transpiration by cooling leaf temperature thereby resulting in more sustainable development under stress and yield gain/maintenance. Measurements depend on weather conditions, plant architecture (height and tiller number) and plant establishment (spacing between plants) (Royo et al. 2002; Winter et al. 1988). CT measurment is an easy and fast selection method for breeding selection in the field

(Reynolds 2012). QTL analyses performed in wheat using CT measurement are promising and several studies have reported co-localisation between QTL associated with yield and with canopy temperature (Bennett et al. 2012c; Olivares-Villegas et al. 2007; Pinto et al. 2010). The positive or negative correlations of such traits complement the arguments pointed out by Tardieu (2012) that a gene/QTL/allele associated with any agronomic trait or related trait could be beneficial in extreme drought and no longer beneficial in moderate drought. Therefore, knowing what kind of stress was applied on the plants is important. Environmental characteristics should include soil composition and depth, temperature, water deficit, light intensity, CO_2 concentration, and the presence or absence of other stresses (biotic stresses).

3. What allele for what environment?

The identification of a genetic region associated with agronomic traits is sometimes controversial especially when genotype by environment interactions occur (Cooper and DeLacy 1994; Cooper et al. 2009). Minor genetic regions (QTL) are rarely consistent and reliable across environments. They are often involved in different physiological and biochemical mechanisms that are interrelated. For this reason, a locus may confer an improvement in one specific environment and be irrelevant or even have a negative effect in other environments. Malosetti et al. (2008) described QTL associated with agronomic traits (yield, ear number and anthesis-silking interval) where allele effects were positive, negative or non-significant depending of the environment. From a theoretical point of view, Chenu et al. (2009) using statistical models in multi-environment analysis, demonstrated the opposite effect of QTL for leaf and silk elongation depending on the environment. This information indicates the complexity of genetic interactions and environmental dependencies (Cooper et al. 2009). However, if a positive effect of an allele is identified within a target population environment it should still be possible to use it as a guide to breeders for selection of consistent wheat varieties that perform better in environment clusters (Chenu et al. 2011).

4. Genes identified for abiotic stresses responses

Plants respond to abiotic stresses such as high temperature and/or drought by inducing cascades of genes involved in complex regulatory and functional processes. Abscisic acid (ABA) signalling components are well-known to be involved in responses to abiotic

stresses, but interconnectivities within the ABA network remain unresolved (Cutler et al. 2010). ABA is one of the most important phytohormones involved in biotic and abiotic stress signalling, interacting synergistically or antagonistically (Atkinson and Urwin 2012). Many genes are induced when abiotic stresses occur. Key genes involved in ABA enzyme biosynthesis (Nambara and Marion-Poll 2005) and other molecules involved in stress responses such as protein kinases/ protein phosphatases (Zhu 2002) *ABI1* and *ABI2* (Merlot et al. 2001), transcription factors (Nakashima et al. 2009) and molecules involved in ubiquitination processes (Lyzenga and Stone 2012) constitute a large and complex network which can enhance or reduce tolerance to biotic or abiotic stresses.

Transcription factors (TFs) are also major gene families regulating gene networks in response to abiotic stresses. Nakashima et al. (2009) dissected the complexity of the regulation network and genes induced by abiotic stresses by comparison of Arabidopsis (*Arabidopsis thaliana* L.) and grasses. A short list of the most important TFs such as NAC (Jensen et al. 2010), DREB (Agarwal et al. 2006), AREB (Fujita et al. 2011) improve the tolerance to abiotic stresses in plant as well as other TF such as C2H2 zinc fingers, WRKY (Rushton et al. 2012) and MYB (Todaka et al. 2012). The most recent research on abiotic tolerance identified *TaNAC2* (Mao et al. 2012), *TaWRKY2* and *TaWRKY19* (Niu et al. 2012) from wheat to confer tolerance to abiotic stress in Arabidopsis.

IV. A QTL on chromosome 3B conferring yield improvement under heat and drought

1. A QTL reported previously

Several QTL on wheat chromosome 3BL contribute to yield improvement under heat and/or drought environments. A genetic region has been first found by Börner et al. (2002) and associated with thousand grain weight where the field trials were conducted under conventional environment conditions in Germany. In one durum wheat population under moderate drought, the same genetic region was found and associated with yield in low-yielding environment (Maccaferri et al. 2008). Most recently the same genetic region was reported to be associated with yield and yield-related component in various studies on bread and durum wheat (Bennett et al. 2012b; Bennett et al. 2012c; Golabadi et al. 2011; McIntyre et al. 2010; Pinto et al. 2010; Wu et al. 2012). Interestingly this region was also reported to be associated with canopy temperature (Pinto et al. 2010; Bennett et al. 2012c).

In this latter study, a QTL called *qYDH3BL* in this thesis, was reported to be responsible for 22% of the genetic variation under multi-environment analysis (heat, drought and well-watered). To get a better understanding of the genetic responses to heat and drought, we were interested in this PhD work in addressing the following questions by using forward genetics (Fleury et al. 2010): Could QTL analysis coupled with positional cloning by using multi-environment analysis and the latest sequencing information help to identify candidate genes for heat and drought tolerance within 3 years of a PhD project? If so, what kind of gene could be related to (1) different physiological mechanisms (transpiration, water-uptake or photosynthesis), (2) agronomic traits such as yield and yield components and (3) any biochemical and signalling pathways already identified to respond to abiotic stresses.

2. The three major components for a successful story

a. Plant material presenting variation

Five parental lines RAC875, Kukri, Excalibur, Gladius and Drysdale, were selected to perform genetic studies of heat and drought tolerance in Australia. Gladius and Drysdale were both reported as drought tolerant (Pers. Comm. Peter Langridge), no information was given on the tolerance to heat for Drysdale. The five lines were used to generate populations including doubled haploid (DH) and recombinant inbred (RI) lines for QTL mapping and gene discovery. Five populations were generated: four originated from a DH (250 to 350 lines) and a RI (3000 lines) population that were developed after crossing RAC875x Kukri and Excalibur x Kukri. A fifth population of 5000 RI lines was produced from a Gladius x Drysdale cross. Three of the parental lines (RAC875, Excalibur and Kukri) were studied in details for their response to drought (Izanloo et al. 2008), where RAC875 and Excalibur showed an higher tolerance to cyclic drought than Kukri. The two DH populations were used for QTL detection under drought and heat conditions. QTL analyses performed on these two populations have been reported in different papers (Bennett et al. 2012a; Bennett et al. 2012b; Bennett et al. 2012c; Edwards 2012; Izanloo 2008). The population of Gladius/Drysdale was created for the purpose of developing better cultivars for two different cropping regions in Australia (New South Wales, NSW and South Australia, SA) presenting different cropping environments (deeper versus shallow soil, Mediterranean versus Sub-tropical climate) with the potential of studying root system.

b. Appropriate statistical tools

Statistical analyses are an important part of QTL detection. To take into account the effect of individual population genetic structure, the whole genome average interval mapping WGAIM approach detailed by Verbyla et al. (2007) and implemented in the wgaim package http://CRAN.R-project.org/package=wgaim (Taylor and Verbyla 2011) written for the R statistical computing environment (R Development Core Team , 2012) was used as a starting point. This extended linear mixed model incorporates a whole genome approach to significantly detect and estimate significant QTL. The modelling of extraneous variation with additional complex modelling of the genotype by environment interaction and whole genome QTL by environment component occurs simultaneously, thus avoiding two stage analyses and cumbersome repeated genome scans, which is the main difference compare to the approaches detailed in part II-7 of this chapter.

c. The wheat genome sequence

The hexaploid wheat (*Triticum aestivum* L.) contains three homologous genomes (A, B and D) with seven chromosomes each and is 40 X the rice genome size. The wheat genome was estimated at 17 000 Mbp. Large genomes often contain repetitive elements and are estimated to constitute 80% of the genome in wheat. The International Wheat Genome Sequencing Consortium (IWGSC, www.wheatgenome.org) was created to obtain a reference sequence of the bread wheat genome to enhance the understanding of genome structure and function and help breeders and scientist to accelerate wheat improvement. The approach used to reduce the complexity of the analysis is a chromosome-chromosome arm based approach. Each chromosome arm represents between 200Mb and 1Gb and therefore enables analysis on a small scale without having to cope with the redundancy of information due to hexaploidy. Using flow cytometry and aneuploid lines, individual chromosome/chromosome arms can be sorted (for a review see (Doležel et al. 2007)) and the DNA can be used to construct BAC libraries and perform shotgun sequencing. The BAC libraries are used to build physical maps that then serve as foundations for sequencing. So far, wheat physical maps have been constructed using two main approaches: the SNaPShot technology (Luo et al. 2003; Paux et al. 2008) and whole genome profiling (Philippe et al. 2012). The first physical map of chromosome 3B was published by Paux et al. (2008). Since then, physical maps were produced for 10 other

chromosomes and recently all chromosomes have been shotgun sequenced to provide information about the gene content and enable *in silico* mapping (www.wheatgenome.org).

3. Objectives of the study

The QTL *qYDH3BL* has been located on the long arm of chromosome 3B and associated with reduced canopy temperature and increased yield under several environmental conditions including, water deficit and/or high temperature (Bennett et al. 2012c). This project aimed to isolate candidate gene(s) for *qYDH3BL* to better understand the molecular basis of heat and drought responses in *Triticum aestivum* for grain yield improvement. The key objectives were to (1) validate the QTL in a recombinant inbred line population, (2) increase the density of markers based on publicly available data as well as design new markers based on recent genomic sequence information from chromosome 3B, (3) fine map the QTL and (4) identify candidate genes underlying the QTL using the sequence annotation of chromosome 3B cv. Chinese Spring. Genetic resources were already available including genetic maps of the two double haploid populations RAC875/Kukri published by Bennett et al. (2012a) and Excalibur/Kukri by Edwards (2012), as well as genetic maps of two populations of Gladius/Drysdale (unpublished). In addition, data from several field trials was available. The ultimate goal is to obtain heat and drought tolerant plants by developing molecular based tools to select elite varieties that carry these specific gene(s) through marker-assisted selection in breeding programs.

Chapter 2

Chapter 2: Multi-environment analysis and improved mapping of a yield-related QTL on chromosome 3B of wheat

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The text, figures, tables and online resources presented in this chapter are exactly the same as the published version except for minor revisions such as the names of tables and figures. The online resources have been changed to appendices in this thesis.

The results published in this chapter were only a part of the data collected and analysed. According to the publication two experiments conducted in 2011 were included. Only thousand grain weight and grain yield were analysed in this chapter. However, several phenotypic data sets were collected. In the meantime, a second round of experiments including two treatments (heat and drought) was conducted in Mexico at the experimental station in Ciudad de Obregon (CIMMYT) in 2012 using a subset of the same material tested in 2011. All the data from these four experiments conducted in 2011 and 2012 were analysed and presented in the Appendix 2.6 for more details.

STATEMENT OF AUTHORSHIP

BONNEAU, J. (Candidate)

Phenotyping and genotyping of population, interpretation of results, planning the experiment conducted in 2009, wrote the manuscript and acted as corresponding author

Certification that the statement of contribution is accurate

Signed
…….Date……
…

TAYLOR, J.

Performed statistical analysis on all data and provided critical evaluation of the manuscript

Certification that the statement of contribution is accurate

Signed
…….Date……
…

PARENT, B

Supervised development of work, planning the experiment conducted in 2009, helped with data interpretation and manuscript evaluation

Certification that the statement of contribution is accurate and permission is given for the inclusion of the paper in the thesis

Signed
…….Date……
…

BENNETT, D

Provided the data of the DH populations for experiments conducted in 2007 and 2008 in Australia and Mexico and provided critical evaluation of the manuscript.

Certification that the statement of contribution is accurate and permission is given for the inclusion of the paper in the thesis

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……Date……
…

REYNOLDS, M.

Supervised development of work in Mexico in each of the years were the material was tested (2007, 2008 and 2011) Provided critical evaluation of the manuscript

Certification that the statement of contribution is accurate and permission is given for the inclusion of the paper in the thesis

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…

FEUILLET, C.

Supervised development of work for the genotyping, helped with data interpretation in terms of genetic mapping and provided critical evaluation of the manuscript.

Certification that the statement of contribution is accurate and permission is given for the inclusion of the paper in the thesis

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LANGRIDGE, P.

Suggested the research topic, supervised development of work, helped with data interpretation and manuscript evaluation

Certification that the statement of contribution is accurate and permission is given for the inclusion of the paper in the thesis

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MATHER, D.

Supervised development of work and contributed significantly to prepare and revise the manuscript.

Certification that the statement of contribution is accurate and permission is given for the inclusion of the paper in the thesis

Signed
…….Date……
…

Chapter 2: Multi-environment analysis and improved mapping of a yield-related QTL on chromosome 3B of wheat

I. Abstract

Improved mapping, multi-environment quantitative trait loci (QTL) analysis and dissection of allelic effects were used to define a QTL associated with grain yield, thousand grain weight and early vigour on chromosome 3BL of bread wheat (*Triticum aestivum* L.) under abiotic stresses. The QTL had pleiotropic effects and showed QTL x environment interactions across 21 diverse environments in Australia and Mexico. The occurrence and the severity of water deficit combined with high temperatures during the growing season affected the responsiveness of this QTL, resulting in a reversal in the direction of allelic effects. The influence of this QTL can be substantial, with the allele from one parent (RAC875) increasing grain yield by up to 12.5% (particularly in environments where both heat and drought stress occurred) and the allele from the other parent (Kukri) increasing grain yield by up to 9% in favourable environments. With the application of additional markers and the genotyping of additional recombinant inbred lines, the genetic map in the QTL region was refined to provide a basis for future positional cloning.

Key words: Genetic analysis / heat / drought / Triticum aestivum / abiotic stress / linear mixed model

II. Introduction

Grain yield is a complex trait that depends on multiple genes and/or gene-network cascades interacting with each other and with the environment (Holland 2007; Shi et al. 2009; Wu et al. 2012). In wheat (*Triticum aestivum* L.), improvements in grain yield have

been achieved through the deployment of major genes such as the *Rht* plant height genes (dwarf and semi-dwarf) (Rebetzke et al. 2012), the *Ppd* photoperiod-sensitivity genes (Beales et al. 2007; Wilhelm et al. 2009) and the *Vrn* vernalisation-response genes (Zhang et al. 2008; Yoshida et al. 2010). However, the grain yield of wheat can be dramatically affected by abiotic stresses such as timing and severity of water deficit and/or high temperature during the crop cycle (Kosina et al. 2007) and by the interaction of these stresses with plant phenology and morphology. The confounding effects of loci affecting plant phenology and morphology can interfere with the detection of other genetic regions that may individually have minor effects but could collectively account for a large proportion of phenotypic variation (Reynolds et al. 2009a). The detection of such loci may be possible with appropriate statistical tools.

Quantitative trait locus analysis has been used to dissect the genetic component of grain yield of wheat grown in favourable environmental conditions (Maccaferri et al. 2008; McIntyre et al. 2010) and with exposure to drought and/or heat (Diab et al. 2008; Mason et al. 2010; Pinto et al. 2010). This enabled the detection of some of the major genes mentioned above, but also the discovery of other genetic regions with minor effects on wheat grain yield. These minor QTL are of great interest but are rarely consistent and reliable across environments. Alleles that confer improvement of grain yield in one set of environments may be irrelevant in other environments (Malosetti et al. 2008). The molecular basis for the minor genes affecting grain yield is also poorly understood. Although positional cloning has been used to identify genes underlying QTL (Krattinger et al. 2009), no QTL for grain yield under heat and drought conditions have been cloned from wheat.

The research reported here focussed on a QTL on chromosome 3BL that had been previously detected in a population of doubled haploid (DH) lines derived from a cross between the drought and heat stress-tolerant wheat breeding line RAC875 and the wheat cultivar Kukri. This QTL was associated with grain yield, thousand grain weight and early vigour in field experiments grown in north-western Mexico (Bennett et al. 2012a). When the same population was evaluated under apparently similar environmental conditions in Australia, the QTL was detected only for the proportion of small grains (*Q.Scr.aww-3B-2*) (Bennett et al. 2012b). The aim of the research reported here was to further investigate the region of interest by improving the genetic map of that region and by evaluating QTL

effects across a diverse range of heat- and drought-related conditions using multi-environment linear mixed model analyses.

III. Materials and Methods

1. Plant Material

The material used in this project includes a set of 368 DH lines derived from the F_1 generation of a cross between the drought tolerant wheat breeding line RAC875 (RAC655/3/Sr21/4*Lance//4*Bayonet) and the cultivar Kukri (76ECN44/76ECN36//Madden/6*RAC177), and a set of 768 recombinant inbred (RI) lines developed from the same cross (Fleury et al. 2010).

2. Marker genotyping

a. Genetic map of the RAC875/Kukri DH population

The genetic map of the target region on 3BL was built using the RAC875/Kukri genetic map and genotypic data published by Bennett et al. (2012a) as a basis. Linkage analysis was performed with simple sequence repeat (SSR) and DArT (Diversity Arrays Technology Pty Ltd, Canberra, Australia) markers assayed on the 368 DH lines. The genetic map was generated using MapManager Version QTXb20 (Manly et al. 2001), with the marker order refined using RECORD (Van Os et al. 2005). Before any further genetic and statistical analysis was conducted, a curation of the genotypic data was conducted and 14 lines were excluded due to inconsistencies that may have been caused by contamination of DNA samples.

b. Polymorphic markers on chromosome 3B

Based on comparison of the linkage map of chromosome 3B with a neighbour genetic map of the same chromosome (Paux et al. 2008) that was developed from 13 genetic maps using the approach described by Cone et al. (2002), fifty-six additional markers (38 SSR and 18 insertion site-based polymorphism (ISBP; Paux et al. 2010; Paux et al. 2011)) were selected for parental screening. For 46 of these markers, primer sequences are publicly available (Appendix 2.1). For the other 10 markers, primer sequences were obtained from Institut fuer Pflanzengenetik und Kulturpflanzenforschung, Gatersleben and TraitGenetics

Ltd (http://www.traitgenetics.com) (markers with the prefix "gwm") and from Genoplante, INRA France (markers with the prefix "gpw"). All of the polymorphic markers were then assayed on each of 368 DH lines using Multiplex-Ready technology (Hayden et al. 2008). The polymorphic markers were added to the map using MapManager Version QTXb20 (Manly et al. 2001). Genetic distances between marker loci were then re-calculated using the hidden Markov algorithm of Lander and Green (1987) available in the R/qtl package (Broman et al. 2010). This package is available from the Comprehensive R Archive Network (CRAN) at http://CRAN.R-project.org/package=qtl. The marker segregation distortion was tested using a chi-squared (χ^2) test ($p < 0.01$). Prior to further statistical analysis the genotype of each DH line for each marker was coded as 1 (homozygous for the RAC875 allele) or -1 (homozygous for the Kukri allele). Missing values were imputed using the flanking marker algorithm of Martinez and Curnow (1992).

c. Selection of recombinant inbred lines for phenotyping

Two marker loci (*barc77* and *gwm114*) that flank the target QTL region were selected based on the results reported by Bennett et al. (2012a). These two markers were assessed on 768 RI lines using Multiplex-Ready technology (Hayden et al. 2008). Lines exhibiting recombination between these two markers (RAC875 genotype at one locus and Kukri genotype at the other one) were screened with a marker for *Ppd-D1* (Beales et al. 2007), using the SSR marker *barc13* that was reported as being closely linked with a QTL for flowering time in the RAC875/Kukri population (*QEet.aww-2B*) (Bennett et al. 2012c) and with other markers that had been mapped between the two SSR loci *barc77* and *gwm114* using the DH population. A total of 109 RI lines were used in this research based on their genotypes at these four markers. The genotype of each RI line for each marker was coded as 1 (homozygous for the RAC875 allele), -1 (homozygous for the Kukri allele) or 0 (heterozygous) prior to analysis.

3. Phenotypic evaluation

Phenotypic data from 21 field experiments (Table 2.1) were used for multi-environment analysis of grain yield, thousand grain weight, and early vigour. Sixteen of the 21 experiments have been described previously by Reynolds et al. (2009a) and Bennett et al. (2012a, b, c). In four of these experiments, the entire population of 368 DH lines was phenotyped. In the other 12 experiments a subset of 260 DH lines was evaluated in experiments conducted in Australia and of these 255 DH were evaluated in experiment

conducted in Mexico. These lines had been selected based on ear emergence time under field conditions, in order to limit confounding effects of phenological variation (Table 2.1).

Five additional experiments were conducted in this work: three in 2009 in Australia under a 4.5 m × 26 m polyurethane tunnel (polytunnel) with removable sides on the Waite Campus of the University of Adelaide (Urrbrae, South Australia, 34°52'S 138°30'E, 48 m above sea level) and two in 2011 in north-western Mexico at a managed-environment field site (CIMMYT, Ciudad de Obregon, 27°25'N 109°54'W, 38 m above sea level).

In the polytunnel experiments, the entries were the parents RAC875 and Kukri, three other wheat cultivars (Gladius, Excalibur and Drysdale) and 46 DH lines that had been selected based on flowering time and on evidence of recombination between *barc77* and *gwm114*.

Table 2.1 Descriptions of the 21 environments in which experiments were conducted; showing locations, water supply from rainfall and/or irrigation, criteria used to select (RAC875/Kukri) lines for inclusion, numbers and types included, sowing densities, mean temperatures around flowering time, and statistical analyses performed.

Environment [a]	Location	Latitude	Longitude	Altitude (M)	Selection criterion [b]	Lines tested [c]	Sowing density seed m^{-2}	Rainfall and/or irrigation mm	Mean temperature around flowering time		Analysis performed [d]	
											MET	Allele effect
									Sept	Oct		
AusRos07_NI_CS	Roseworthy (SA)	34°57' S	138°36' E	68	All	368 DH	200	153	21.8	25.4	✓	✓
AusRos08_NI_CS	Roseworthy (SA)	34°57' S	138°36' E	68	All	368 DH	200	223	20.8	25.2	✓	✓
AusPie07_NI_CS	Piednippie (SA)	32°68' S	134°31' E	35	Flowering time	260 DH	200	113	22.4	24.1	✓	✓
AusPie08_NI_CS	Piednippie (SA)	32°80' S	135°15' E	35	Flowering time	260 DH	200	212	22	25.6	✓	✓
AusHor08_NI_CS	Horsham (VIC)	35°21' S	138°74' E	132	Flowering time	260 DH	200	187	18.5	23.7	✓	✓
AusBoo07_NI_CS	Booleroo (SA)	32°88' S	138°35' E	342	Flowering time	260 DH	200	159	20.8	24.2	✓	✓
AusMin07_NI_CS	Minnipa (SA)	32°80' S	135°15' E	165	Flowering time	260 DH	200	86	23.9	26.1	✓	✓
AusStr08_NI_CS	Streaky Bay (SA)	32°80' S	134°22' E	27	Flowering time	260 DH	200	95	22	25.6	✓	✓
AusRob07_NI_CS	Robinvale (VIC)	34°61' S	142°81' E	89	Flowering time	260 DH	200	99	21.4	25.2	✓	✓
AusNun08_NI_CS	Nunjikompita (SA)	32°27' S	134°31' E	73	Flowering time	260 DH	200	96	22	25.6	✓	✓
									Feb	**March**		
MexObr07_DI_CS	Ciudad de Obregon	27°28' N	109°56' W	38	All	368 DH	200	150	25.2	28.6	✓	✓
MexObr07_FI_CS	Ciudad de Obregon	27°28' N	109°56' W	38	All	368 DH	200	750	25.2	28.6	✓	✓

MexObr08_FI_CS	Ciudad de Obregon		27°28' N	109°56' W	38	Flowering time	255 DH	200	750	24.4	27	✓	✓
MexObr09_DI_CS	Ciudad de Obregon		27°28' N	109°56' W	38	Flowering time	255 DH	200	50	25.6	26.9	✓	✓
MexObr11_DI_CS	Ciudad de Obregon		27°28' N	109°56' W	38	Marker genotypes	34 DH / 77 RI	200	150	24.5	29.3	•	✓
										April	**May**		
MexObr08_FI_LS	Ciudad de Obregon		27°28' N	109°56' W	38	Flowering time	255 DH	200	900	30.7	35	✓	✓
MexObr09_FI_LS	Ciudad de Obregon		27°28' N	109°56' W	38	Flowering time	255 DH	200	1050	30.5	35.6	✓	✓
MexObr11_FI_LS	Ciudad de Obregon		27°28' N	109°56' W	38	Marker genotypes	34 DH / 109 RI	200	1050	30.7	34.8	•	✓
										Oct	**Nov**		
AusUrr09_SI-S_LS	Urrbrae (SA)		34°57' S	138°36' E	225	Recombinant	46 DH	133	N/A	21.8	27.5	✓	✓
AusUrr09_SI-W_LS	Urrbrae (SA)		34°57' S	138°36' E	225	Recombinant	46 DH	133	N/A	21.7	27.5	✓	✓
AusUrr09_SI-D_LS	Urrbrae (SA)		34°57' S	138°36' E	225	Recombinant	46 DH	133	N/A	21.7	27.5	✓	✓

a Aus Australia, Mex Mexico, 07-11 2007 to 2011, DI drip irrigation , NI for not irrigated but rainfed, FI for flooding irrigation, SI for sprinkler irrigation, D drought, S Saturated, W Well watered, LS late sowing, CS conventional sowing.
b Marker genotypes: recombinant lines between the two loci *barc77* and *gwm114* and genotyped for *PPd-D1* and *barc13*
c DH doubled haploid lines, RI recombinant inbred lines.
d Black dot indicates that phenotypes from only the doubled haploid lines were included in the analysis.

These experiments were sown in early September, which is much later than the normal commercial sowing time (April/May) for wheat in South Australia. The late sowing ensured the plants would be exposed to high temperatures and drought stress during flowering and grain filling. Within each experiment, there were two plots of each entry, arranged in a completely randomised design. Each plot consisted of two rows of eight plants (0.6 m x 0.2 m), with data collected on 12 of these plants. The experiments were irrigated every second day from sowing to flowering, using sprinklers. One experiment (designated AusUrr09_SI-S_LS, for Australia – Urrbrae (location) 2009 – sprinkler irrigation – saturated - late sowing) was supplied with sufficient water to maintain the leaf water potential close to 0 MPa. The second experiment (AusUrr09_2_SI-W_LS, where W denotes well-irrigated) received sufficient water that the leaf water potential was -0.2 MPa at flowering time. In the third experiment (AusUrr09_SI-D_Ls, where D denotes moderate drought) water supply was withheld after anthesis, decreasing water potential to -0.6 MPa. The soil was shallow (40 to 60 cm deep) and rich in clay.

In Mexico, one experiment (MexObr11_DI_CS for Mexico - 2011 – drip irrigation – conventional sowing) was sown in December 2010. During the first two months, this experiment received around 150 mm of water from a drip irrigation system. This experiment included 114 entries: 34 DH lines (20 of which were common with the three experiments conducted in Australia in 2009), 77 RI lines, RAC875, Kukri, and one control (cultivar Sokoll). A second experiment in Mexico (designated MexObr11_FI_LS where FI denotes flood irrigation and LS late sowing) was sown in March so that it would be exposed to very high temperatures at flowering time (average T_{max} > 32°C). This experiment received around 1050 mm of water by flood irrigation. This experiment included 146 entries: the same 34 DH lines as in MexObr11_DI_CS, 109 RI lines (including the 77 RI lines that were in MexObr11_DI_CS), RAC 875, Kukri and other cultivars (Sokoll and Weebil 1).

All of the DH and RI lines included in these two experiments were selected based on their genotypes for the SSR locus *barc13* (representing the photoperiod-sensitivity locus *Ppd-B1* that underlies the QTL *QEt.aww-2B*), the *Ppd-D1* locus for the photoperiod-sensitivity and the SSR loci *barc77* and *gwm114*. Each of the selected lines carried one of the parental allelic combinations at *barc13* and *Ppd-D1* (i.e. the photoperiod insensitivity allele *Ppd-B1a* and the photoperiod sensitivity allele *Ppd-D1b* as in RAC875, or the photoperiod sensitivity allele *Ppd-B1b* and the photoperiod insensitivity allele *Ppd-D1a* as in Kukri)

and a non-parental (recombinant) allelic combination for the markers *barc77* and *gwm114*. In each of these experiments, a two-replicate alpha-lattice design was used, with each plot consisting of a raised bed with 2 m rows separated from each other by 30 cm. The soil type at the experimental station has been described by Olivares-Villegas et al. (2007) and Deckers et al. (2009).

Early vigour was scored on a scale from 1 (lowest vigour) to 9 (highest vigour) in each of eight experiments AusRos08_NI_CS, AusPie07_NI_CS, AusBoo07_NI_CS, AusMin07_NI_CS, AusRob07_NI_CS, MexObr07_FI_CS, MexObr08_FI_CS, MexObr08_FI_LS. In Mexico, grain was machine harvested, whereas in Australia spikes were manually harvested and threshed. For five experiments (AusUrr09_SI-S_LS, AusUrr09_SI-W_LS, AusUrr09_SI-D_LS, MexObr11_DI_CS and MexObr11_FI_LS), a seed counter (Pfueffer GmBH, Germany) was used to count out dry samples (10% moisture content) of between 250 and 500 grains, which were then weighed to estimate thousand grain weight. Mid-day leaf water potential (MPa) was measured using a Scholander pressure chamber (Scholander et al. 1964).

4. Statistical analysis

a. Single environment single trait analysis

Initially for each environment, the traits grain yield, thousand grain weight, and early vigour were analysed using a linear mixed model that partitioned and accounted for genetic and non-genetic or extraneous variation. Where $\boldsymbol{y} = (y_1, \ldots, y_n)$ is a vector for trait observations, the single environment linear mixed model was defined as

$$\boldsymbol{y} = \boldsymbol{X\tau} + \boldsymbol{Zu} + \boldsymbol{Z_g g} + \boldsymbol{e} \quad (1)$$

where $\boldsymbol{X\tau}$ is the fixed component of the model containing an indicator term that differentiates the DH lines from control lines (parents and cultivars) as well as marker information for *Ppd-D1* and *barc13* to control for differences in phenology. This term was also used to capture possible covariate information or linear trends that might exist across rows or columns of the field experiment. The term $\boldsymbol{Zu}$ is a random component that captures extraneous non-genetic variation possibly existing in the environment. Most importantly, $\boldsymbol{Z_g g}$ is a random component that models the genetic effects among DH lines grown in the environment. The genetic effects were assumed to have a distribution

$\boldsymbol{g} \sim N(\mathbf{0}, \sigma_g^2 \boldsymbol{I}_g)$ where σ_g^2 is the genetic variance and $\boldsymbol{I}_g$ is the identity matrix. The residual error, $\boldsymbol{e}$, was assumed to be distributed $\boldsymbol{e} \sim N(\mathbf{0}, \sigma^2 \boldsymbol{R})$ where σ^2 is the residual variance and $\boldsymbol{R}$ is a matrix that typically contains a parameterization for a separable AR1 x AR1 process (AR1 = auto-regressive process of order 1) to capture the correlation between observations due to the proximity of neighbouring plots in the environment. The effects $\boldsymbol{u}$, $\boldsymbol{g}$ and $\boldsymbol{e}$ were considered to be independent. The terms of this model varied according to the experimental layout and the numbers of DH and control lines sown at each location.

The best linear unbiased predictions (BLUPs) of the genetic effects for each line were extracted from each model and a generalized heritability (h_g^2) was calculated using the formula developed by Cullis et al. (2006) and Oakey et al. (2006), namely

$$h_g^2 = 1 - \frac{PEV}{2\sigma_g^2}$$

Where PEV is the average pairwise prediction error variance of the BLUPs and σ_g^2 is the genetic variance.

b. Multi-environment single trait analysis

The multi-environment trial (MET) model for a single trait can be considered as an extension of the single environment single trait model defined in (1). In this extended model the vector of trait observations is $\boldsymbol{y} = (\boldsymbol{y}_1, \ldots, \boldsymbol{y}_t)$ where t is the number of environments involved in the analysis. The terms $\boldsymbol{X\tau}$ and $\boldsymbol{Zu}$ were partitioned according to the fixed effects and non-genetic random effects at each location. The vector of residual errors was $\boldsymbol{e} = (\boldsymbol{e}_1, \ldots, \boldsymbol{e}_t)$ which at the jth site, was assumed to have distribution $\boldsymbol{e}_j \sim N(\mathbf{0}, \sigma_j^2 \boldsymbol{R}_j)$; $j = 1, \ldots, t$. In this model $\boldsymbol{e}_i$ and $\boldsymbol{e}_j$ for all $i \neq j$ were considered independent. Thus, phenotypically, the environments were deemed to be unrelated.

An important aspect of the MET model is the parsimonious modelling of the variance-covariance structure for the genotype by environment interaction. Typically, the genetic effects are assumed to have a distribution $\boldsymbol{g} \sim N(\mathbf{0}, \boldsymbol{\Sigma} \otimes \boldsymbol{I}_g)$ where $\boldsymbol{\Sigma}$ is an unstructured $(t \times t)$ variance-covariance matrix that reflects the relationships of the DH and control genetic effects between environments and $\otimes$ represents the direct product or Kronecker operator. Computationally, the estimation of $\boldsymbol{\Sigma}$ is difficult and an approximation was

sought that models the genetic effects using the factor analytic (FA) approach of Smith et al. (2001, 2005). Under this approximation, the variance of the genetic effects becomes

$$\text{var}(\boldsymbol{g}) = (\boldsymbol{\Gamma\Gamma}^{\text{T}} + \boldsymbol{\Psi}) \otimes \boldsymbol{I_g}$$

where $\boldsymbol{\Gamma}$ is a $(t \times k)$ matrix of factor loadings, k is the number of factors involved in the approximation and $\boldsymbol{\Psi}$ is a diagonal matrix of environment specific variances. This FA approximation reduces the number of parameters required for estimation from $t(t+1)/2$ in the unstructured case, to tk.

If $\boldsymbol{D}$ represents $(t \times t)$ a diagonal matrix of genetic variances extracted from the diagonal values of $(\boldsymbol{\Gamma\Gamma}^{\text{T}} + \boldsymbol{\Psi})$ then the correlation matrix for the genetic effects can be calculated using:

$$\text{cor}(\boldsymbol{g}) = \boldsymbol{D^{-1/2}}(\boldsymbol{\Gamma\Gamma}^{\text{T}} + \boldsymbol{\Psi})\boldsymbol{D^{-1/2}} \otimes \boldsymbol{I_g} \quad (2)$$

The expression on the left hand side of the Kronecker operator was used to summarize the genetic correlation of the trait across the environments used in the analysis.

c. Multi-environment QTL analysis

For multi-environment QTL analyses, the MET model for each trait was extended by partitioning the multi-environment genetic effects:

$$\boldsymbol{g} = (\boldsymbol{a}_i \otimes \boldsymbol{I_m})(\boldsymbol{m}_i \otimes \boldsymbol{1}_t) + \boldsymbol{p} \quad (3)$$

where $\boldsymbol{a}_i = (a_{i1}, \ldots, a_{it})$ is a vector for the fixed QTL by environment marker effects for the ith marker, $\boldsymbol{m}_i$. In this expression, $\boldsymbol{p}$ represents the residual component of the genetic model not captured by the marker. Analogous to the MET linear mixed model, these become residual genotype by environment interaction effects and are modelled using the FA approach of Smith et al. (2001, 2005). Each marker of chromosome 3B was then considered in turn and a separate MET QTL model was fitted for each marker in the linkage group.

To detect putative multi-environment QTL on chromosome 3B an appropriate hypothesis test was used. From each MET QTL model the single marker multi-environment QTL effects were tested simultaneously under the null hypothesis $H_0: a_{i1} = a_{i2} = \cdots = a_{it} = 0$ and a Wald statistic was calculated (Kenward and Roger 1997). Using the Wald statistics a

multi-environment QTL profile, analogous to a LOD (logarithm base 10 of odds) QTL profile, spanning the linkage group 3B was calculated for each trait. Significant QTL regions were identified as peaks exceeding a Wald statistic threshold that was calculated through back transformation of an adjusted Bonferroni corrected p-value with significance level $\alpha = 0.05$ (Li and Ji 2005). This threshold differed for each trait due to the number of sites involved in the multi-environment QTL analysis.

d. Multi-environment allele effects with DH and RI

Using the QTL profiles of all three traits from the multi-environment QTL analysis, a set of four markers on chromosome 3B were chosen as representing a specific QTL region for further investigation. For this analysis genetic marker information for each of the four markers was combined across DH lines and RI lines, with missing marker scores set to zero. The MET QTL model for each trait was then refitted for each of the four markers. For each trait by marker combination, the marker effect was estimated and its significance was tested using a Wald statistic.

e. Computations

All single and multivariate site analyses were performed using the linear mixed modelling package ASReml-R (Butler et al. 2009; software at http://www.vsni.co.uk) available in the R Statistical Computing Environment. ASReml-R uses a residual maximum likelihood (REML) approach of Patterson and Thompson (1971) for estimation of the model parameters. It also provides useful diagnostic tools such as variograms and residual plots to help determine environmental trends possibly existing in the field.

IV. Results

1. Grain yield means and trait heritability

The mean grain yield for the RAC875/Kukri DH population varied considerably across environments, ranging from 0.32 t/ha to 6.53 t/ha (Table 2.2). Across experiments, the mean grain yield of RAC875 was significantly (p = 0.03) higher than that of Kukri. Heritability of grain yield (Table 2.2) was moderate to high (ranging from 0.58 to 0.87) for most experiments, but low (0.23 and 0.30) for two of the polytunnel experiments. The heritability of thousand grain weight was moderate to high (0.60 to 0.92) for all

Table 2.2 Mean grain yields of RAC875, Kukri and the RAC875/Kukri lines, and heritability estimates for grain yield, early vigour and thousand grain weight for each environment in which data were collected.

Environment [a]	Lines evaluated [b]	Mean grain yield t/ha			Heritability		
		Population	RAC875	Kukri	Grain yield	Early vigour	Thousand grain weight
AusRos07_NI_CS	368 DH	2.36	2.94	2.59	0.87	-	0.81
AusRos08_NI_CS	368 DH	2.18	2.86	2.17	0.86	0.65	-
AusPie07_NI_CS	260 DH	0.32	0.45	0.24	0.79	0.52	0.92
AusPie08_NI_CS	260 DH	1.43	1.47	1.36	0.67	-	0.92
AusHor08_NI_CS	260 DH	0.96	0.94	0.89	0.77	-	-
AusBoo07_NI_CS	260 DH	1.57	1.60	1.64	0.68	0.43	0.89
AusMin07_NI_CS	260 DH	0.41	0.45	0.35	0.77	0.67	0.89
AusStr08_NI_CS	260 DH	0.65	0.67	0.55	0.75	-	0.84
AusRob07_NI_CS	260 DH	0.55	0.60	0.53	0.65	0.48	0.79
AusNun08_NI_CS	260 DH	0.53	0.60	0.50	0.83	-	0.92
MexObr07_DI_CS	368 DH	1.46	1.39	0.87	0.62	-	0.6
MexObr07_FI_CS	368 DH	4.40	5.57	4.86	0.58	0.29	0.83
MexObr08_FI_CS	255 DH	5.14	5.40	5.50	0.67	0.63	0.83
MexObr09_DI_CS	255 DH	2.23	2.75	2.04	0.71	-	0.85
MexObr11_DI_CS	34 DH / 77 RI	1.11	1.33	1.10	0.74	-	0.84
MexObr08_FI_LS	255 DH	1.54	2.04	1.56	0.84	0.69	0.42
MexObr09_FI_LS	255 DH	2.27	2.41	2.68	0.75	-	0.77
MexObr11_FI_LS	34 DH / 109 RI	2.79	3.84	3.05	0.75	-	0.87
AusUrr09_SI-S_LS	46 DH	6.53	7.08	7.09	0.61	-	0.71
AusUrr09_SI-W_LS	46 DH	5.88	5.46	4.68	0.23	-	0.61
AusUrr09_SI-D_LS	46 DH	2.00	1.84	2.22	0.3	-	0.81

a Aus Australia, Mex Mexico, 07-11 2007 to 2011, DI drip irrigation , NI not irrigated but rainfed, FI flooding irrigation, SI Sprinkler irrigation, D drought, S saturated, W well watered, LS late sowing, CS conventional sowing.
b DH doubled haploid lines, RI recombinant inbred lines

experiments except for Mex08_FI_LS (0.41). The heritability of early vigour was between 0.43 and 0.65 for seven of the eight experiments in which this trait was scored, but was only 0.29 for MexObr07_FI_CS.

2. Multi-environment analysis

For each of the traits, a sequential set of FA models was fitted for the genotype by environment interaction component of the MET model (Table 2.3). The most parsimonious model for each trait was then chosen by minimizing the model selection criterion, the Bayesian Information Criterion (BIC) developed by Schwarz (1978). For early vigour, the FA2 (factor analytic model of order 2) was found to be the most appropriate (Table 2.3). Thousand grain weight and grain yield required FA3 and FA4 models, respectively (Table 2.3).

Table 2.3 Genotype by environment interaction models used in multi-environment analysis.

Trait (number of environments)	Model	Number of parameters	Log-likelihood	BIC[b]	Efficiency %
Yield (21)	FA5[a]	119	9990.13	-18878.72	78.4
	FA4	100	9957.11	-18988.55	78.0
	FA3	81	9866.99	-18984.19	50.7
	FA2	62	9740.44	-18906.97	40.5
	FA1	42	9543.25	-18697.71	22.9
Thousand grain weight (19)	FA4	91	8057.50	-15285.93	84.2
	FA3	73	8012.04	-15359.01	80.3
	FA2	56	7867.67	-15225.13	73.3
	FA1	38	7717.46	-15088.71	59.2
Early vigour (8)	FA3	29	-336.76	919.12	73.6
	FA2	23	-342.67	880.13	71.7
	FA1	16	-409.14	953.79	40.6

a FA5: Factor analytic model of order k=5
b Bayesian information criterion

For these traits, the need for more factors was due to the diverse range and number of environments used in the MET analysis. For each trait, the estimated multi-environment genetic variance-covariance matrix was extracted from the MET analysis and converted to a multi-environment genetic correlation matrix using equation (2). An empirical threshold of r = 0.4 was used to help discriminate environments. For grain yield, five groups of

experiments Gya to Gye were identified (Figure 2.1). The group Gya consists of the three polytunnel experiments (Table 2.2), which were poorly correlated ($r < 0.4$) with the 18 other experiments. The experiments conducted in Mexico formed three overlapping groups (Gyb, Gyc and Gyd in Figure 2.1). The groups Gyb and Gyc included all experiments conducted in Mexico that received flood irrigation except for MexObr09_DI_CS. The group Gyd consisted of four experiments conducted in Mexico (including an environment in which drought occurred earlyin the growth cycle) and the three lowest yielding experiments conducted in 2007 in Australia. Two of these environments had high temperatures at flowering time (Table 2.2). All of the experiments conducted under rainfed conditions in Australia grouped together (group Gye). For thousand grain weight, the multi-environment genetic correlation plot identified four groups of experiments (Appendix 2.2) one of which consisted of just one experiment from Mexico and three of which each contained experiments from both Australia and Mexico. For early vigour, the multi-environment genetic correlation exhibited two distinct groups: Gva (three experiments in Mexico) and Gvb (five experiments in Australia) (Appendix 2.2).

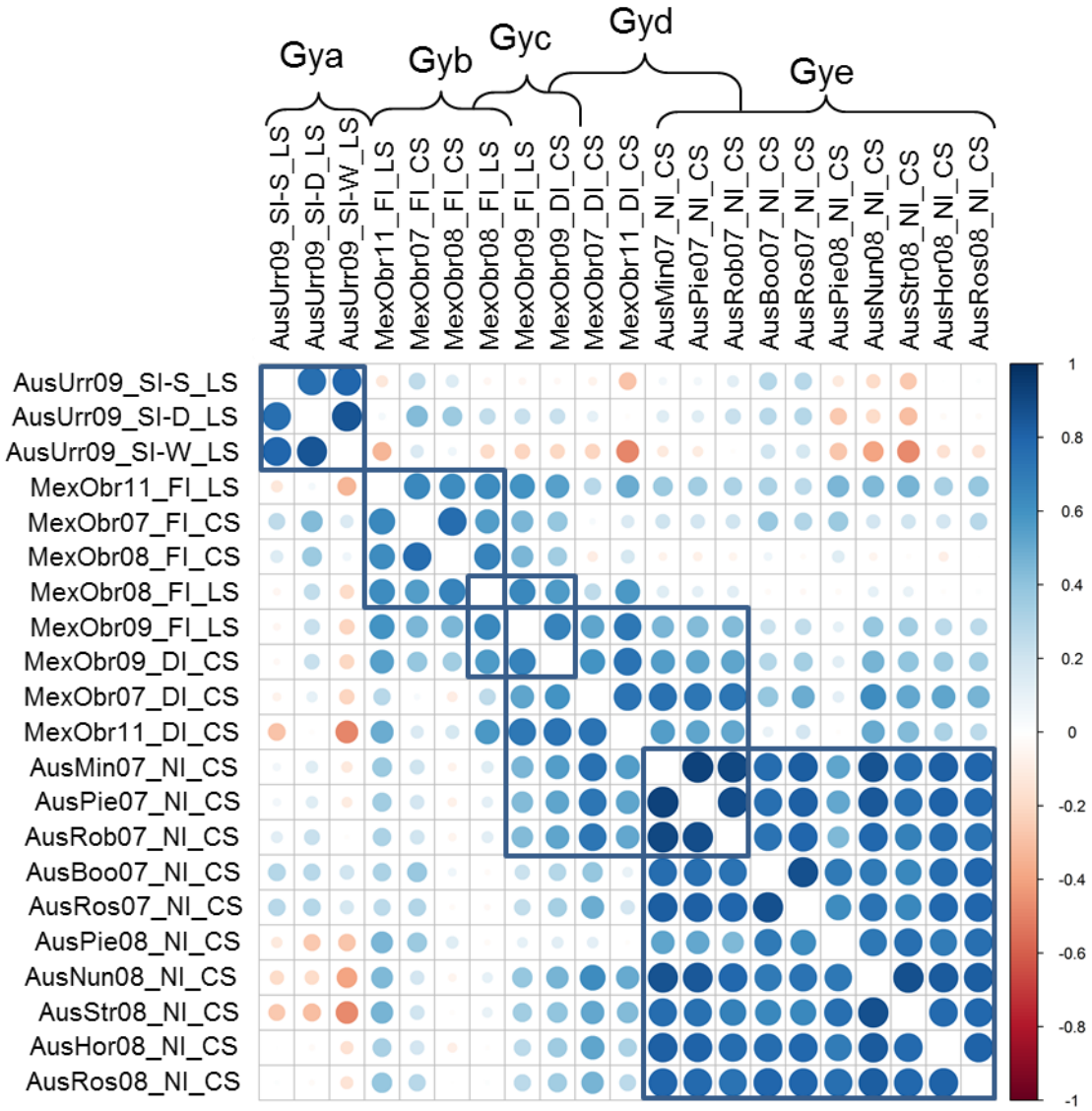

Figure 2.1 Multi-environment genetic correlation for grain yield extracted from the associated FA4 model. Each circle represents a pairwise correlation coefficient between environments of 0.4 or more, with the diameter of the circle proportional to the absolute value of the correlation coefficient and the colour of the circle indicating whether the correlation is positive (blue) or negative (red). Groups of genetically correlated environments are outlined by squares and labelled at the top of the figure.

3. Map of chromosome 3B

Twenty molecular markers (Figure 2.2) were added to the QTL region on chromosome 3BL in the RAC875/Kukri genetic map, including 9 markers (5 SSR and 4 ISBP) between *wPt-4401* and *gwm114*, the markers reported previously by Bennett et al. (2012a) as delimiting the 95% confidence interval for the QTL. Comparison of the resulting genetic map with a neighbour genetic map of chromosome 3B (Paux et al. 2008) indicated a similar order of markers. None of the markers mapped on chromosome 3B exhibited significant segregation distortion.

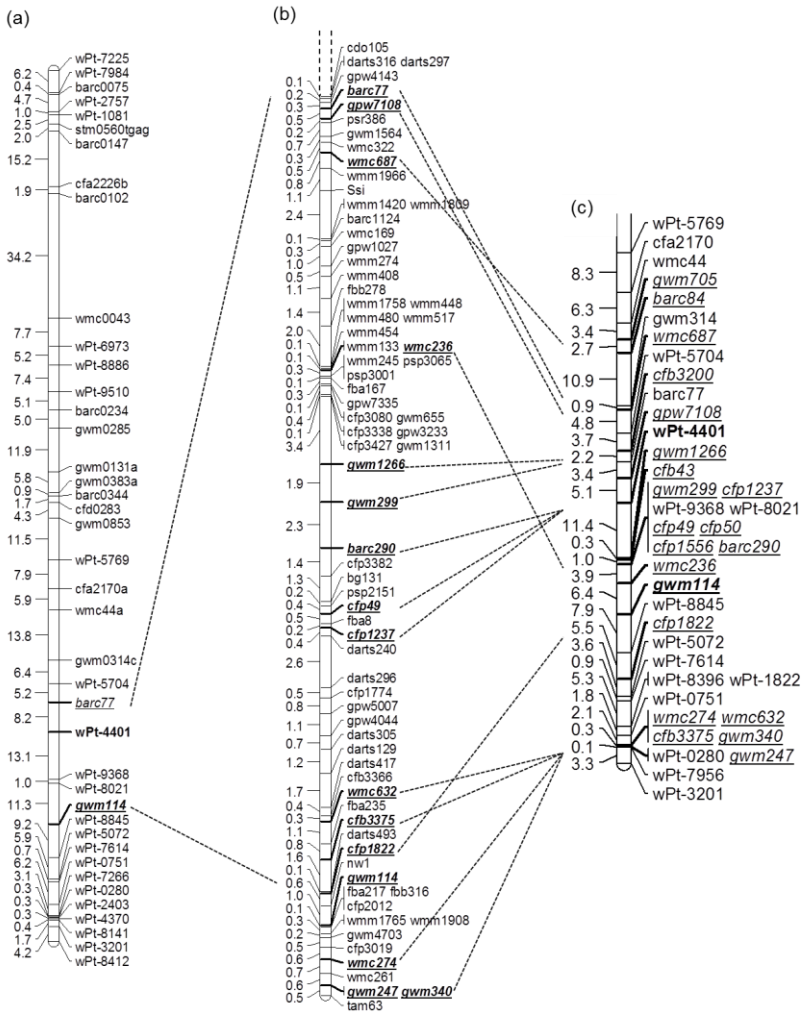

Figure 2.2 Relationships between three genetic maps of chromosome 3BL: (**a**) the map of chromosome 3B (RAC875/Kukri) published by Bennett et al. (2012a), (**b**) part of a neighbour map of chromosome 3B adapted from Paux et al. (2008), (**c**) the region of interest of chromosome 3BL (RAC875/Kukri). Common markers between the three maps are indicated with lines. The new markers mapped here, are shown in italic font and underlined (**b** and **c**). The two markers that flank the interval found by Bennett et al. (2012a) are shown in bold font (**a** and **c**).

4. Multi-environment QTL analysis

Extension of the MET model for each trait to individually incorporate each of the 47 markers that had been mapped on chromosome 3B resulted in Wald statistic profiles for each trait (Figure 2.3). For each trait there was a highly significant peak in the 5.2 cM chromosome region that includes the loci *gwm1266*, *cfb43*, *gwm299*, and *wmc236* and several minor peaks elsewhere on the chromosome.

a. Allele effects of single marker across all environments

When the MET QTL model for grain yield and thousand grain weight were refitted with the combined genetic information from the DH and the RI lines (Table 2.2), it was found that the magnitudes and directions of allelic effects at the four marker loci in the QTL peak region varied among environments (Figure 2.4). For grain yield, there were statistically significant effects in 10 of 21 experiments, with the favourable allele coming from RAC875 in eight experiments (all of group Gyc, most of group Gyb and Gyd and part of group Gye) and a favourable effect from Kukri (at marker locus *wmc236* only) in two of the three experiments in group Gya (polytunnel). For thousand grain weight, there were statistically significant effects in seven of 19 environments, with the favourable allele coming from RAC875 in four experiments (group Gyb, Gyc and Gyd) and from Kukri in three experiments (groups Gya and Gyb). For six of the eight experiments in which early vigour was rated, there were significant positive allelic effects ($p<0.05$), indicating that RAC875 carries an allele improving early vigour (Appendix 2.3).

b. Allele effects using recombinant inbred lines

To further investigate allele effects in the chromosomal region of interest a simplified bi-environment model was carried out on the two experiments containing 109 RI lines (MexObr11-DI-CS and MexObr11-FI-LS). In this analysis the complete phenotypic information for the DH and RI lines was used to ensure environmental and design effects were appropriately modelled but the genetic marker information of the DH lines was ignored.

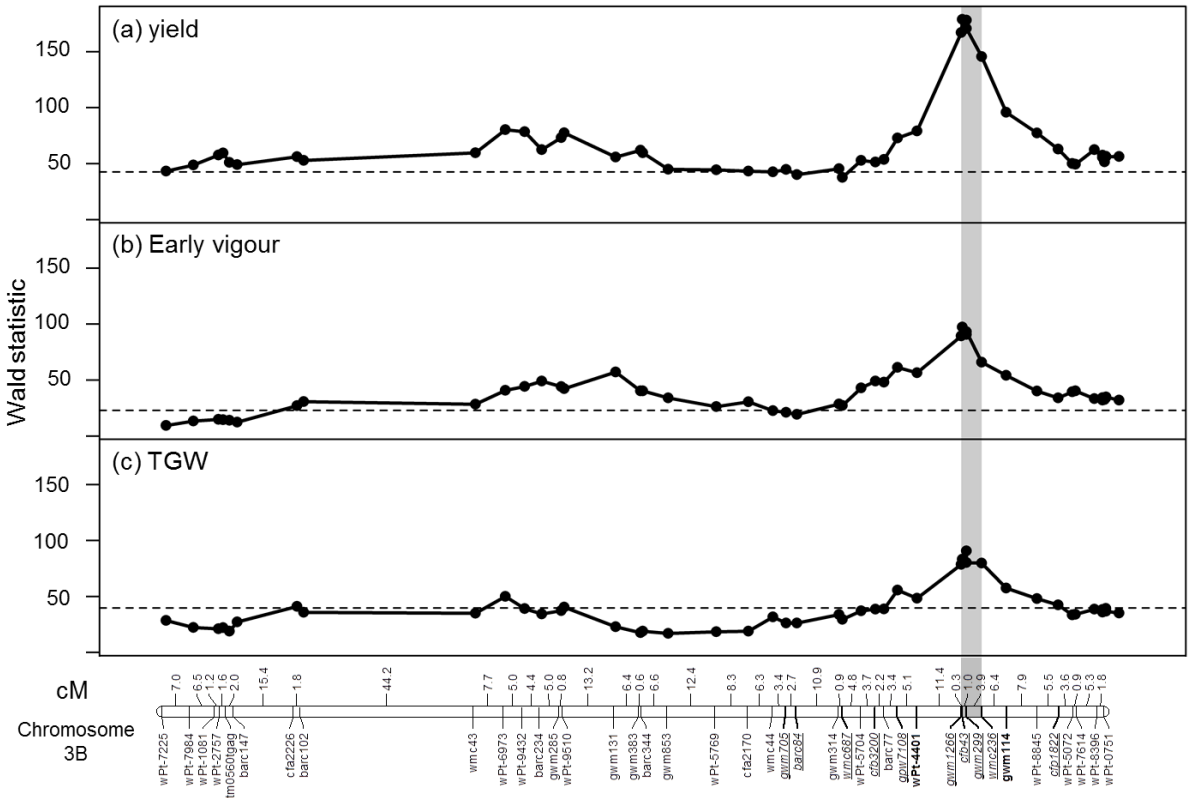

Figure 2.3 Wald-statistic profiles on chromosome 3B based on a multi-environment QTL scan. The profiles correspond to three traits: (**a**) grain yield (21 environments), (**b**) early vigour (eight environments), and (**c**) thousand grain weight (19 environments). The two markers that flank the interval found by Bennett et al. (2012a) are shown in bold font. Collocated markers have been omitted from the map displayed below the profiles.

The results show the RAC875 allele was favourable in MexObr11_FI_LS ($p<0.05$) at three loci (*gwm1266*, *cfb43* and *cfp49*), increasing grain yield by 4% and for thousand grain weight in MexObr11_DI_CS with a maximum of 6.2% increase at *cfp1556* locus (Figure 2.5).

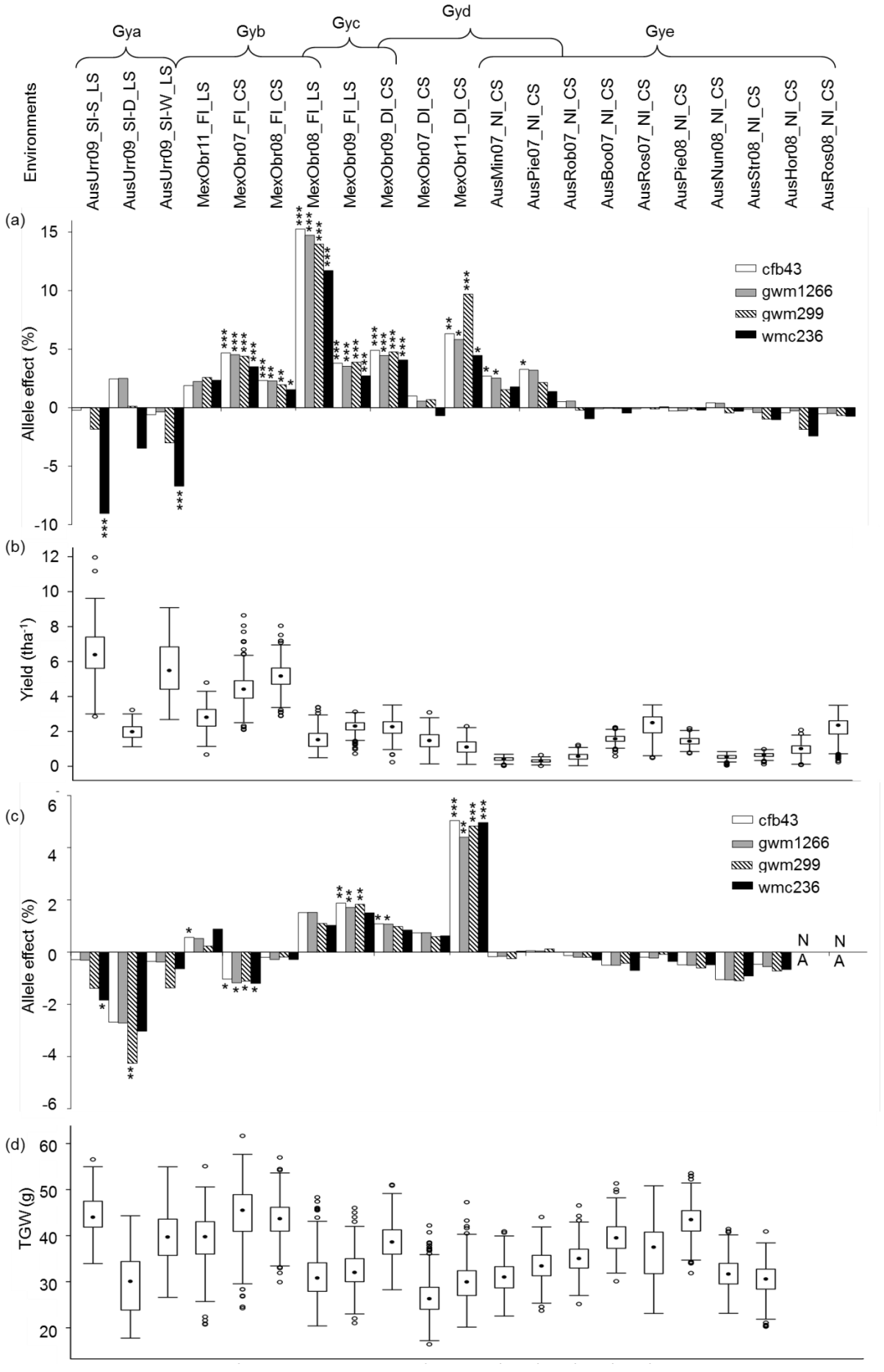
Gya
Gyb
Gyc
Gyd
Gye
Environments
AusUrr09_SI-S_LS
AusUrr09_SI-D_LS
AusUrr09_SI-W_LS
MexObr11_FI_LS
MexObr07_FI_CS
MexObr08_FI_CS
MexObr08_FI_LS
MexObr09_FI_LS
MexObr09_DI_CS
MexObr07_DI_CS
MexObr11_DI_CS
AusMin07_NI_CS
AusPie07_NI_CS
AusRob07_NI_CS
AusBoo07_NI_CS
AusRos07_NI_CS
AusPie08_NI_CS
AusNun08_NI_CS
AusStr08_NI_CS
AusHor08_NI_CS
AusRos08_NI_CS
(a)
Allele effect (%)
cfb43
gwm1266
gwm299
wmc236
(b)
Yield (tha-1)
(c)
Allele effect (%)
cfb43
gwm1266
gwm299
wmc236
N A
N A
(d)
TGW (g)

Figure 2.4 Allele effects (**a** and **c**) of four loci (*cfb43*, *gwm1266*, *gwm299* and *wmc236*) on chromosome 3B and trait means (**b** and **d**) for: grain yield (**a** and **b**) and thousand grain weight (**c** and **d**) in each environment. Allele effects are expressed as percentage relative to the trait mean. A positive effect indicates that the RAC875 allele increased the trait value while a negative effect indicates that the Kukri allele increased the trait value. Levels of significance are represented by: * p <0.05, ** p <0.01, *** p <0.001. In the boxplots (**b** and **d**), the black dot indicates the median value, the box encloses the second and third quartiles, the whiskers extend to ±1.5 times the inter-quartile range, and the empty dots indicate outliers. The groups Gya (Group yield a) to Gye (Group yield e) were selected based on multi-environment correlation analysis with an empirical threshold of r = 0.4 (Figure 2.1).

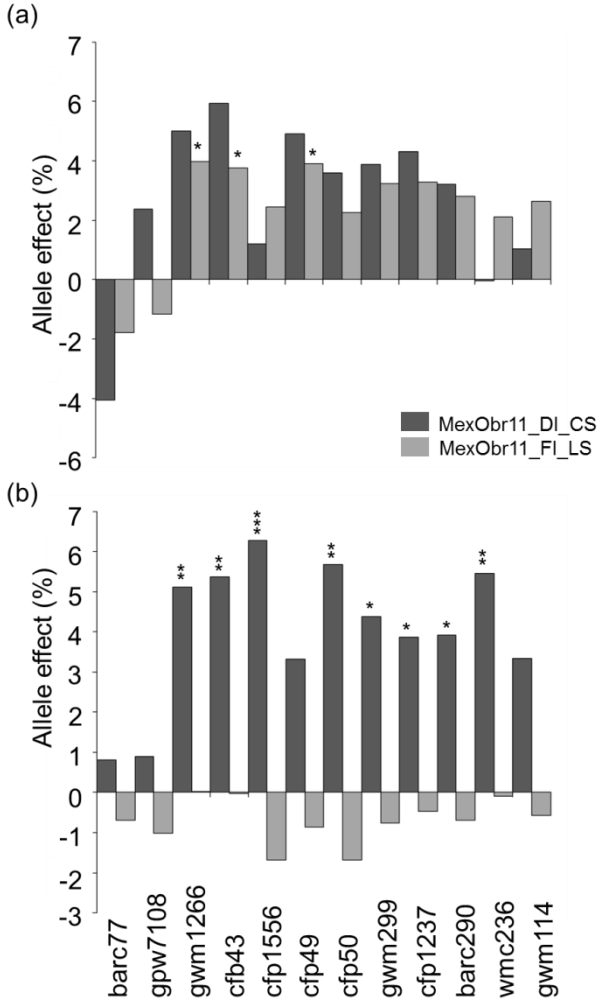

Figure 2.5 Allele effects at 12 loci on chromosome 3B using a bi-environmental analysis of: (**a**) grain yield and (**b**) thousand grain weight of for 77 recombinant inbred lines evaluated in the drought-stressed Mex11_DI_CS experiment (dark grey) and for 109 recombinant inbred lines in the late-sown Mex11_FI_LS experiment in which high temperatures were experienced during grain filling. (light grey). Allele effects are based on genetic marker information from recombinant inbred lines only. A positive effect indicates that the RAC875 allele increased the trait value while a negative effect indicates that the Kukri allele increased the trait value. Levels of significance are represented by: * p <0.05, ** p <0.01, *** p <0.001.

V. Discussion

Crop yield under stress is determined by complex interactions between the genetic make-up of the plant and the nature and timing of the environmental stress with respect to the plant's development. In a previous study using the RAC875/Kukri population of wheat a region on chromosome 3B was found to be associated with yield under drought and heat stress (Bennett et al. 2012a). The key objectives of the current study were to define the yield response across different environments and provide a basis for fine mapping and ultimately cloning the gene(s) underlying the QTL. The first component of the study involved an assessment of the performance across environments of the DH lines that were used for the initial mapping. This provided an improved map of the target region and a basis for identifying additional molecular markers. A second larger population of RI lines was then used to confirm the location of the QTL.

Most of the environments in which experiments were grown experienced moderate or severe stress. The severity of stress can be assessed by comparing the average grain yields across the experiments. Under the most favourable growing conditions (AusUrr09_SI-S_LS) some lines yielded more than 10 t/ha. In the experiments used in the present study, the average site yields ranged from 0.32 to 6.53 t/ha indicating that the stress levels were reducing yields by up to 95% of the potential. Yields of less than 1 t/ha are generally regarded as non-commercial, however in certain parts of the world such as South Australia, a grain yield lower that 1 t/ha is still considered useful (Hunt and Kirkegaard 2011).

The grouping of environments used in this study follows a pattern that reflects the importance of the nature and pattern of drought exposure. The experiments conducted in Mexico separated into those where flood irrigation was used as opposed to drip irrigation. Given the deep soils at the Obregon site, flood irrigation followed by drought is expected to provide an advantage to lines that are able to track moisture as it retreats down the soil profile. In contrast, the drip irrigation system would imposed a drought stress more similar to the sites in Australia, where intermittent and declining rainfall provides adequate moisture during earlier stages of the crop cycle but can result in strong terminal drought stress. Consistent with this, the drip-irrigated experiments conducted in Mexico showed considerable genetic correlation with the most severely droughted experiments in

Australia. The differences in performance of lines between flood-irrigated experiments and the drip-irrigated and rainfed experiments may well be related to the genetic control of root architecture, an aspect worthy of further experimentation. The high temperatures experienced in all of the experiments conducted in Mexico may have added significantly to the stress severity, yet these experiments still showed reasonable grain yield (Table 2.2).

The three experiments conducted in a polytunnel (AusUrr09) extended the range of environmental conditions under which the RAC875/Kukri materials were evaluated. The polytunnel experiments all received adequate moisture throughout vegetative growth. This was in contrast to the conditions experienced in field environments in both Mexico and Australia, where plants would have encountered moisture limitations early in their development. The very high grain yields achieved in the saturated (AusUrr09_SI-S_LS) and well-watered (AusUrr09_SI-W_LS) polytunnel experiments were likely attributable to the pre-anthesis establishment of a strong source of photosynthates prior to anthesis, combined with continuation of an adequate water supply throughout grain filling. These high yields were achieved despite exposure to very high temperatures during grain filling; mean temperatures of 27°C in November, with 5 (AusUrr09_SI-W_LS) to 10 (AusUrr09_SI-S_LS) days with maximum temperatures exceeding 35°C). With imposition of post-anthesis drought (AusUrr09_SI-D_LS), grain yield was substantially reduced.

Malosetti et al. (2008) reported that the directions of allelic effects for grain yield, ear number, and anthesis-silking interval in maize (*Zea mays* L.) depended on the environmental conditions (drought and/or nitrogen supply) under which a mapping population was investigated. In our study in wheat, one parent (RAC875) contributed the favourable QTL allele for grain yield in almost all of the experiments conducted in Mexico and in two low-yielding experiments in Australia (AusPie07_NI_CS and AusMin07_NI_CS), whereas the other parent (Kukri) contributed the favourable allele for grain yield under the more favourable environmental conditions in Australia (Figure 2.4). In seven out of ten environments in which significant effects were detected for grain yield, there were also effects on thousand grain weight. In six of these environments, the allele that increased grain yield also increased thousand grain weight. The one exception was MexObr07_FI_CS, where the RAC875 allele increased grain yield but decreased thousand grain weight. That allele must have had a favourable effect on one or both of the other two yield components, the number of tillers per m^2 and/or the number of grains per tiller. Lines

with the RAC875 allele were apparently better able to exploit the favourable conditions of this irrigated environment to establish and retain more tillers and/or grains, increasing yield without affecting grain size.

In addition to heat and drought stress, several crop management factors could explain the opposite allelic effects including: (1) sowing density (200/m^2 in Australia versus 133/m^2 in Mexico) which could affect competition among plants, (2) water supply (sprinkler versus flooding irrigation), (3) soil composition, with a light fertile soil in Australia (McCord and Payne 2004) and a deep fertile soil in Mexico (Northwest of Mexico, Valle del Yaqui, CIMMYT) (Deckers et al. 2009), (4) field management (raised beds to avoid anoxia in Mexico versus flat plots in Australia) (5) biotic stresses (pathogens, pests or weed competition), or abiotic stresses other than drought and heat (e.g salt or heavy metals) and (6) differences in photoperiod due to differences in latitude and/or sowing date. Some of these differences could have provided contrasting advantages for different types of root systems (Palta et al. 2011). For example, the deep soil and flood irrigation in Mexico could have benefited plants with deep roots whereas this trait was unlikely to provide significant advantages on the shallow soils and with intermittent rainfall in Australia.

Both water deficit and high temperature can affect the water flow through the plant. These stresses (individually or combined) are reflected in the water status of the plants (Tardieu and Davies 1993). Canopy temperature is also a good indicator for water status; differences in canopy temperature have been associated with the same genetic region on 3BL in RAC875/Kukri population by Bennett et al. (2012a) and also in another wheat population (Pinto et al. 2010). Other measurements such as stomatal conductance or leaf water potential can also represent plant water status. In other crops, such as rice (*Oryza sativa* L.), QTL associated with both leaf water potential and grain yield have been identified under different water regimes (Qu et al. 2008). Jongdee et al. (2002) proposed leaf water potential as a selection criterion for improving drought tolerance. In the present study, based on measurement of daytime leaf water potential taken at flowering time in five experiments (AusUrr09_SI-S_LS, AusUrr09_SI-W_LS, AusUrr09_SI-D_LS, MexObr11_DI_CS and MexObr11_FI_LS) the grain yield advantage provided by the RAC875 allele was greatest in environments with severe water stress (-2 to -3.8 MPa in Mexico versus -1.4 to -0.6 MPa in Australia) (Appendix 2.4).

In the RAC875/Kukri population, the early vigour QTL was collocated with the grain yield QTL in stressed and non-stressed environments. Early vigour has been reported to be a beneficial adaptive trait for warm and dry climates (Ludwig and Asseng 2010), leading to strong plants throughout the growing season. Early vigour in shoots has been found to increase root growth (Watt et al. 2005, Richards et al. 2007) and nitrogen uptake (Liao et al. 2006). However, early vigour does not necessarily lead to high yield. It is generally associated with high biomass, which can be unfavourable if drought occurs during grain filling (terminal drought) since large leaf area can lead to high rate of water loss under high temperatures.

The multi-environment QTL analysis methods used here combine a multiplicative FA approach to model the genotype by environment interaction of grain yield and yield related traits whilst simultaneously estimating individual marker effects across environments. By accurately capturing the genetic correlation of the DH and RI lines across the environments this approach becomes a powerful tool for estimation of QTL, such as the QTL studied here, as well as assisting in determining the correct parental allele for target environments.

From the Wald statistic profile along chromosome 3B it was clear that a narrow genetic region had pleiotropic effects on grain yield, thousand grain weight and early vigour (Figure 2.3). The effects of each marker within this region were then evaluated across 21 experiments (environments) using data from both DH lines and RI lines (Figure 2.4 and Figure 2.5). The analysis showed that this region plays an important role in determining grain yield in a range of environments and is likely to be a useful target for selection in breeding programs. The MET analysis incorporated new loci (*gwm1266*, *cfb43*, *gwm299*, and *wmc236*) that were not available for the analysis conducted by Bennett et al. (2012a). These new markers revealed positive effects of the RAC875 allele on grain yield in two experiments in Australia (AusPie07_NI_CS and AusMin07_NI_CS) and a negative effect of the RAC875 allele on thousand grain weight in Mexico (MexObr07_FI_CS) (Fig 4).

After initial genetic analysis using the mapping population of DH lines, selected RI lines were phenotyped for validation of allele effects, to narrow down the genetic region and to permit the identification of candidate genes for functional analysis. The starting point of this analysis was a region of over 20 cM (Bennett et al. 2012a). The inclusion of additional markers in the multi-environment analysis allowed the region to be narrowed down to

around 5 cM. The analyses of stress responses of RI lines confirm the significance of the region. While the nature and the function of the gene underlying this QTL remain unknown, addition of new markers to the linkage map has been useful to select the associated genetic region and to track it with reliable molecular markers (Figure 2.3 and Figure 2.4).

Despite evidence for substantial genetic variation in response to water deficit (Reynolds et al. 2009b), it is difficult to attribute this variation to specific genes. Many studies have attempted to define genomic regions associated with drought and/or heat responses in Triticeae crops and there is now a substantial body of literature describing regions of potential relevance; in bread wheat (Mathews et al. 2008; Mason et al. 2010; Alexander et al. 2012), in durum wheat (Peleg et al. 2009) and in barley (von Korff et al. 2008; Chen et al. 2010). The region of chromosome 3BL that contains the marker locus *gwm299* has often been reported as associated with quantitative traits related to tolerance to abiotic stress (heat and/or drought) or biotic stress (pathogenic fungi or nematodes) (Appendix 2.5). This collocation of QTL may be due to multiple important genes in this region or to gene(s) with pleiotropic effects. Improvements in marker technologies and in methods for analysis of genetic and environmental data have allowed us to revisit this chromosome region.

These results, coupled with ongoing wheat whole-genome sequencing (http://www.wheatgenome.org/) provides a promising foundation for positional cloning of the QTL affecting grain yield under moderate to extreme drought and heat stress. Isolation of putative candidate gene(s) underlying this QTL will require a better understanding of gene content and order in this region by using the ongoing gene annotation of chromosome 3B sequence (http://www.wheatgenome.org/content/view/full/407). Further, dissection of the genotype by environment interactions and the effects of alleles might be obtained through additional field experiments with precise quantification of the environmental characteristics including soil factors (moisture, composition and depth), drought severity and air temperatures. Careful genetic characterisation of the impact of this chromosome region on grain yield across a range of environments will permit assessment of the usefulness of this locus for wheat improvement through marker assisted selection.

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Chapter 3

Chapter 3: Fine mapping of a yield QTL on chromosome 3B in Gladius/Drysdale bread wheat population

I. Introduction

Chapter 2 reported a QTL associated with grain yield under heat and drought conditions. Another bi-parental bread wheat recombinant inbred line population was tested under various environmental conditions in Australia and in Mexico (Table 3.1). A previous QTL analysis (Lance Maphosa, Pers. Comm. ACPFG) showed a QTL in the same genetic region as reported in Chapter 2 on chromosome 3B. Interestingly, the location of the QTL was similar.

The research described here focussed on a QTL located on chromosome 3BL. The genetic region seemed to coincide with results previously detected in various populations of bread and durum wheat (Chapter 2, Pinto et al. 2010, Golabadi et al. 2011) grown under diverse environmental conditions including heat and drought stress. A recombinant inbred line (RIL) population derived from a cross between two drought-tolerant wheat cultivars Gladius and Drysdale was used for drought and heat QTL detection. Little is known on heat tolerance of Drysdale although Gladius has been described as heat-tolerant by breeders (Jefferies, Pers. Comm.). This wheat population has the potential to provide information that could be relevant for increasing yield around Australia since the cultivars represent two wheat production regions where Gladius is from South Australia and Drysdale from New South Wales. The environmental differences such as shallow versus deep soil and the contrast of Mediterranean climate versus warm temperate climate could potentially generate varieties tolerant to multiple abiotic stresses. Phenotypic data on grain yield, thousand grain weight and grain morphology traits were kindly provided by Dr Matthew Reynolds (CIMMYT), Dr Livinus Emebiri (New South Wales Department of Primary Industries) and Lance Maphosa (Australian Centre for Plant Functional Genomics). The aims of the work described in this chapter were to investigate further this region by increasing the density of markers, assigning markers to physical contigs and run a MET QTL analysis which could help validate this QTL and investigating further the genetic region.

II. Materials and Methods

1. Plant Materials

The materials used was two sets of 250 wheat (*Triticum aestivum* L.) recombinant inbred lines (RIL) of a cross between wheat cultivar Gladius (RAC875/Krichauff//Excalibur/Kukri/3/RAC875/Krichauff/4/RAC875//Excalibur/Kukri) and the wheat cultivar Drysdale (Hartog*3/Quarrion). The lines were selected from a total population of 5000 lines (Fleury et al. 2010). The first population included 250 randomly selected lines. A SNP genetic map was used to complement information in genetic mapping. Within these two sets of 250 lines, 130 lines were in common.

a. Three genetic maps of chromosome 3B

Map1 was revised by Lisle and Eckermann (Pers. Comm., SAGI) based on the first set which included 250 lines. This map was built based on genotypic data from simple sequence repeat (SSR) and DArT (Diversity Arrays Technology Pty Ltd, Canberra, Australia) markers that were assayed across the population. Map 2 was constructed using genotypic data from the Wheat 9k SNP assay chip assayed on another set of 250 lines. The Wheat 9k SNP assay including marker names and sequences is available at http://wheat.pw.usda.gov/. The construction and curation of the Map 2 was performed by Taylor Julian (Pers. Comm., SAGI). Map 3 combined Map 1 and Map 2 which includes 113 common lines. Map 3 was generated using IciMapping software developed by Li et al. (2008) available at http://www.isbreeding.net. The ordering of markers was performed and the genetic distances were re-evaluated using the Kosambi algorithm.

b. Photoperiod and vernalization genes

The lines from the first set of 250 lines from the Gladius/Drysdale RIL population studied in this research were screened for allelic variation for four segregating loci (*Ppd-D1*, *Ppd-B1*, *Vrn-A1* and *Vrn-D1*). The genotyping data was kindly provided by Lance Maphosa (Australian Centre for Plant Functional Genomics) and implemented in the statistical model to reduce the confounding effect of phenology as described in Chapter 2 of this thesis.

c. Polymorphic markers on chromosome 3B

Based on comparison of the "neighbour map" of chromosome 3B (Paux et al. 2008) additional markers were selected for parental screening. For some of these markers, primer sequences were not publicly available. For these markers, aliquots of primers were obtained from INRA (Pierre Sourdille, Pers. Comm., markers with the prefix "gpw" and "wmm"). Each marker that was detected as polymorphic between the parental lines (Appendix 3.1), was then assayed on each of the 250 RIL (Pop1) to enhance the density of markers on 3BL using the M13-tailed primer method developed by Oetting et al. (1995). Each marker screened across this population was then added to the map using IciMapping software. With this software the order was re-performed and the genetic distance was re-calculated using Kosambi algorithm.

d. Contig assignment

In *silico*, the sequences of six SNP markers named m6930, m4311, m4312, m8185, m6273 and m3159 (Appendix 3.2, Matthew Hayden, Pers. Comm.) and three DArT markers (wPt-1870, wPt-0021 and wPt-2391) (available at http://www.triticarte.com.au/) were compared to the entire database of 3B sequences using BLASTN to assign these markers on physical contig. The physical map that includes the contig details using the wheat physical map viewer v4 is available at http://urgi.versailles.inra.fr/Species/Wheat. The limit of acceptance of assignment was based on the percentage of similarity (> 96%) and the final percentage of matches (80 to 100%) between the query (the SNP or the DArT sequences) and the hit from the 3B sequence database. The function blastall from Blast 2.2.20 (http://www.ncbi.nlm.nih.gov/) was used for this purpose. The molecular markers not assigned in the genetic neighbour map were screened by PCR on the minimal tiling path (MTPv2) described by Rustenholz et al. (2011). Multi-locus markers (showing more than one amplicon) were ignored to facilitate contig assignment therefore only single locus markers were screened.

e. Marker design

Using the physical map of chromosome 3B published by Paux et al.(2008) a total of 31 markers (cfb500 to cfb530) were designed on contig sequences anchored in the region of interest to confirm the position of the contig and/or to anchor them in the genetic map of the population of Gladius/Drysdale Pop1. The program SSR*finder* ("homemade script" by

Frederic Choulet, INRA Clermont-Ferrand) was used to design SSR markers based on the genomic sequence from Chinese Spring used in the chromosome 3B sequencing project (http://urgi.versailles.inra.fr/Projects/3BSeq; unpublished data).

2. Description of Environments and phenotyping

Phenotypic data from the five experiments (Table 3.1) described in this study were used for single environment QTL and multi-environment QTL analysis. In each of these experiments, the entire population (Pop1) of 250 RILs was phenotyped. In north-western Mexico at a managed-environment field site (CIMMYT, Ciudad de Obregon, 27°25'N 109°54'W, 38 m above sea level), the experiment (MexObr10_DI_CS for Mexico – 2010 – drip irrigation – conventional sowing) was sown in December 2009. During the first two months, the plants received around 150 mm of water from a drip irrigation system. This experiment included 264 entries including the population (250), the two parental lines (Gladius and Drysdale) and 12 released varieties (Samayoa_C2005, Kukri, Excalibur, Espada, Mace, Krichauff, Ventura, Waagan, Janz, Diamondbird, EGAGregory and Sokoll). A two-replicate alpha-lattice design was used where each plot consisted of a raised bed with two-meter rows separated from each other by 30 cm. The soil type at the experimental station is coarse sandy clay, mixed montmorillonitic type calciorthid, with low organic matter and light alkaline where the effective soil depth is around 120 cm (Deckers et al. 2009; Olivares-Villegas et al. 2007).

The four experiments conducted at the Leeton experimental station (New South Wales) included 285 entries in both experiments conducted in 2009 (AusLee09_NI_CS and AusLee09_NI_LS) and 270 entries in 2010 (AusLee10_NI_CS and AusLee10_NI_LS). All experiments included Gladius and Drysdale repeated several times as well as 12 varieties (Excalibur, Espada, Mace, Krichauff, Ventura, Waagan, Janz, Diamondbird, Gregory and Wentworth).

The experiments in 2009 and 2010 (AusLee09_NI_LS and AusLee10_NI_LS) were sown late in the season (August) to study the effect of heat on grain yield and grain quality (Lance Maphosa, Pers. Comm.). The two experiments AusLee09_NI_CS and AusLee10_NI_CS were sown conventionally (Mid-June) and conducted under irrigation by flooding. The soil at the experimental station in Leeton (NSW) is classified as grey, self-mulching clay, described as a vertisol under the Australian Soil Classification (Isbell 1996), with a risk of acidity. The

Table 3.1 Description of environments with their abbreviations, localisation, number of lines tested, sowing density, rainfall for Gladius/Drysdale population Pop1

Environment[a]	**Location**	**Latitude**	**Longitude**	**Altitude (M)**	**Lines tested** [b]	**seeds density** [c]
MexObr10_DI_CS	*Ciudad de Obregon (Mexico)*	27°28' N	109°56' W	38	250 RIL	200
AusLee09_NI_CS	*Leeton (NSW)*	34°57' S	146°41' E	140	250 RIL	200
AusLee09_NI_LS	*Leeton (NSW)*	34°57' S	146°41' E	140	250 RIL	200
AusLee10_NI_CS	*Leeton (NSW)*	34°57' S	146°41' E	140	250 RIL	200
AusLee10_NI_LS	*Leeton (NSW)*	34°57' S	146°41' E	140	250 RIL	200

a Meaning of abbreviations : Aus Australia, Mex Mexico, 09-10 2009 to 2010, DI drip irrigation , NI irrigated and rain fed, D drought, LS late sowing, CS conventional sowing.
b recombinant inbred line
c number of seeds per square meter

soil is also described as fertile with effective soil depth around 40-60 cm. The mean annual rainfall in Leeton is 432 mm (www.bom.gov.au). Five traits were selected for the purpose of this study including grain yield, thousand grain weight, grain length, grain roundness and grain thickness. All grain measurements were described in Maphosa (2012).

3. Statistical Analysis

Prior to statistical analysis the genotype of each RIL for each marker was coded as 1 (homozygous for the Gladius allele), -1 (homozygous for the Drysdale allele) and 0 (heterozygous for both Gladius and Drysdale allele). Missing values were imputed using the flanking marker algorithm of Martinez and Curnow (1992).

a. Single environment single trait analysis

The methods used to analyse the data from the field followed the models outlined by Glimour et al. (1995) to reduce spatial effects in field experiments. For each trait in each environment, a mixed linear model using the residual maximum likelihood was performed in program R using ASReml (package) as described in Chapter 2 of this thesis. Single environment, single trait QTL analysis was performed using an extension of the mixed linear model using the R package WGAIM developed by Taylor and Verbyla (2011). Through this model the heritability (h_g^2) for each trait was calculated following the formula developed by Cullis et al. (2006) and Oakey et al. (2006).

$$h_g^2 = 1 - \frac{PEV}{2\sigma_g^2}$$

Where PEV correspond to the average pairwise prediction error variance of the BLUPs and σ_g^2 to the genetic variance. To reduce confounding effects of major genes that co-segregate in this population, the genotype information for *Vrn-A1*, *Vrn-D1* and *Ppd-D1* and *Ppd-B1* were added to each of the models as fixed effects.

b. Multivariate environment single marker QTL analysis

The QTL analysis was performed using an extension of the mixed linear model including the whole genome scan as well as the interactions of QTL with environment. The method used in this chapter followed the same method used in Chapter 2. The only exception was the integration of the four flowering genes (instead of two) in the model as fixed factors to reduce their effects in the analysis.

III. Results

1. Grain yield means and trait heritability, correlation between traits among environments

The mean grain yield for the population including 250 RIL (Pop1) tested in four environments varies from 1.79 to 5.51 t/ha where the lowest yield was in the late sowing environment (AusLee09_NI_LS) and the highest grain yield was in the irrigated experiment conducted in 2010 in NSW (AusLee10_NI_CS) (Table 3.2). The heritability for all traits (grain yield, thousand grain weight, grain roundness, thickness and length) was high and varied from 0.76 to 0.93 (Table 3.3).

The correlation of grain yield between environments showed a weak correlation between AusLee10_NI_CS and the three other environments. AusLee09_NI_LS and MexObr10_DI_CS were highly correlated to each other with a correlation of 0.73. The two experiments conducted in Leeton in 2009 (AusLee09_NI_LS and AusLee09_NI_CS) showed a correlation close to 0.5. Thousand grain weight presented a high correlation among the three experiments including AusLee09_NI_CS, AusLee10_NI_CS and MexObr10_DI_CS (Table 3.3). The grain characteristic traits, grain length, thickness and roundness, were highly correlated between the two sites where the data was collected (AusLee09_NI_CS and AusLee10_NI_CS).

Table 3.2 Lines of Gladius/Drysdale evaluated for mean grain yields of the population, parental line performance, and heritability estimates for grain yield and other traits in each of environments where the data were collected.

Environment[a]	Lines Evaluated	Mean grain yield kg ha^{-1}			Heritability[c]				
		Population	Gladius	Drysdale	Grain yield	Grain roundness	Grain thickness	Grain length	Thousand grain weight
MexObr10_DI_CS	250 RIL	2.87	2.84	3.45	0.82	-	-	-	0.81
AusLee09_NI_CS	250 RIL	4.28	5.06	4.47	0.80	0.84	0.78	0.93	0.83
AusLee09_NI_LS	250 RIL	1.52	1.79	1.49	0.67	-	-	-	-
AusLee10_NI_LS	250 RIL	3.31	3.61	3.57	0.81	-	-	-	-
AusLee10_NI_CS	250 RIL	5.37	5.51	5.47	0.73	0.76	0.82	0.89	0.81

a Meaning of abbreviations : Aus Australia, Mex Mexico, 09-10 2009 to 2010, DI drip irrigation , NI irrigated and rain fed, D drought, LS late sowing, CS conventional sowing.

Table 3.3 Genetic correlation across environments where the phenotypic data were collected

Trait	**Environment**	*MexObr10_DI_CS*	*AusLee10_NI_LS*	*AusLee09_NI_LS*	*AusLee10_NI_CS*
Yield	MexObr10_DI_CS	1			
	AusLee10_NI_LS	0.504779	1		
	AusLee09_NI_LS	0.416324	0.559332	1	
	AusLee10_NI_CS	*0.308748*	*0.23857*	*0.224132*	1
	AusLee09_NI_CS	0.736536	0.546496	0.466052	0.424476
Thousand grain weight	MexObr10_DI_CS	1			
	AusLee10_NI_LS	-	1		
	AusLee09_NI_LS	-	-	1	
	AusLee10_NI_CS	0.766312	-	-	1
	AusLee09_NI_CS	0.838742	-	-	0.661697
Thickness	MexObr10_DI_CS	1			
	AusLee10_NI_LS	-	1		
	AusLee09_NI_LS	-	-	1	
	AusLee10_NI_CS	-	-	-	1
	AusLee09_NI_CS	-	-	-	0.696084
Roundness	MexObr10_DI_CS	1			
	AusLee10_NI_LS	-	1		
	AusLee09_NI_LS	-	-	1	
	AusLee10_NI_CS	-	-	-	1
	AusLee09_NI_CS	-	-	-	0.894774
Length	MexObr10_DI_CS	1			
	AusLee10_NI_LS	-	1		
	AusLee09_NI_LS	-	-	1	
	AusLee10_NI_CS	-	-	-	1
	AusLee09_NI_CS	-	-	-	0.907732

The correlation between grain characteristic traits showed that grain roundness and grain thickness were negatively correlated in both environments (AusLee09_NI_CS and AusLee10_NI_CS) whereas the length was positively correlated to grain roundness but uncorrelated to grain thickness (Appendix 3.3). The analysis of temperature along the crop cycle for the two irrigated experiment in 2009 and 2010 showed two periods when the average temperature per day was higher than 30°C (Appendix 3.4).

2. Single site analysis using WGAIM

The single site QTL analysis associated five genetic locations with at least one trait including loci on chromosomes 3A, 3B, 4A, 4B and 6A (Figure 3.1). Three QTL for grain yield in the experiment AusLee09_NI_CS were found on 4B, 3B and 6A. Only one grain yield QTL was detected in the environment MexObr10_DI_CS located on the long arm of chromosome 3B. Two QTL for grain roundness where found on 4B and 6A in AusLee09_NI_CS only. The QTL on 6A for grain yield and grain roundness co-located with the locus *GW2*. Finally grain thickness was found only on chromosome 3A in AusLee09_NI_CS. No QTL (> LOD 3) were found for either grain length or thousand grain weight. In both experiments AusLee09_NI_LS and AusLee10_NI_CS where either thousand grain weight, grain yield, grain roundness, grain thickness and grain length were measured, no QTL were found.

3. Multi-environment single marker whole genome QTL analysis

The Wald statistic profile of single marker whole genome QTL analysis across environments where the trait were measured (Figure 3.4) identified one QTL for grain yield on chromosome 3BL where the genetic interval based on the Wald Statistic threshold correspond to the genetic region of 4 cM between loci *wmm1758* and *cfb511* ((a) of Figure 3.4). For thousand grain weight, two QTL where found on 4A and 6B; one QTL for grain thickness on 6A; two QTL for grain length on 1B2 and 7B and finally two QTL for grain roundness on 2B and 6A.

4. Allele effect on chromosome 3B

Focusing on chromosome 3B for the purpose of this chapter, the allele effect showed a positive contribution of the Drysdale allele for grain yield, thousand grain weight, and

thickness (Figure 3.3). However, the Gladius allele showed a positive contribution to grain roundness. The allele effect for grain yield showed one locus, *wPt-1870,* which increased grain yield from 4.1 to 7.5% in four environments AusLee09_NI_CS, AusLee09_NI_LS, AusLee10_NI_LS and MexObr10_DI_CS. The allele effect for thousand grain weight presented two regions along chromosome 3B in MexObr10_DI_CS, where the region on the long arm of the chromosome 3B was similar to the region found in the AusLee09_NI_CS environment. The highest allele effect corresponds to the same locus as grain yield, *wPt-1870*. The allele effect for grain roundness and grain thickness showed the same region being associated with both traits using a bi-environmental analysis but with opposite effect. The Gladius allele was positively contributing to a higher roundness score while the Drysdale allele contributed higher grain thickness. The locus that had the highest association with both traits was *wmc236.* The analysis was performed across the whole genome where the allele effect was calculated for each marker, each trait in each environment (not shown).

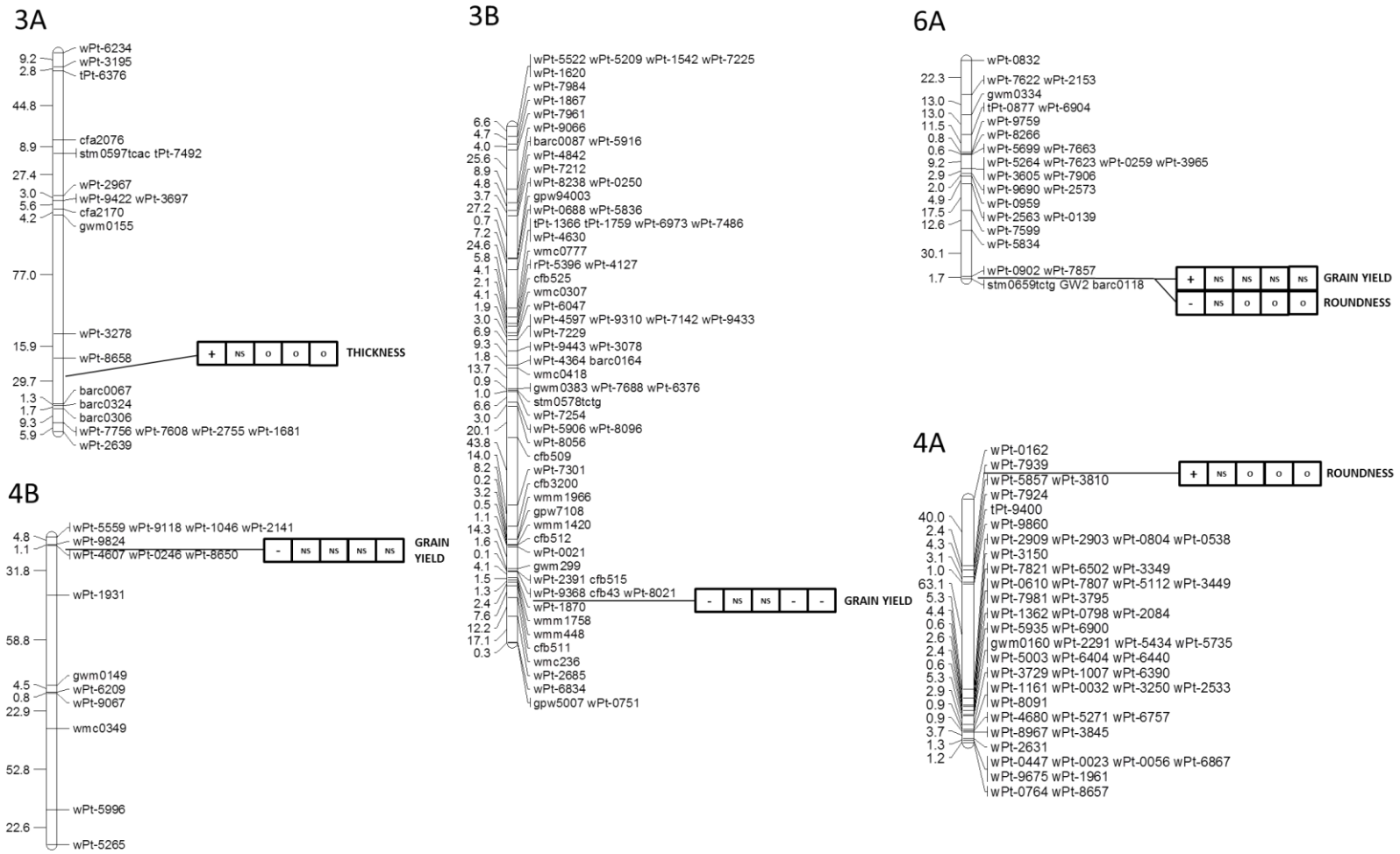


Figure 3.1 QTL detected across single site analysis using WGAIM on chromosome 3A, 3B, 4A, 4B and 6A. The five squares represent the environments in the following order (1) AusLee09_NI_CS, (2) AusLee09_NI_LS, (3) AusLee10_NI_CS and (4) MexObr10_DI_CS (5) AusLee10_FI_LS. The positive and negative sign indicates the favourable allele carried by Gladius and Drysdale respectively, NS means non-significant and finally 'o' means the data were not collected.

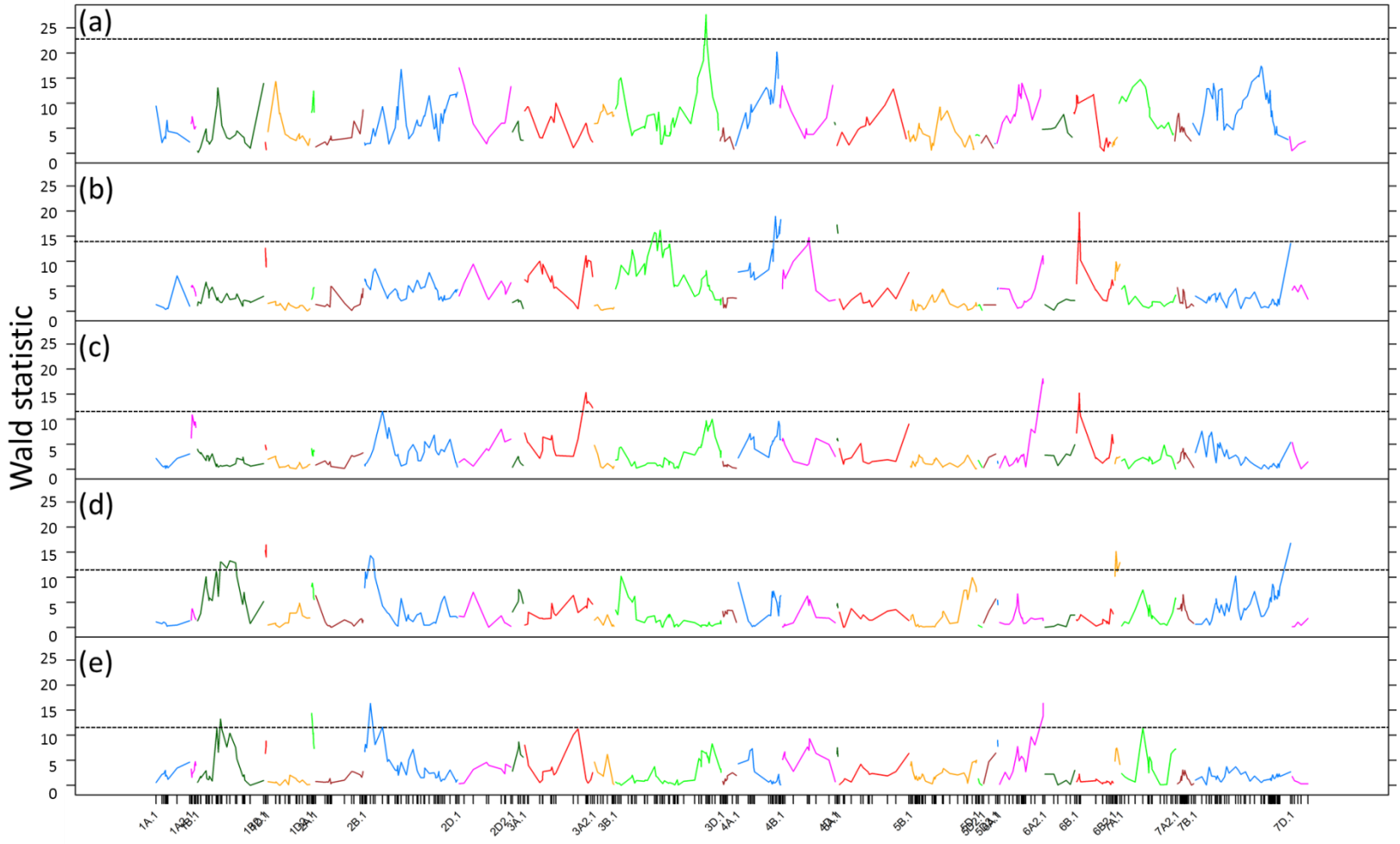


Figure 3.2 Wald statistic profile across environments at the whole genome scale for the five traits (a) grain yield including 4 environments, (b) thousand grain weight including 3 environments, and grain characteristics in only two environments (c) grain thickness, (d) grain length and (c) grain roundness. The different individual linkage groups are indicated on the X axis

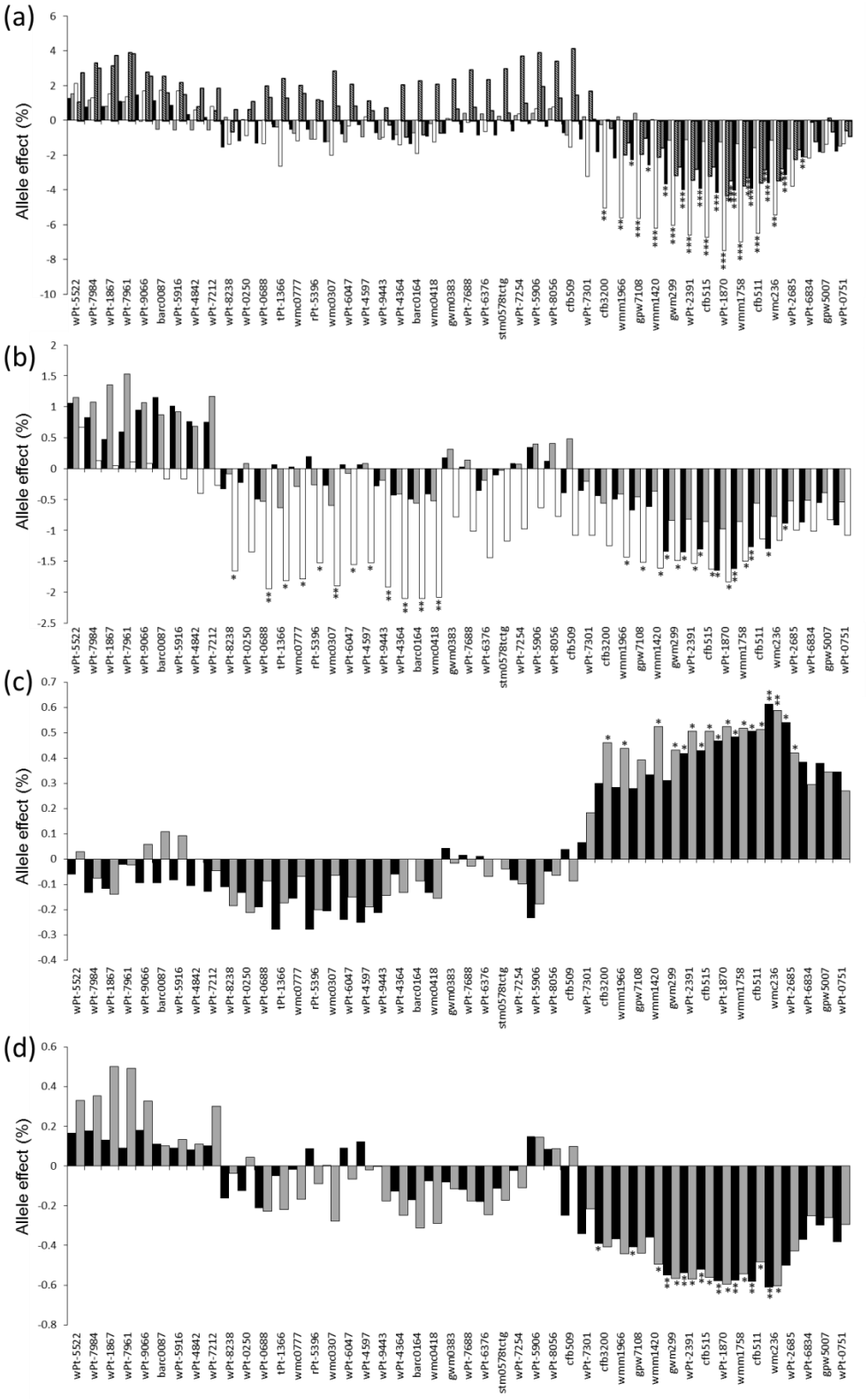


Figure 3.3 Allele effects of all loci along chromosome 3B for: (a) grain yield (b) thousand grain weight (c) grain roundness and (d) grain thickness. Allele effects are expressed as percentage relative to the trait mean. A positive effect indicates that the Gladius allele increased the trait value while a negative effect indicates that the Drysdale allele increased the trait value. Levels of significance are represented by: * p <0.05, ** p <0.01, *** p <0.001.

5. Mapping of additional markers on chromosome 3B

Given the emphasis on chromosome 3B, 15 new markers were added (Figure 3.4). The loci *cfb512*, *cfb515* and *cfb511* confirmed the location of the three contigs ctg1094, ctg730 and ctg1689 based on the neighbour updated map available at http://urgi.versailles.inra.fr/Species/Wheat. The genetic map of chromosome 3B which includes the three types of markers SSR, SNP and DArT allowed visualization of the physical assignment for 13 physical contigs representing the region between loci *cfb3200* and *wmc326* of approximately 10.6 Mbp with a genetic distance of 27.7 cM equivalent to 0.4Mbp per cM.

6. Contig assignment

Five additional contigs were anchored to the genetic map by comparison in *silico* of the six SNPs and the three DArT sequences on 3B. These included ctg2680, ctg61, ctg274 and ctg2430/ctg1793 (Table 3.4 and Figure 3.4). The sequences used for alignment using BLASTN were covered by 83.8% to 100%. The six SNPs and the three DArTs markers were mapped within the region of interest and the physical assignment resulted in the addition of three different contigs summarized in Table 3.4. Two SNPs (m8185 and m6273) were assigned to a contig already located to the region of interest, ctg1691. As well as these two SNPs, *wPt-2391* was assigned to a previous contig, ctg660. Finally, the two SNP (m4311 and m4312) were designed from the same sequence, wsnp_Ex_c56591, and were therefore assigned on the same contig, ctg61. The marker *gpw7108* was assigned on the same contig as *wmm1966*, ctg1216. Finally, *wmc236* was assigned to three contigs; ctg647 (1.5 Mbp), and two small contigs ctg975 and ctg1648 (0.23 and 0.21 Mbp respectively, not shown). In total 15 contigs ranging from 217 kb (ctg1689) to 1.500 kb (ctg647) were identified covering a region of at least 11 Mb of the region studied.

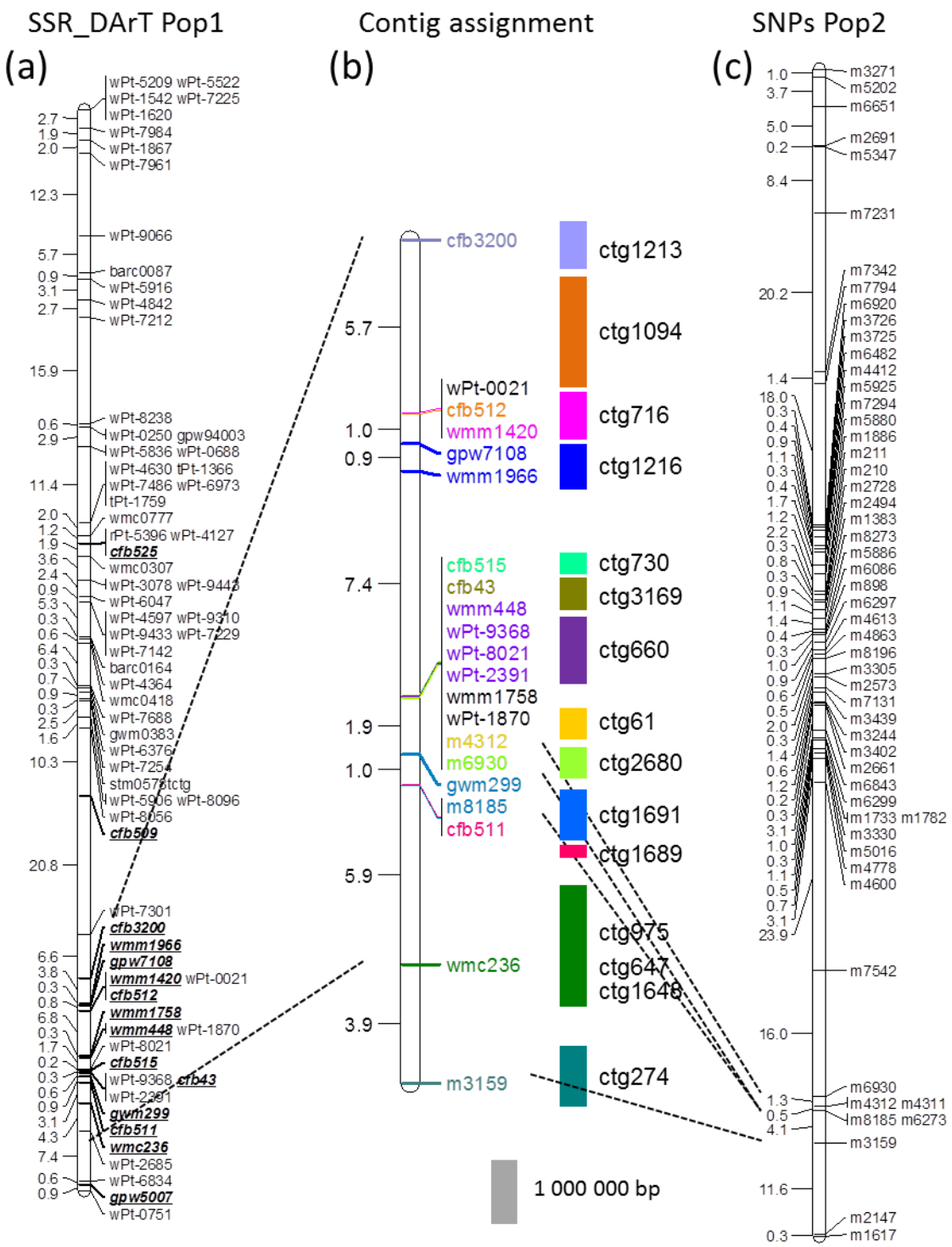


Figure 3.4 Three genetic maps of chromosome 3B: (a) Implemented map of chromosome 3B from Pop1 including new markers (bolded) from the literature and new SSR primers 'cfb5- -', (b) partial genetic map of 3B including 113 genotypes where SSR, DArT and SNP where screened and assigned to physical contigs (ctg) the colour code correspond to physical assignment of markers to their respective contigs , (c) SNP genetic map of 3B including SNP markers from the Wheat 9k SNP assay chip (the sequence names are in Appendix 3.2).

Table 3.4 Summary of the comparison of the 6 SNPs (sequence name in Appendix 3.2) and three DArTs sequences to the 3B sequence database using BLASTN

Mapping names	Contig sequence	contig	percentage of similarity	length of alignment	NOM[a]	NOG[b]	size of alignment	e value	percentage covered	Total
m6930	LG_03	ctg2680	99.82	549	1	0	548	0	39.28	
m6930	LG_03	ctg2680	97.3	407	9	2	406	0	29.10	
m6930	LG_03	ctg2680	99.72	362	1	0	361	0	25.88	94.26
m4311/m4312	BCN_02	ctg61	99.14	463	4	0	462	0	64.25	
m4311/m4312	BCN_02	ctg61	100	129	0	0	128	4.00E-66	17.80	
m4311/m4312	BCN_02	ctg61	100	127	0	0	126	6.00E-65	17.52	99.58
m8185	IV_01	ctg1691	98.18	548	7	2	547	0	26.32	
m8185	IV_01	ctg1691	99.33	298	2	0	297	7.00E-163	14.29	
m8185	IV_01	ctg1691	96.12	309	12	0	308	2.00E-144	14.82	
m8185	IV_01	ctg1691	99.2	250	2	0	249	5.00E-133	11.98	
m8185	IV_01	ctg1691	97.85	186	4	0	185	5.00E-90	8.90	
m8185	IV_01	ctg1691	98.63	146	2	0	145	6.00E-71	6.98	
m8185	IV_01	ctg1691	100	108	0	0	107	5.00E-53	5.15	
m8185	IV_01	ctg1691	96.52	115	4	0	114	1.00E-47	5.48	
m8185	IV_01	ctg1691	98.13	107	2	0	106	1.00E-47	5.10	99.04
m8185	IV_01	ctg1691	89.16	83	7	1	80	8.00E-15	**overlap**	
m6273	IV_01	ctg1691	99.26	542	1	2	538	0	85.80	
m6273	IV_01	ctg1691	100	91	0	0	90	2.00E-43	14.35	100.15
m6273	IV_01	ctg1691	89.16	83	7	1	80	2.00E-15	**overlap**	
m3159	DD_04	ctg274	100	476	0	0	475	0	30.37	
m3159	DD_04	ctg274	99.75	398	0	1	396	0	25.32	
m3159	DD_04	ctg274	100	167	0	0	166	3.00E-88	10.61	
m3159	DD_04	ctg274	100	116	0	0	115	7.00E-58	7.35	
m3159	DD_04	ctg274	100	108	0	0	107	4.00E-53	6.84	
m3159	DD_04	ctg274	100	99	0	0	98	1.00E-47	6.26	
m3159	DD_04	ctg274	100	91	0	0	90	6.00E-43	5.75	
m3159	DD_04	ctg274	100	51	0	0	50	4.00E-19	3.20	
m3159	DD_04	ctg274	100	38	0	0	37	2.00E-11	2.36	
m3159	DD_04	ctg274	100	37	0	0	36	9.00E-11	**overlap**	
m3159	DD_04	ctg274	100	35	0	0	34	1.00E-09	2.17	100.25
wPt-0021	BCF_12	ctg2430	100	514	0	0	514	0	100	
	BCQ_01	ctg1793	99.81	514	1	0	514	0	100	
wPt-2391	ER_06	ctg660	99.15	586	5	0	586	0	100	
wPt-1870	BCN_03	Ctg61	83.8	463	49	7		4.00E-81	83.8	

a Number of mismatches
b number of gaps

IV. Discussion

The timing and nature of environmental stresses such as heat and/or drought interact with the complex genetic control of plant life cycle to determine grain yield. In previous studies in wheat, QTL have been reported for loci associated with grain yield and yield-related components under diverse environmental conditions (summarised in Appendix 2.5 from Chapter 2 of this thesis). The objectives of the study reported here were:

- Identify QTL associated with total grain yield and grain characteristics such as thousand grain weight, grain length, thickness and roundness. In particular common QTL were sought as a route to simplifying phenotyping.
- Increase the density of markers in the genetic region of interest, anchor genetic markers to the physical contig and deliver marker information for marker assisted selection.
- Provide additional information for fine mapping as a basis for positional cloning of QTL associated with increase yield under heat and/or drought-stress.

1. Multivariate single marker QTL analysis

The region of interest on 3BL was associated with grain yield in four environments, including drought conducted in Mexico, irrigated and heat trials conducted in Leeton, New South Wales in 2009. The absence of the QTL in this region in the experiment conducted in Leeton in New South Wales in 2010 could be associated by good growing conditions in this trial reflected by the grain yield average of 5.37 t/ha. This contrasts to the conditions in 2009 trial where yield was reduced by more than 1 t/ha. This suggests that the allelic difference at this locus does not play an important role under good growing conditions but does exert an effect if plants are grown under stress such as heat AusLee09_NI_LS, AusLee10_NI_LS or drought MexObr10_DI_CS. The presence of the QTL effect in AusLee09_NI_CS is interesting. Under well irrigated conditions, the expected result should have been the same as in AusLee10_NI_CS. However the average temperature for the growth season in 2009 was unusual with the average daily temperature maxima in September and October sometimes higher than 30 degrees. Overall the average temperature at the Leeton experimental station around flowering time and grain filling was much higher in 2009 than in 2010 (http://www.bom.gov.au/climate/), up to 10 degrees.

Since the optimal growth temperature for wheat is between 18 to 24°C (Stone and Nicolas 1994). The plants grown in AusLee09_NI_CS were therefore subject to heat stress, suggesting that the QTL on chromosome 3B is not only drought but also heat responsive. The same QTL was observed in the same year for the experiment where the lines were sown late to expose them to high temperatures late in the growing season. Thousand grain weight showed the same response as grain yield where the allele from Drysdale was favourable. Not surprisingly, thousand grain weight contributed to the final grain yield. Unfortunately thousand grain weight was not measured for the AusLee09_NI_LS trials. This could have provided useful confirmation of the QTL being expressed in stressed environments with the favourable allele carried by Drysdale. This conclusion assumes that AusLee09_NI_CS received high temperature stress.

The results for thousand grain weight measurements revealed two regions controlling this trait in the MexObr10_DI_CS trial indicating the possibility of two loci from Drysdale improving thousand grain weight in droughted environment. The same region on the long arm of chromosome 3B was detected in AusLee09_NI_CS which could indicate its value under well watered condition but where a heat stress occurs during the crop cycle. Finally no allele effect were found from the measurements made in the AusLee10_NI_CS trial indicating that the QTL only plays an important role in this population if the plants are subject to heat or drought conditions. The allele effects on grain roundness and thickness were studied only in AusLee09_NI_CS and AusLee10_NI_CS. It appeared that the region controlling both traits is similar but opposite in terms of allele effect. In both environments, the Gladius allele was favourable for roundness whereas the Drysdale allele was favourable for thickness. This result indicates the conflict of traits for seed characteristics and end-uses. The different alleles appear to have the capability of increasing grain the thickness and reducing roundness or vice versa.

2. A QTL previously reported

Agronomic QTL in this region of 3B reported previously include tolerance to both biotic and abiotic stresses. (Diab et al. 2008) published a co-located QTL for (Canopy temperature depression) CTD under different environments (irrigated, rain fed), chlorophyll content, carbon isotope discrimination and finally quantum yield that corresponded to photosynthetic efficiency. (Pinto et al. 2010) along with (Bennett et al. 2012b) reported on a QTL for canopy temperature in this region. Two studies found a co-

located QTL associated with grain weight per ear (Huang et al. 2004) and thousand grain weight (Golabadi et al. 2011). This last paper reported that the molecular marker gwm299-3B explained 10% of the genetic variation for thousand grain weight in a durum population under terminal drought stress. A QTL for grain yield was also observed in the same genetic region by Pinto et al. (2010), Maccaferri et al. (2008) and Kumar et al. (2007). Other studies that identified interesting QTL in this region include, harvest index in a high yielding environment was reported by McIntyre et al. (2010) as well as screenings (high and low yielding environments) and finally water soluble carbohydrates in low yielding environment. These results to confirm the importance of this region for yield improvement in diverse climatic conditions. However, it is not clear if the different studies actually identified the same QTL.

3. Importance of increasing the density of markers

From the original map of Pop1 the density of markers was increased by mapping 13 markers to the target region on 3B (between *cfb3200* and *wmc236*). The genetic map of the 113 lines containing the three types of markers (Map (b) in Figure 3.4) improved the physical mapping of the region by physically assigning three DArT and the 6 SNP markers on contigs. The deployment of these markers allowed the assignment of contigs across the whole region. However, the sizes of the gaps between contigs remains unknown and missing contigs are likely to represent missing information on genes. This problem can only be addressed through a better understanding of the structure and detailed organization of the 3B chromosome pseudomolecule. The existing project of the sequencing of chromosome 3B may provide the solution (http://urgi.versailles.inra.fr/Projects/3BSeq; unpublished data). In this chapter, increasing markers density and physical assignment of the 3B region produced a preliminary base to fine map the genetic region of interest. Based on research done previously on chromosome 3B, the region is located in deletion bin 3BL7-0.63-1.00 with a size of 208 Mbp (Saintenac et al. 2009). The region studied here corresponds to 5% of the deletion bin. The gene annotation for chromosome 3B has started but only 13 physical contigs have been completed (Choulet et al. 2010). The completion of the pseudomolecule for chromosome 3B will help to fill gaps and identify candidate genes.

4. Grain characteristics

Improving grain yield is challenging especially in climates where heat and drought stress occur. In addition to grain yield the seed characteristics are important, such as the shape of the grain represented by length, roundness, thickness and width but also by the weight represented by as thousand grain weight. Grain morphology is related to the final grain yield (Kesavan et al. 2012). In this context it is important that the QTL on 3BL was associated with improving grain yield through an influence on thousand grain weight and grain shape. This locus appears to be particularly important in warm and/or dry environments. In wheat, two genes associated with grain size and shape have been identified. The gene *TaGW2* influences the width, weight and ratio of grain length to width and is located on chromosome 6A. This gene encodes a RING-type protein with E3 ubiquitin ligase function (Su et al. 2011). A second candidate gene *TaCKX6-D1* (cytokinin oxidase/dehydrogenase) influences thousand grain weight in wheat and is located on the long arm of chromosome 3D (Zhang et al. 2012). Interestingly, this gene is in the distal region of chromosome 3D and could be an orthologous gene to the chromosome 3B locus. This class of gene was also reported in barley (Zalewski et al. 2010)to increase plant productivity when HvCKX1 was silenced.

A QTL for grain shape has been reported in wheat on 3BL (Gegas et al. 2010) and was associated with thousand grain weight, the area ratio of the grain and the width in 2007 in one DH population (Avalon x Candenza). This QTL is close to the locus *wPt-8021* (mapped in the population studied here).

5. Grain morphology traits for better and faster screening

The presence of the QTL for grain morphology traits such as grain roundness and thickness in the same location as grain yield under stress, could potentially improve the phenotyping since it could be evaluated across a larger number of samples than grain yield. Due to the strong genetic association of these traits, a validation of this QTL could be achieved by simply screening a sample of seeds using the method described by Gegas et al. (2010).

Chapter 4

Chapter 4: In the direction of positional cloning of *qDHY.3BL* for heat and drought tolerance on wheat chromosome 3B

Grain yield improvement under heat and drought is challenging. QTL analysis, fine mapping and wheat genome sequencing information are essential in forward genetics; one concept to tackle the challenge. Here, we report a preliminary list of candidate genes as well as a possible deletion/insertion of a chromosome segment potentially involved in grain yield improvement under high temperature and water deficit. The candidate genes include genes potentially involved in biological pathways (ubiquitination and ABA signalling) that respond to abiotic stresses including heat and drought. By combining information from fine QTL mapping and the physical map and sequence annotation from chromosome 3B significant progress has been achieved towards cloning *qDHY.3BL*.

I. Introduction

Bread wheat (*Triticum aestivum* L.) is one of the most important crops worldwide. It is on about 217 million hectares globally and with 653 million tonnes produced in 2010, it ranked third after maize (840 million tonnes) and rice (696 million tonnes) (www.fao.org).

Wheat production is affected worldwide by many factors, including biotic (bacteria, fungi, nematodes, insects, viruses) and abiotic stresses (including drought, heat, frost, salt, heavy metals…). Farmers, breeders and scientists now also must deal with the effects of climate change, a major factor underlying the stagnation of wheat yields (Brisson et al. 2010; Lobell et al. 2011; Lobell et al. 2012). Climates characterized by heat and/or drought events during crop cycle can dramatically reduce production (Kosina et al. 2007). Therefore, improving grain yield under drought and heat stress is becoming essential. Grain yield is a complex trait due to its genetic architecture (Holland 2007). It is driven by many genes that interact with each other but also interact with the environment (genotype by environment interaction) resulting in low heritability. Consequently, improving and/or maintaining yield requires a good understanding of the underlying genetic control and interactions with the environment (Cooper et al. 2009). QTL analysis and positional cloning of genes involved in drought and heat tolerance has a potential for improving yield under such unfavourable environmental conditions. Several studies in wheat have used QTL analysis to identify genetic regions that can contribute to yield (see recent examples: Alexander et al. 2012; Bennett et al. 2012a; Wu et al. 2012). However, even though it is

possible to use QTL analysis to map chromosomal regions affecting traits, positional cloning of the genes themselves is a real challenge in bread wheat (*T. aestivum* L.).

In the past decades, international efforts have helped in developing genetic and genomic resources to support gene discovery and breeding programs. However, until recently, progress in genomics of bread wheat was slow due to the size of its genome (17Mbp, 40X larger than that of rice (*Oryza sativa* L.)) and its complexity (polyploid). The wheat genome comprises three homologous genomes A, B and D including 7 chromosomes each. The origin of the 3 homologous genomes through successive hybridizations between diploid and tetraploid genomes is relatively well known; for review see (Matsuoka 2011). One of the other challenging aspects is the high amount (>80%) of transposable elements and repetitive elements that increase the difficulty in sequencing and assembling the whole genome. Sequencing minimal tiling paths (Green 2001) of individual chromosomes using next generation sequencing technologies is the strategy adopted by the International Wheat Genome Sequencing Consortium (IWGSC, www.wheatgenome.org) to deliver a reference sequence of the bread wheat genome. The chromosome-based approach relies on the isolation of individual chromosomes and chromosome arms by flow sorting (Doležel et al. 2012) to build BAC libraries. The BAC libraries are then used to construct physical maps that serve as a platform for accelerating map-based cloning. Physical maps are built after fingerprinting and assembly of the BAC clones into contigs. Two fingerprinting methods have proven efficient for building wheat physical maps: the SNaPshot technology (Luo et al. 2003; Paux et al. 2008) and more recently whole genome profiling (Philippe et al. 2012).

Positional cloning in wheat is challenging, and so far less than a dozen of genes have been cloned in wheat (Krattinger et al. 2009) including phenology genes (*VRN1* (Yan et al. 2003), *VRN2* (Yan et al. 2004) and *VRN3* (Yan et al. 2006)), disease resistances genes (*Lr1* (Qiu et al. 2007), *Lr10* (Feuillet et al. 2003), *Lr21* (Huang et al. 2003), *Pm3* (Yahiaoui et al. 2004)), a gene related to polyploidy (*Ph1*) (Griffiths et al. 2006), a gene controlling nutrient content and senescence (*Gpc-B1*) (Uauy et al. 2006) and finally the *Q* gene related to threshing and spike morphology (Faris et al. 2003). In most cases, gene isolation was achieved only after about 10 years of research mainly because of the difficulty of performing chromosome walking in a genome composed mostly of repeated elements. Wheat genome physical mapping and sequencing projects should dramatically improve gene discovery by accelerating the identification of target regions and candidate genes. The

first physical map of chromosome 3B was published by Paux et al. (2008) and the sequencing recently achieved (C. Feuillet, Pers. Comm.). About 40 genes and QTLs have been identified on chromosome 3B in the wheat gene catalogue (Catherine Feuillet, Pers. Comm.) and several projects are now benefiting from these resources to achieve map-based cloning.

The locus *qDHY.3BL* was recently mapped on chromosome 3BL of hexaploid wheat (*Triticum aestivum*) by Bennett et al. (2012b) and Chapter 2, in the deletion bin 3BL7-0.63-1.00. It is associated with yield and yield-related components under heat and drought or a combination of both. The same QTL position was found in diverse wheat populations (*Triticum aestivum* and *Triticum turgidum* subsp. *durum*) (Appendix 2.5). The allelic version of *qDHY.3BL* carried by a drought tolerant line RAC875 was found to contribute to grain yield increase under abiotic stresses by up to 12.5%. We took advantage of the availability of the 3B physical map and the first draft of the reference sequence to initiate positional cloning of *qDHY.3BL*. Candidate physical contigs located under the QTL peak were selected after performing multi-environment inferred marker QTL analysis. Candidate genes were selected based on their putative function, the variation of their expression under drought and gene sequence polymorphisms.

II. Materials and Methods

1. Plant Materials

Five cultivars showing variability under high temperature and/or water deficit were used to generate wheat populations for reverse genetics including QTL detection and positional cloning (Fleury et al. 2010).

(1) One set of 368 DH lines derived from the F_1 generation of a cross between the drought tolerant wheat breeding line RAC875 (RAC655/3/Sr21/4*Lance//4*Bayonet) and the cultivar Kukri (76ECN44/76ECN36//Madden/6*RAC177), and one set of 768 recombinant inbred (RI) lines developed from the same cross (Fleury et al. 2010). Two abbreviations were applied to facilitate the description of these populations; 'RK_DH' for the DH population and 'RK_RI' for the RI population.

(2) Two sets (Pop1 and Pop2) of 250 wheat (*Triticum aestivum* L.) recombinant inbred (RI) lines derived from the F7 generation of a cross between the drought tolerant

wheat cultivar Gladius (RAC875 / Krichauff // Excalibur / Kukri / 3 / RAC875 / Krichauff / 4 / RAC875 // Excalibur / Kukri) and the wheat cultivar Drysdale (Hartog*3/Quarrion) which include 5000 lines (Fleury et al. 2010). The Pop1 and Pop2 contained 250 lines. Within these two sets (Pop1 and Pop2) of 250 lines, 115 lines were common (Pop3). Three abbreviations were applied to facilitate the identification of these sets of lines: GD_RI1, GD_RI2 and GD_RI3.

(3) One set of 233 DH lines derived from the F_1 generation of a cross between the drought tolerant wheat cultivar Excalibur (RAC177(Sr26)/Uniclum-492//RAC311-S) and the drought sensitive cultivar Kukri (76ECN44/76ECN36//Madden/6*RAC177) (Fleury et al. 2010). It was abbreviated as 'EK_DH'.

2. Genetic mapping of *qDHY.3BL* on chromosome 3BL

Six genetic maps were used in this chapter. The two partial 3BL genetic maps of RK_DH and RK_RI that were published originally and described in Chapter 2 of this thesis, the three genetic maps of chromosome 3B of GD_RI1, GD_RI2 and GD_RI3 that are described in Chapter 2. Finally, the genetic map of the EK_DH was first described by Edwards (2012). The six maps were re-evaluated using the IciMapping software developed by Li et al. (2008) which is available at http://www.isbreeding.net. Marker ordering was performed using the RECORD algorithm (Os et al. 2005). The genetic distances were calculated using Kosambi algorithm (Kosambi 1943). The visualization of the genetic maps was performed using MapChart (Voorrips 2002).

Using MergeMap online program (http://138.23.178.42/mgmap/), a genetic consensus map was generated to combine the three maps RK_DH, EK_DH and GD_PRI3. The weight of each map was determined on the confidence of marker order and population size relative to each map. The weight for each map was: 100 for RK_DH, 80 for GD_RI3 and 60 for EK_DH.

3. QTL analysis

A single site QTL analysis was performed on data from one location where the EK_DH population was evaluated (AusPie07_NI_CS_2, Appendix 4.1). QTL detection for this specific site was done using the WGAIM (whole genome average interval mapping) (Taylor and Verbyla 2011) approach described in Chapter 2 of this thesis. To help with the localisation of *qYDH.3BL* an enhancement of the multi-environment QTL analysis was

performed, using a multi-environment inferred marker QTL analysis (MEIM-QTL) involving a modelling process similar to that described in Chapter 2 of this thesis. This consisted of (1) identification of the most appropriate genotype by environment multi-environment statistical model (2) calculation of inferred marker genotypes at regular intervals in the chromosome region using the flanking marker method described by Whittaker et al. (1996) (3) refitting the multi-environment model incorporating each inferred marker in turn and sequentially scanning along the chromosome region. For each inferred marker, inferred marker coefficients simultaneously tested for all environments using a Wald statistic (Kenward and Roger 1997).

Three MEIM-QTL were performed on the RK_DH, RK_RI and GD_RI1 including twenty-one, four and five environments where all experiments were conducted in Mexico and Australia. The environments are summarised in Appendix 4.1.

4. Genotyping

The "neighbour map" of chromosome 3B (Paux et al. 2008) built using the integrated map approach described by Cone et al. (2002) in maize, was used to identify additional markers for parental screening and mapping. All markers were assayed following one of the three methods, Multiplex-Ready technology developed by Hayden et al. (2008), the M13 tailed marker developed by Oetting et al.(1995), and the high-resolution melting (HRM) curve analysis (Wittwer et al. 2003) using Roche LightCycler 480 (LC480). Each marker screened across each population was then added to the map using IciMapping software (Li et al. 2008).

5. Molecular marker design

Using the "neighbour map" of chromosome 3B published by Paux et al. (2008) and the second version of the physical map of chromosome 3B (Rustenholz et al. 2011), a total of 79 SSRs (named as cfb5--), 61 ISBP (named as cfp60--) and 15 SNP (named as cfs60--) were designed using BAC-ends, contig sequences and gene sequences to: (1) Confirm the genetic position of physical contigs, where no polymorphic marker where found based on the neighbour map; (2) Anchor physical contig in the genetic map of either populations RK_DH, EK_DH and GD_RI3; (3) Genetically map genes which contained SNP detected between the parental lines. The sequences of markers are available in Appendix 3.2.

ISBP and SSR markers were designed using the ISBP*finder* (Paux et al. 2010) and SSR*finder* (Frédéric Choulet, Pers. Comm.) programs run on the Chinese Spring genome sequence from chromosome 3B (http://urgi.versailles.inra.fr/Projects/3BSeq; unpublished data). The TREP database (http://wheat.pw.usda.gov/ITMI/Repeats/) was concatenated with the additional repeat element sequences published by Choulet et al. (2010) designated as TREPplus and run prior to primer design. SNPs were designed on genomic sequences for gene mapping using Primer3 published by Rozen and Skaletsky (2000). Primers were designed to flank one or two SNP and to amplify products of 200 bp to 250 bp, for detection by high-resolution melting (HRM) curve (Wittwer et al. 2003).

6. Marker assignment on physical contig

The molecular markers that were not assigned in the genetic neighbour map were screened by PCR (Paux et al. 2008) on the minimal tiling path (version 2) which includes 9216 clones covering 99% of chromosome 3B (Rustenholz et al. 2011). Only single locus markers were used for physical assignment. The sequences of SNP markers (m6930, m4311, m4312, m8185, m6273 and m3159 – Matthew Hayden, Pers. Comm.) and two DArTs (wPt-1870 and wPt-2391 - available at http://www.triticarte.com.au/) were assigned *in silico* by comparing their sequences to the chromosome 3B sequence database (C. Feuillet, Pers. Comm.) using BLASTN (default parameters) to assign them on physical contig.

7. Annotation

Annotation of all the physical contigs was performed using the TriAnnot pipeline developed by Leroy et al. (2012). The annotation consists of masking transposable elements, annotating coding regions with a quality index and identifying non-coding sequences and molecular markers.

8. Putative function of annotated genes

To define the putative functions for all TriAnnot derived genes, BLASTX (default parameters) was used to compare the CDS sequence against: (1) rice protein sequences – MSU v7 (http://rice.plantbiology.msu.edu/), and (2) *Brachypodium* 8x release proteins (http://www.brachypodium.org/).

9. Presence of genes in databases

The CDS of each gene anchored in the target region was selected and further screened for their presence in the following databases using BLASTN (default parameters, E-value cut-off 1e-50): (1) the TC Harvard database available at http://compbio.dfci.harvard.edu/; (2) the Triticeae sequence database (The Triticeae Full-Length CDS Database (TriFLDB) (Mochida et al. 2009)); (3) ACPFG resources of transcript assemblies of wheat cultivars Kukri published by Schreiber et al. (2012) where the same method used by Schreiber et al. (2012) was applied with transcriptome of RAC875, Drysdale, Excalibur and Waagan. Additionally, a BLASTN search (default parameters, E-value cut-off $1e^{-20}$) was performed against a database of gene probes (68 to 73bp) (microarray platform Wheat Long Oligo Chip, Ute Baumann, ACPFG, unpublished data) which were studied for differential expression between three parental lines (Excalibur, Kukri and RAC875). Only the profile of gene expression corresponding to RAC875 and Kukri were considered in this chapter. The parental lines were tested with cyclic drought as described in Izanloo et al. (2008) where the sampling was performed on seedling plants at days 5, 9, 14, 23 and 25. This last resource was kindly provided by the Australian Centre for Plant Functional Genomics.

10. SNP discovery

The strategic wheat initiative in Australia has generated an important dataset where the five parents studied here were survey sequenced (RAC875, Kukri, Gladius, Drysdale and Excalibur) (10 times coverage) generating a dataset of genomic sequence reads of 100bp (Edwards et al. 2012). Each gene sequence (1000bp prior the first exon, all introns/exons and 1000 bp after the last exon) annotated on physical contigs within the target region was compared to respective parental genomic sequences (RAC875 (719), Kukri (963), Gladius (870), Drysdale (834) and Excalibur (810) million reads) using the software Bowtie 2 (http://bowtie-bio.sourceforge.net/) (Parameters: --very-sensitive --mp 30,25 --rfg 15,13 --rdg 15,13 --np 15 --no-unal --no-mixed, and all remaining parameters were kept as default). A semi-automated procedure was used to detect and select SNP based on two criteria: coverage at the position was required to be greater than 5 for each parent and the SNP had to be unique between both parents without mismatches. The software Tablet developed by Milne et al. (2010) was used to visualize the output.

III. Results

1. Fine mapping at the *qDHY.3BL* QTL in 4 populations

The *qDHY.3BL* QTL on 3BL was detected previously in the four populations. It was associated with grain yield in one environment in the EK_DH population located between *wPt-8021* and *gwm114* (Edwards 2012). The environment was characterized by terminal drought and warm temperature. Here, we improved the resolution of the genetic map by mapping seven new markers in the *qDHY.3BL* region which previously contained only five markers.

A new single QTL analysis was then performed using WGAIM on one site (AusPie07_NI_CS_2). This resulted in the detection of a weak peak association (LOD < 2.4) of an 1.3 cM marker interval between loci *cfb43* and *wPt-8021* (co-located with *cfb539*). The QTL analysis combined with marker indicated the most significant interval of 1.3 cM. The favourable allele carried by Excalibur was contributing to increase grain yield by 4.2 %.

qDHY.3BL was also detected for grain yield under heat and drought conditions in the RK_DH and RK_RI populations (Chapter 2). Based on the genetic information detailed in Chapter 2, the genetic resolution in the *qDHY.3BL* region was improved by mapping an additional nineteen and twenty new markers in the RK_DH and RK_RI populations, respectively. After performing MEIM-QTL of grain yield trait in the RK_DH population from the 21 environments analysed (Chapter 2), the Wald statistic profile showed a most likely QTL peak between loci *wmm448* and *cfp6008* (Figure 4.1). The allele from RAC875 contributed positively to grain yield in eight environments but negatively in two environments. The Wald statistic profile obtained after MEIM-QTL analysis of grain yield in the RK_RI population tested in four environments conducted in Mexico (2011 and 2012), showed a QTL peak between loci *cfp6009* and *cfp6018* (Figure 4.2). The allele carried by RAC875 increased grain yield from 4 to 8% in four environments in which high temperature and water deficit were individually applied in the two years of the experiment (2011 and 2012).

Finally, the same QTL was also detected in GD_RI1 (Chapter 2) for grain yield under drought and heat environmental conditions. The genetic map of GD_RI1 is detailed in Chapter 3, from this map only one marker was added. After performing MEIM-QTL of grain yield from the five environments in which the GD_RI1 population was tested, the

Wald statistic profile showed a QTL peak between loci *wPt1870* and *cfb43* (Figure 5.3). The Drysdale allele contributed positively to grain yield in three environments (MexObr10_DI_CS, AusLee09_FI_CS and AusLee10_FI_LS).

Thus, our results showed that in 4 populations, under 29 environments, a QTL peak was observed systematically on chromosome 3BL in an interval of 1 cM comprising 16 markers based on the consensus map (from *wmm1758* to *cfb539*). Interestingly, four loci *cfs6009*, *gwm1266*, *cfb43* and *wPt-8021* were found in common between the 4 genetic maps and no major differences marker order were observed between the four genetic maps (not shown).

The results using single-site QTL analysis for EK_DH and using MEIM-QTL for RK_DH, RK_RI and GD_RI1 showed a positive contribution to grain yield when the allele from RAC875, Drysdale or Excalibur is carried.

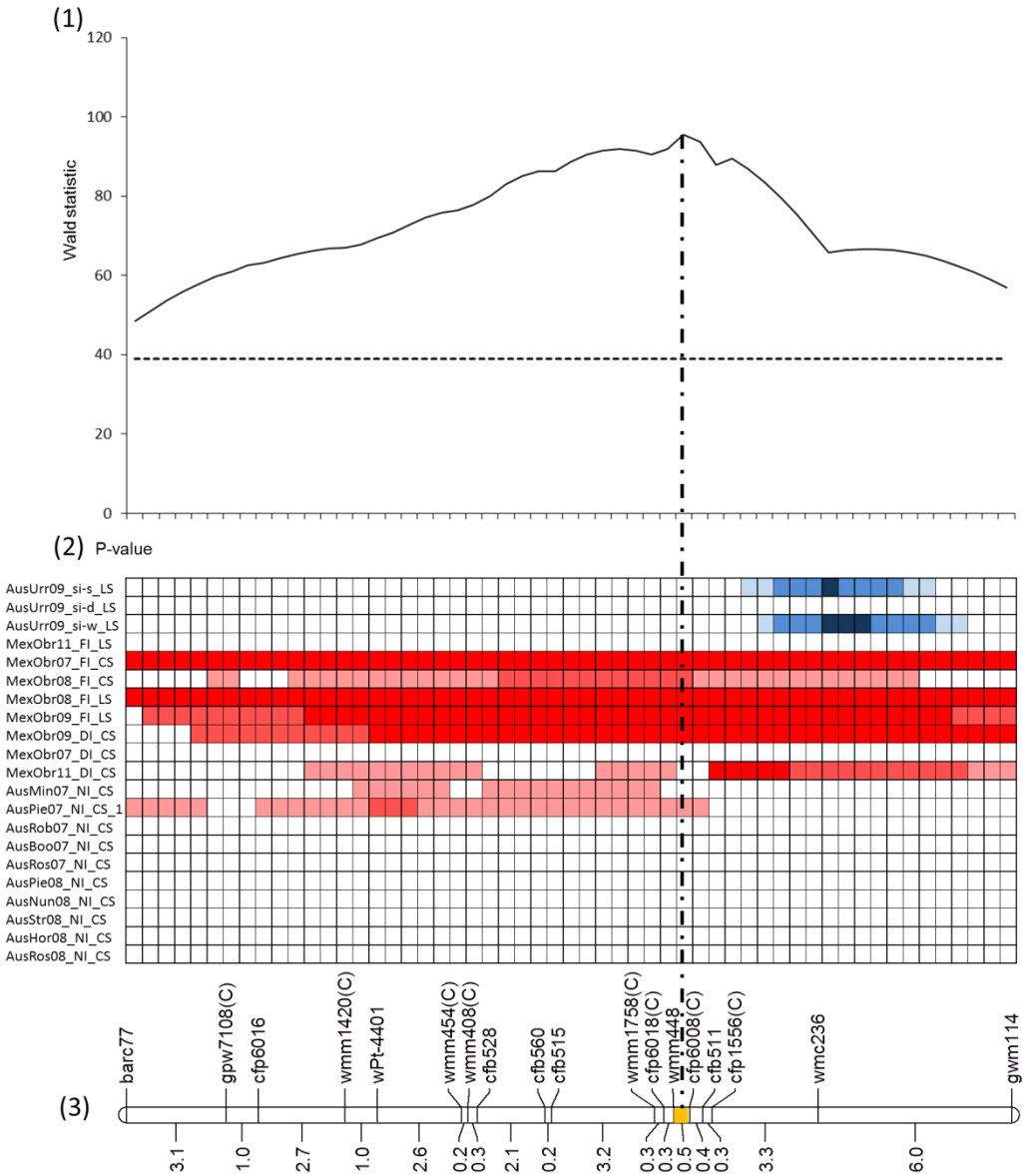


Figure 4.1 Multi-environment inferred marker QTL analysis of *qYDH.3BL* on the RAC875/Kukri DH population (RK_DH) using the grain yield trait from the twenty-one environments (a detailed description of the 21 environments is in supplementary Appendix 4.1: (Aus) Australia, (Mex) Mexico, (DI) drip irrigation, (FI) flooding irrigation, (CS) convention sowing, (LS) late sowing, (SI) sprinkler irrigation, (D) drought, (W) well-watered, (S) saturated).
(1) Wald statistic profile with inferred markers, the dotted line corresponds to the Wald statistic threshold; (2) allele effect per environment expressed in p-values where the red (RAC875) and blue (Kukri) colour indicate the positive contribution to grain yield per environment. Levels of significance are represented by: light p <0.05, medium p <0.01, strong colour p <0.001; (3) Partial genetic map of chromosome 3BL of the doubled haploid population RAC875/Kukri, co-located markers are simplified by (C).

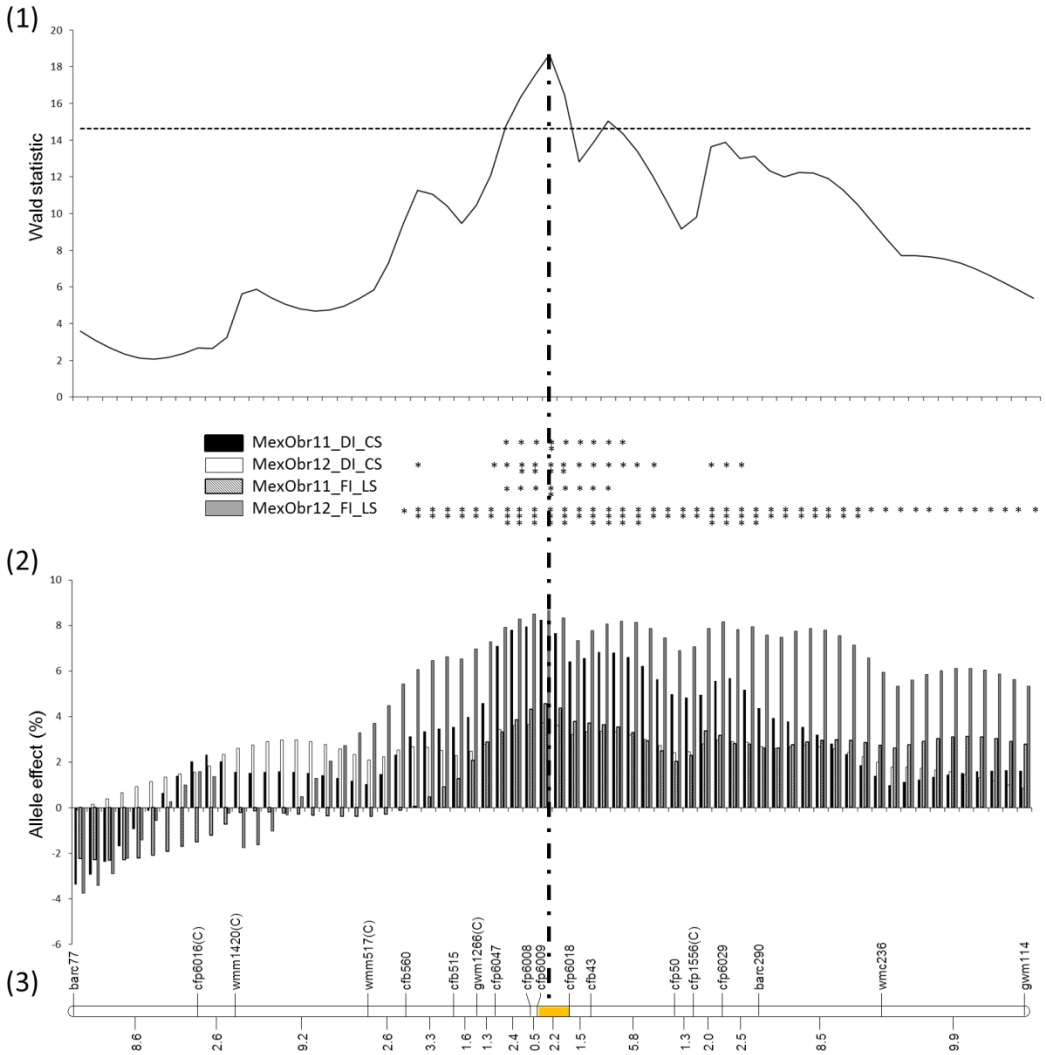


Figure 4.2 Multi-environment inferred marker QTL analysis of *qYDH.3BL* on the RAC875/Kukri RI population (RK_RI) using the grain yield trait from the four environments (MexObr11_DI_CS, MexObr12_DI_CS, MexObr11_FI_LS and MexObr12_FI_LS where the environment were described with Mexico (Mex), Ciudad de Obregon (Obr), drip irrigation (DI), flooding irrigation (FI), convention sowing (CS), late sowing (LS)) where 77, 40, 109 and 40 recombinant inbred were tested respectively. (1) Wald statistic profile with inferred markers, the dotted line corresponds to the Wald statistic threshold; (2) allele effect per environment expressed as percentage relative to the trait mean. Levels of significance are represented by: * p <0.05, ** p <0.01, *** p <0.001; (3) Partial genetic map of chromosome 3BL of 109 recombinant inbred lines (RAC875/Kukri).

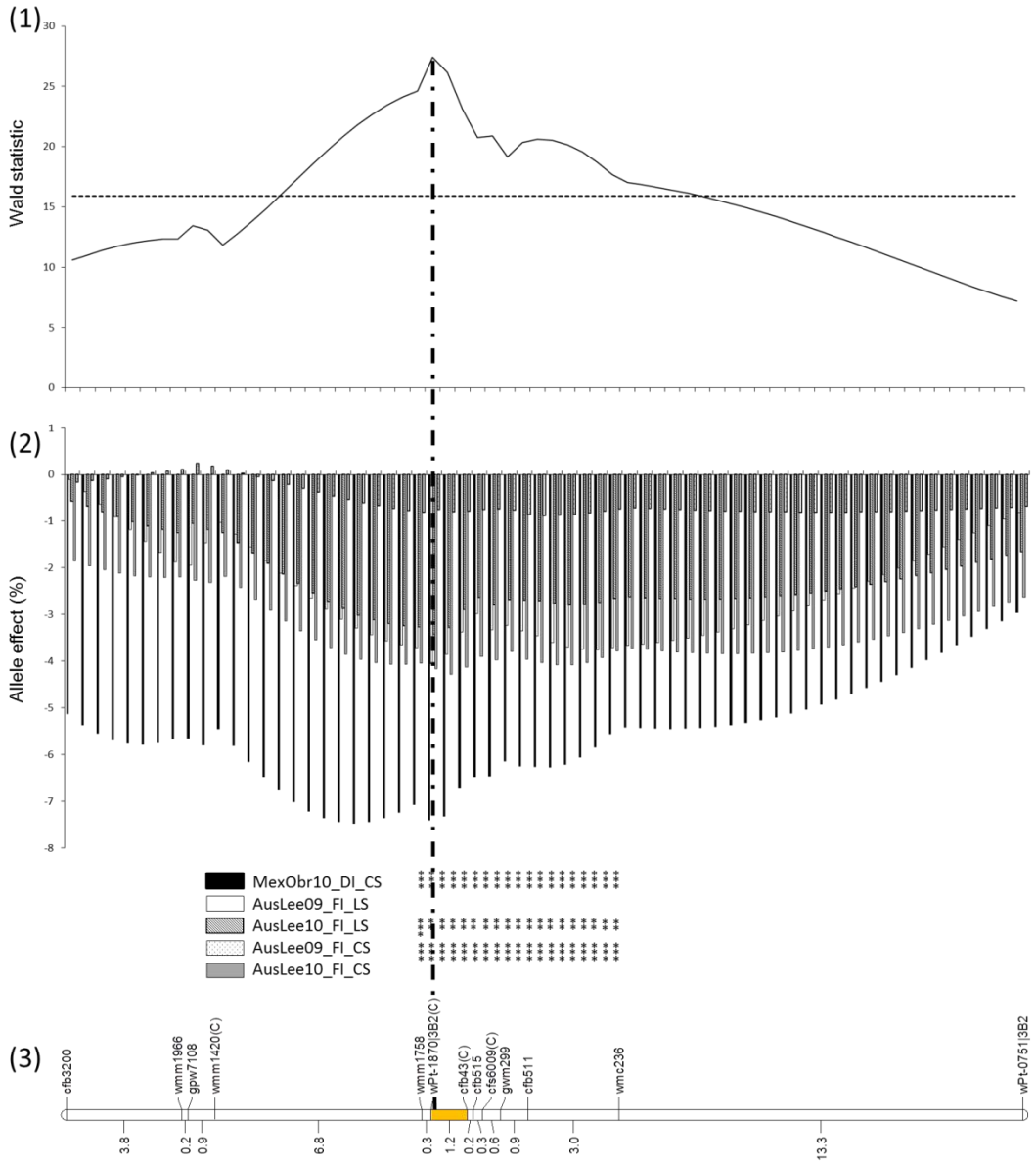


Figure 4.3 Multi-environment inferred marker QTL analysis of *qYDH.3BL* on the Gladius/Drysdale RI population (GD_RIPop1) using the grain yield trait from the five environments (MexObr10_DI_CS, AusLee09_FI_CS, AusLee09_FI_LS, AusLee10_FI_CS and AusLee10_FI_LS where the environment were described with (Mex) Mexico, (Obr) Ciudad de Obregon, (Aus) Australia, (Lee) Leeton, (DI) drip irrigation, (FI) flooding irrigation, (CS) convention sowing, (LS) late sowing) where the population was tested respectively.
(1) Wald statistic profile with inferred markers, the dotted line corresponds to the Wald statistic threshold; (2) allele effect per environment expressed as percentage relative to the trait mean. Levels of significance are represented by: * p <0.05, ** p <0.01, *** p <0.001; (3) Partial genetic map of chromosome 3BL of the recombinant inbred lines (Gladius/Drysdale), co-located markers are simplified by (C).

2. Physical contig assignments

To establish a physical map spanning the *qDHY.3BL* locus, 3 dimensional pools of the minimal tiling path of chromosome 3B (Rustenholz et al. 2011) were screened by PCR with 5 markers located between *cfb560* and *gwm114* (Table 4.1 and (underlined markers (a) Figure 4.4)). It identified 5 physical contigs (ctg173, ctg3169, ctg660, ctg1691 and ctg647) ranging from 0.39 Mb to 1.89 Mb for a total size of 4.63 Mb. The sequences of 6 SNPs (mapped in GD_RI2, m4311 (not shown) co-locating with m4312, m6273 (not shown) co-locating with m8185, m6930 and m3159) and two DArT sequences (wPt-2391 and wPt-1870) mapped in GD_RI1 were compared with the sequences derived from sequencing the minimal tiling path MTP of chromosome 3B (C. Feuillet, Pers. Com.). This "*in silico"* mapping resulted in the identification of 5 contigs (ctg660, ctg61, ctg2680, ctg1691 and ctg274) ranging from 0.5 Mb to 1.03 Mb for a total size of 2.88 Mb. Ctg274 was ignored because of its position distal from the QTL. Thus, in total the PCR and *in silico* mapping allowed the assignment of 13 markers spanning the QTL to eight different physical contigs representing a total of 5.674 Mb of sequence and a genetic interval of 5.3 cM. (Table 4.1 and Figure 4.4).

The marker *cfb43* located at 0.2 cM from marker *wmm448* on the RK_DH population was found by PCR on four BACs belonging to four contigs (ctg660 (1.03 Mb), ctg3169 (500 kb), ctg3168 (78 kb) and ctg633 (151 kb)). These two markers were assigned by PCR to the same BACs TaaCsp3BF057F10 assembled on contig ctg660. This result allowed us to merge ctg3169 and ctg660. In addition, an ISBP, *cfp6018,* designed on ctg3169 was mapped genetically in the RK_DH and RK_RI populations confirming the anchoring of ctg3169. Thus, these results show that the fused ctg3169-ctg660 carry markers that are under the QTL peak. Ctg3169 was therefore considered of higher interest than ctg633 and 3168.

The marker *wmc236* located at 3.5 cM at the distal region of the QTL based on the consensus map has been assigned by PCR to three BAC belonging to three contigs: a large contig ctg647 (1.990 kb) and two small contigs ctg975 (277 kb) and ctg1648 (257 kb). The same marker was assigned *in silico* to the same three contigs with 100% match, more specifically on the scaffold AIK_02 of ctg647. The SSR marker *cfb539* designed on ctg647 scaffold AIK_01 mapped in the QTL region in the EK_DH population together

Table 4.1: Assignment of molecular markers to contigs of the 3B physical map. The molecular markers mapped under *qYDH.3BL* and the method used to assign them is provided. PCR screening was performed on the minimal tiling path MTPv2 by Rustenholz et al. (2011) and *in silico* mapping was performed by sequence alignment against the sequence of the 3B physical contigs.

Markers of the genetic region	***Assignment method***	***Type of marker***	***BAC clone[a]***	***Contig assignment***	***Deletion Bin***
cfb515	Designed on contig sequence	SSR	-	ctg730	3BL7-0.63-1.00
gpw3233	MTPv2	SSR	TaaCsp3BF159F15	ctg173	3BL7-0.63-1.00
cfb43	MTPv2	SSR	TaaCsp3BF278D04	ctg3169	-
			TaaCsp3BF167L17	ctg3168	-
			TaaCsp3BF142L07	ctg633	-
			TaaCsp3BF057F10	ctg660	3BL7-0.63-1.00
cfp6018	Designed on BAC-end sequence	ISBP	-	ctg3169	3BL
wmm448	MTPv2	SSR	TaaCsp3BF057F10	ctg660	3BL7-0.63-1.00
			TaaCSP3BF109C05	ctg660	
cfp6008	Designed on BAC-end sequence	ISBP	TaaCsp3BF240I03	ctg660	3BL7-0.63-1.00
cfp6009	Designed on BAC-end sequence	ISBP	TaaCsp3BF268K03	ctg660	3BL7-0.63-1.00
cfs6009	Designed on gene sequence	SSR	-	ctg660	3BL7-0.63-1.00
wPt9368	(Paux et al. 2008)	DArT	TaaCsp3BF078D14	ctg660	3BL7-0.63-1.00
wPt8021	(Paux et al. 2008)	DArT	TaaCsp3BF078D14	ctg660	3BL7-0.63-1.00
wPt2391	*In silico*	DArT	-	ctg660	3BL7-0.63-1.00
wPt1870	*In silico*	DArT	-	ctg61	-
cfp6047	Designed on contig sequence	ISBP	-	ctg61	-
m4311/m4312	*In silico*	SNP		ctg61	-
m6930	*In silico*	SNP	-	ctg2680	-
cfp49	(Paux et al. 2008)	ISBP	TaaCsp3BF064D10	ctg1691	3BL7-0.63-1.00
cfp50	(Paux et al. 2008)	ISBP	TaaCsp3BF099C14	ctg1691	3BL7-0.63-1.00
barc290	-	SSR	-	-	-
cfp1237	(Paux et al. 2008)	ISBP	TaaCsp3BF064D10	ctg1691	3BL7-0.63-1.00
cfp1556	(Paux et al. 2008)	ISBP	TaaCsp3BF099C14	ctg1691	3BL7-0.63-1.00
cfp6029	Designed on BAC-end sequence	ISBP	TaaCsp3BF204G05	ctg1691	3BL7-0.63-1.00
m8185/m6273	*In silico*	SNP	-	ctg1691	3BL7-0.63-1.00
gwm299	MTPv2	SSR	TaaCsp3BF027L08	ctg1691	3BL7-0.63-1.00
cfb511	Designed on contig sequence	SSR	-	ctg1689	
cfb539	Designed on contig sequence	SSR	-	ctg647	
wmc236	MTPv2 and *in silico*	SSR	TaaCsp3BF290G18	ctg647	3BL9-0.38-0.50
			TaaCsp3BF140I19	ctg975	3BL7-0.63-1.00
			TaaCsp3BF371M16	ctg1648	-
m3159	*In silico*	SNP	-	ctg274	-
gwm114	-	SSR	-	-	-

a annotation of BAC clones according to http://urgi.versailles.inra.fr/gb2/gbrowse/wheat_phys_pub/

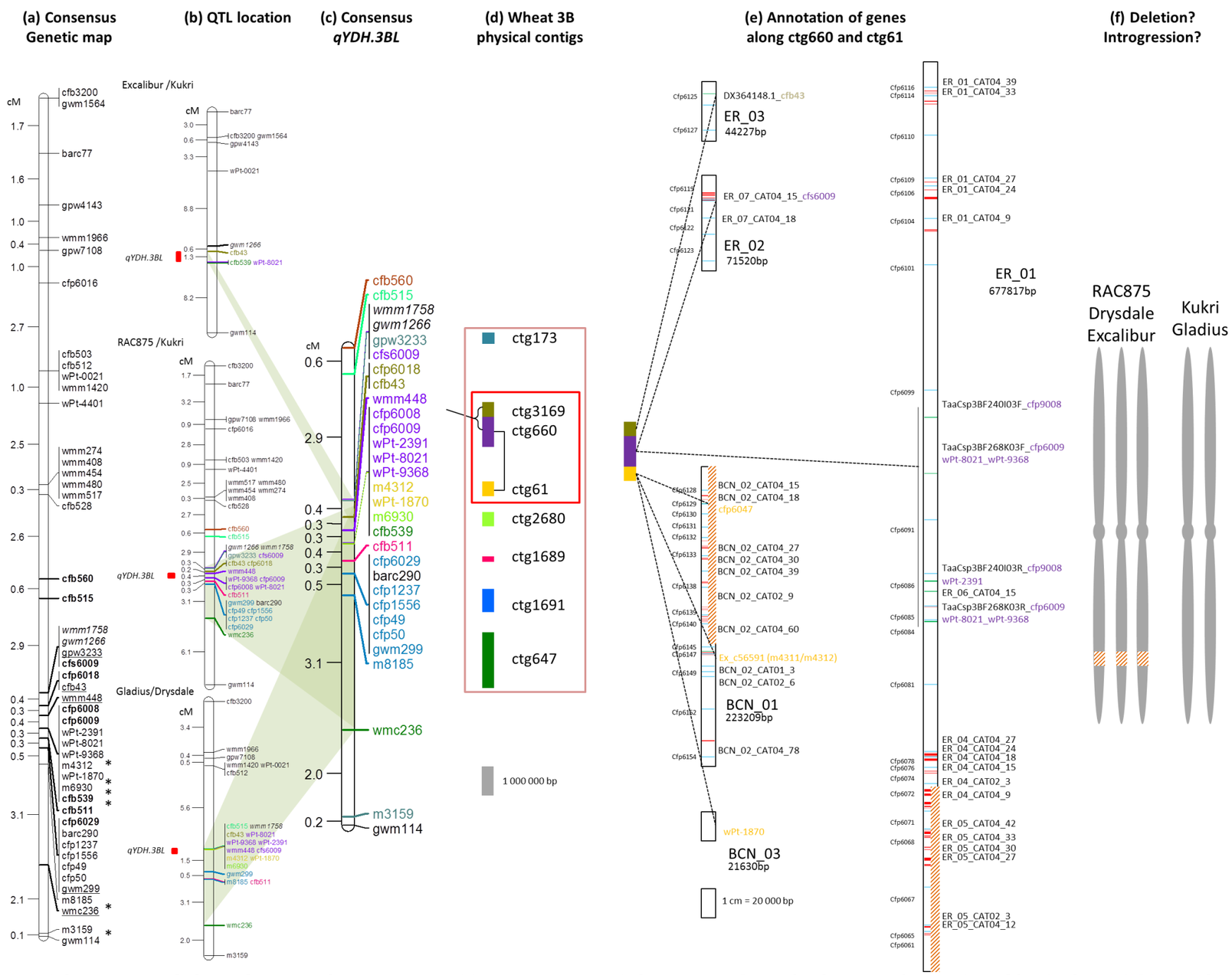


Figure 4.4 Schematic representation of the genetic and physical maps at the *qYDH.3BL* . (a) consensus map of chromosome 3B in the three populations (Excalibur/Kukri, RAC875/Kukri and Gladius/Drysdale). The underlined markers were assigned by PCR, the markers with a star were assigned *in silico.* Markers in bold were designed on either BAC, contig or gene sequence. (cfb for SSR, cfp for ISBP and cfs for SNP). (b) representation of the three regions of chromosome 3BL in the three populations. The colour code for the markers on the maps corresponds to contig assignment. The region of the *qYDH.3BL* QTL detected in each population is represented by the red bar. (c) Magnification of the consensus region underlying *qYDH.3BL*. (d) physical contig assignment (e) annotated BAC, markers, and genes identified on physical contigs ctg660 (ER_01, ER_02 and ER_03) and ctg61 (BCN_01 and BCN_03) (horizontal bars correspond to genes (red), markers and BAC (green), new ISBP designed on scaffold sequences named cfp6--- are indicated on the left side of contigs sequences (blue), (f) regions showing a presence/absence (PAV presence/absence variation) polymorphism in the three parental lines (RAC875, Drysdale and Excalibur) are represented by the orange hashed bars.

with marker *wPt-8021*. As marker *wPt-8021* flanked the QTL in EK_DH therefore ctg647 was kept for further analyses. In addition, one SSRs and five ISBPs designed from BAC-end and contig sequences confirmed the position of seven contigs (ctg3169 (*cfp6018*), ctg660 (*cfp6008, cfp6009*), ctg61 (*cfp6047* only mapped in RK_RI population), ctg1691 (*cfp6029*) and ctg647 (*cfb539*). One SNP (*cfs6009*) confirmed the anchoring of contig ctg660. Additionally, one SSR *cfb511* designed from ctg1689 was mapped in the RK_DH and RK_RI populations (this contig was previously assembled with ctg1691 in the first physical map of 3B published by Paux et al. (2008) and named ctg1036).

Marker *gpw3233* co-segregated with marker *cfs6009* (ctg660), therefore the contig ctg173 was kept in the short list of contigs. Marker *cfb539* was designed from contig ctg647 and co-located with *wPt-8021* therefore the contigs ctg61, ctg2680, ctg1689 and ctg1691 were also kept for further investigation of candidate genes.

Thus, in total eight contigs (Figure 4.4) ranging from 0.217 Mb to 1.990 Mb were identified covering a region of at least 6.320 Mb under *qYDH.3BL*. Based on the combination of the QTL analyses two categories of contigs were distinguished: ctg3169, ctg660 and ctg61 were considered as high confidence based on the MEIM-QTL analysis results whereas the flanking contigs ctg173, ctg2680, ctg1689, ctg1691 and ctg647 were considered with a lower confidence. Annotation of all the contigs was obtained from INRA Clermont-Ferrand and candidate genes were investigated.

3. Candidate genes for *qYDH.3BL*

Annotation of contigs ctg173, ctg660, ctg61, ctg2680, ctg1689, ctg1691 and ctg647 located under *qYDH.3BL* using the TriAnnot pipeline (Leroy et al. 2012) revealed eighty-five genes whereas no gene was annotated on ctg3169. Putative homologous genes in rice and *Brachypodium* were identified by selecting the first hit using BLASTx alignment (Appendix 4.3). A total of 30% genes were located on chromosome 1 in rice and almost 50% on chromosome 2 in *Brachypodium* which are the syntenic chromosomes to chromosome 3B in wheat. Homologs of the remaining genes were located randomly across either genomes (rice and *Brachypodium*) indicating that more than 50% of the predicted genes under the QTL *qYDH.3BL* are not found in syntenic regions.

To identify putative candidate genes among the eighty-five predicted genes, we performed different kind of analyses. First, we looked whether the eighty-five predicted genes have

records in any databases including TriFDB, TC Harvard and in-house transcript DB (ACPFG) (Figure 5.5). Based on this selection criteria, sixty-five genes showed a hit with an e-value lower than the cut off of 1.00E-50 (Appendix 4.4) and were kept considering that the other genes may be false positives. The 65 genes were split in 2 groups based on their physical contig location. Twenty genes belong to high confidence contigs whereas 45 were assigned to low confidence contigs. A second filter was then applied to the 65 genes (Figure 4.5): (1) genes were mapped using individual genomic reads obtained from the five parental lines (genomic reads provided by Ute Baumann, ACPFG); (2) transcriptomic data obtained in cyclic drought experiment between RAC875 and Kukri were used to analyse the putative differential expression of the candidate.

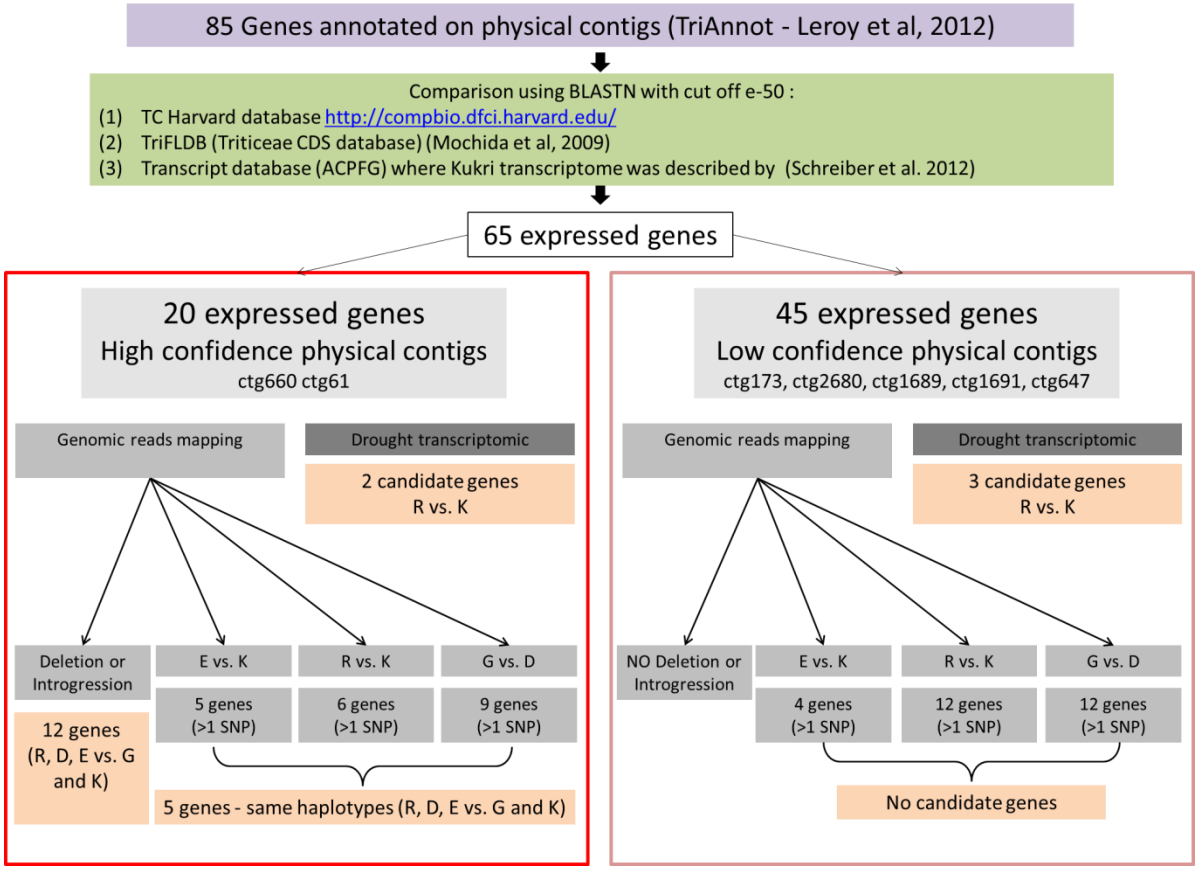


Figure 4.5 Summary of candidate gene selection from the original eighty-five predicted genes (purple) annotated in the *qYDH.3BL* region. The first step is illustrated by the green box with the selection of genes with high hits with transcript sequences. The two approaches with high (red box) and low confidence (pink box) physical contigs correspond to the two groups visualized in Figure 4.4. Genomic read mapping was performed on the respective genes of the two groups (light grey). The sequence comparison between the predicted genes of each group with cyclic drought transcriptomic is represented in dark grey. E, K, R, G and D correspond to Excalibur, Kukri, RAC875, Gladius and Drysdale.

4. Candidate genes located in high confidence physical contigs

The two contigs ctg660 (1.03 Mb) and ctg61 (543 kb) were comprised of three sequence scaffolds each (ER_ for ctg660 and BCN_ for ctg61). Ten markers located under the QTL, including four DArTs (*wPt-1870, wPt9368, wPt8021* and *wPt2391*), one SSR (*wmm448*), three ISBPs (*cfp6008*, *cfp6009* and *cfp6047*) and three SNPs (*cfs6009, m4311* and *m4312*) were found on these sequence scaffolds. Most of the markers found in ctg660 are located in the middle of the longest sequence ER_01 whereas the SNP cfs6009 and *cfb43* were found on scaffold ER_02 and ER_03 (Figure 4.4). The four markers on ctg61 were found on two sequence scaffolds where the ISBP (*cfp6047*) and the two SNPs (*m4311* and *m4312*) were on BCN_01 whereas the DArT (*wPt-1870*) was found on a small scaffold BCN_03. All the predicted genes were annotated along the scaffold sequences of ctg660 and ctg61. No genes or markers were found on contig sequence BCN_02.

Favourable alleles for *qYDH.3BL* originated from RAC875, Drysdale and Excalibur versus Gladius and Kukri. The Chinese Spring sequence of the 20 genes including 3'-UTR (1000bp), exons, introns and 5'-UTR (1000bp) were used to map genomic reads obtained after whole genome shotgun sequencing of the five parental lines at 10 times coverage. Five, six and nine genes showed at least one SNP along their sequences between the parent pairs Excalibur vs. Kukri, RAC875 vs. Kukri and Gladius vs. Drysdale, respectively (Figure 4.5). A total of five genes had the same haplotypes in the tolerant parents RAC875, Drysdale, Excalibur vs. Gladius and Kukri (Table 4.2). Gene ontology analysis was used to identify further potentially interesting candidate genes. A gene (ER_01_CAT04_15) located on ctg660 was syntenic with a gene in *Brachypodium*, Bradi2g61450.1, coding for a RING-finger protein containing a C3HC4-type domain that are known to be involved in ubiquitination. It contained three exons with 1 SNP in the promoter region (position 570 bp), 8 SNP in intron, and one SNP in the third exon changing a histidine into an arginine (position 2623) (Table 4.3, Figure 4.6). The same haplotype was found in RAC875, Drysdale and Excalibur and the other one in Kukri and Gladius.

The alignment of sequence reads along the twenty genes resulted in an even coverage of the gene sequence including 5'UTR, all introns/exons and 3'UTR. However, twelve genes were only covered by Kukri and Gladius reads whereas almost none of the Excalibur, RAC875 and Drysdale reads matched these genes thereby suggesting that they were absent in those lines. One present/absent gene group was found on ctg660 and coded for disease

resistance genes whereas the second group was coding for three glutathione S-transferases (GSTs) including 1 GST on ctg660 and 2 GSTs on ctg61, one glycosyl hydrolases family 17 on each contig and one protein containing a F-box domain (Table 4.4). The extremely low coverage of these twelve genes in RAC875, Drysdale and Excalibur indicated a presence/absence polymorphism related to a possible deletion of introgression of genes associated with *qYDH.3BL*.

Two predicted genes annotated on ctg660 and ctg61 were found to match one gene from the transcript database of cyclic drought experiment between RAC875 and Kukri (Figure 4.5), however no significant statistical difference in the expression between the two parents was found.

Table 4.2 Putative functions of the five genes presenting common SNPs in the three parental pairs (Excalibur vs. Kukri, RAC875 vs. Kukri and Gladius vs. Drysdale). The gene function was found by homology with rice and *Brachypodium* genes. The blue color indicates synteny between wheat and *Brachypodium*. The gene of interest for abiotic stress tolerance is bolded and underlined. The italic e-value was lower than the cut off e^{-20}.

Annotation TriAnnot	***Rice homolog***	***Putative function***	***E-value***	***Score***	***Brachypodium homolog***	***Putative function***	***E-value***	***Score***
BCN_02_CAT01_3	LOC_Os02g17280.1	gamma-secretase subunit APH-1B	3.00E-93	339	Bradi3g10110.1	endopeptidase activity	7E-122	347
BCN_02_CAT02_6	LOC_Os10g33910.1	mitochondrial import inner membrane translocase subunit	8.00E-32	132	Bradi2g61480.1		8E-54	164
ER_07_CAT04_15	LOC_Os03g62250.1	***zinc finger, C3HC4 type domain containing protein***	*0.001*	40.8	Bradi2g61450.1	***RING, subfamily zinc finger (C3HC4-type RING finger) protein***	4E-44	145
ER_07_CAT04_18	LOC_Os05g25310.1	acyl-CoA synthetase protein	8.00E-73	270	Bradi3g03730.1	fatty-acyl-CoA synthase activity	2E-87	271
ER_01_CAT04_27	LOC_Os05g25430.1	receptor-like protein kinase	6E-21	99.4	Bradi2g31490.1	CrRLK1L	*2E-13*	70.5

Table 4.3 Summary of the SNP position along the genomic sequence of the candidate genes coding for a RING-finger protein containing a C3HC4 domain including 1000bp prior the first exon and 1000bp after the last exon. The number in parenthesis corresponds to the number of reads representing the parental lines. The underlined parental lines correspond to the same haplotype. The SNP located in exon are bolded.

position	*570*	*1147*	*1707*	*2013*	*2073*	*2278*	***2623***	*2782*	*3603*
RAC875	A(5)	C(13)	T(23)	C(3)	-	T(7)	**G(7)**	-	-
Kukri	G(29)	T(19)	C(19)	G(3)	A(17)	G(14)	**A(34)**	-	-
Gladius	G(9)	T(7)	C(10)	G(17)	A(25)	G(16)	**A(7)**	C(7)	T(3)
Drysdale	A(12)	C(14)*	T(12)	C(12)	G(11)	T(12)	**G(8)**	T(8)	G(4)
Excalibur	A(4)	C(18)	T(9)	C(26)	G(8)	T(2)	**G(4)**	T (1)	-

* Out of the 14 reads from Drysdale, 2 reads didn't have the (C) SNP at the position 1147. These two reads had another SNP further down the sequence. There were considered either sequencing errors of homologues.

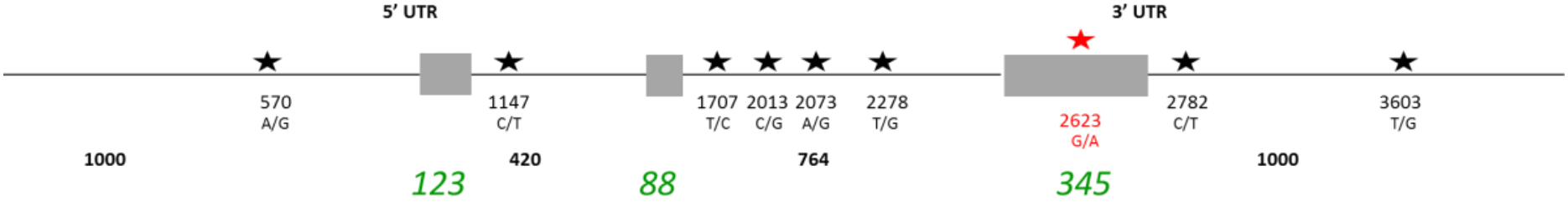


Figure 4.6 Schematic representation of RING finger protein containing a zinc C3HC4-type domain annotated on contig ctg660 with respective SNP positions along the sequences. The grey boxes correspond to exons and the stars to SNP. The red stars correspond to SNP within exon. The size of each segment is indicated in black bold for intron, 5' UTR and 3'UTR and green for exons.

Table 4.4 Summary of the genomic read mapping of the twenty predicted genes from cv. Chinese Spring annotated on ctg660 and ctg61 with a high hit in transcriptome analysis. The gene name refers to the annotation in Figure 4.4, the gene length corresponds to 1000bp upstream, exons, introns and 1000bp downstream. The number of reads covering the gene is indicated for each of the five parental lines (RAC875, Drysdale, Excalibur, Kukri and Gladius). The highlighted section in the table corresponds to the genetic region which was not found in the three parental lines (RAC875, Drysdale, Excalibur).

genes	***putative function rice homologous***		***RAC875***	***Drysdale***	***Excalibur***	***Kukri***	***Gladius***
		Gene Length	**Reads**	**Reads**	**Reads**	**Reads**	**Reads**
ER_07_CAT04_15	zinc finger, C3HC4 type domain containing protein	3738	254	380	260	498	388
ER_07_CAT04_18	acyl-CoA synthetase protein, putative	4615	196	426	246	494	578
ER_01_CAT04_39	OsFBX27 - F-box domain containing protein	4185	714	868	782	2400	862
ER_01_CAT04_27	receptor-like protein kinase	8765	1984	2116	1630	1818	1454
ER_01_CAT04_24	TKL_IRAK_CrRLK1L-1.3 - The CrRLK1L-1 homolog	3578	164	224	184	330	462
ER_01_CAT04_9	expressed protein	3042	764	512	928	2546	672
ER_04_CAT04_24	verticillium wilt disease resistance protein	3900	0	2	0	352	322
ER_04_CAT04_15	von Willebrand factor type A domain	2378	6	44	10	104	134
ER_04_CAT02_3	verticillium wilt disease resistance protein	6729	12	6	12	520	808
ER_04_CAT04_9	verticillium wilt disease resistance protein	7740	0	0	0	556	832
ER_05_CAT04_42	verticillium wilt disease resistance protein	7658	0	0	0	700	990
ER_05_CAT04_30	TKL_IRAK_CrRLK1L-1.3 - The CrRLK1L-1 homolog	4166	4	8	0	362	414
ER_05_CAT04_27	OsFBX219 - F-box domain containing protein	3470	18	20	10	390	378
ER_05_CAT02_3	glycosyl hydrolases family 17	3603	38	22	14	332	352
ER_05_CAT04_12	glutathione S-transferase	5317	20	10	10	466	530
BCN_02_CAT04_30	glycosyl hydrolases family 17	3899	0	2	0	264	258
BCN_02_CAT04_39	glutathione S-transferase	2748	0	2	8	254	358
BCN_02_CAT02_9	glutathione S-transferase	2523	4	4	0	242	288
BCN_02_CAT01_3	gamma-secretase subunit APH-1B	5043	268	468	286	528	542
BCN_02_CAT02_6	mitochondrial import inner membrane translocase	3865	172	344	200	334	368

5. Low confidence physical contigs

The forty-five predicted genes annotated on the lower confidence physical contigs (ctg173, ctg2680, ctg1689, ctg1691 and ctg647) were compared to the database of probes used in drought transcriptomic experiment (Ute Baumann, ACPFG, unpublished data). The experiment consisted of applying two cyclic droughts on seedling and collecting tissues at 5 time point along the first 25 days (Figure 4.7). A total of 4 genes were found to match a probe. Of those, only three genes (Figure 4.5), located on contigs ctg1691 and ctg647 were differentially expressed between RAC875 and Kukri for at least one time point (Figure 4.7). These genes have the following putative functions: a chlorophyllide a oxygenase protein (gene 1), a protein phosphatase 2C (gene 2) and a basic helix-loop-helix DNA binding protein (gene 3). The difference of gene 1 expression occurred at the second drought and persisted after re-watering. Gene 2 was under-expressed in Kukri after the first re-watering before being up-regulated after the second re-watering. By contrast RAC875 maintained its expression until the second re-watering. The profile of gene 3 was similar between the two parental lines until the second re-watering where RAC875 was down-regulating its expression. The expression of these two genes differed between RAC875 and Kukri under cyclic drought, therefore are considered as candidate genes. No genes were found in common between the parent pairs Excalibur vs. Kukri, RAC875 vs. Kukri and Gladius vs. Drysdale. No deletions or introgressions were detected for these forty-five predicted genes (Figure 4.5).

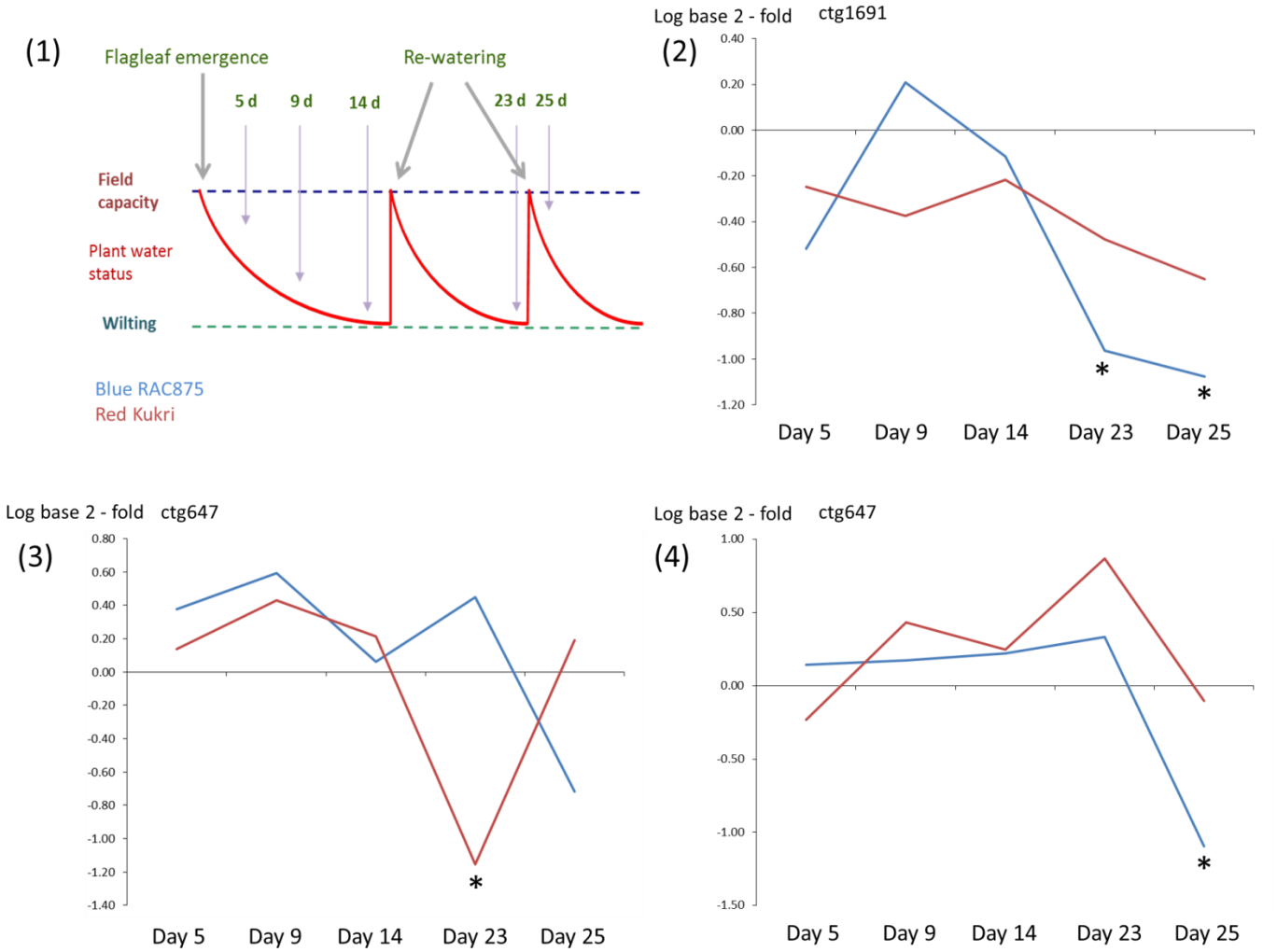


Figure 4.7 Expression profiles of 3 candidate genes in cyclic drought transcriptomic experiments. (1) experimental design as described by Izanloo et al. (2008) with five time points (day 5, day 9, day 14, day 23 and day 25) (2) chlorophyllide a oxygenase gene on contig ctg1691, (3) protein phosphatase 2C gene on contig ctg647, (4) basic helix-loop-helix DNA binding domain containing gene on contig ctg647. The expression of each gene for each parent is coloured: in blue for RAC875 and red for Kukri

IV. Discussion

1. Fine mapping in four populations significantly improves the detection of the drought and/or heat tolerance *qYDH.3BL* QTL

Overall, *qYDH.3BL* is heat and drought responsive where the gene carried by RAC875, Drysdale and Excalibur contribute positively to grain yield confirming the use of this QTL in breeding programs. Grain yield in cereals is determined by complex interactions between the genetic back ground and the timing, period and severity of environmental stress applied along the growth cycle of the plant. Genes underlying QTL involved in grain yield under abiotic stress responses (heat and drought) have not been identified yet in wheat. However, in species where the genome sequence is available like rice gene annotation and QTL analysis for abiotic stress tolerance such as drought have been summarized in a QTL database Q-TARO available at http://qtaro.abr.affrc.go.jp/. The use of multiple populations increase the chance to fine polymorphisms compare to the use of a single bi-parental population.

Mapping the same trait in four different bi-parental populations (RK_DH, RK_RI, EK_DH and GD_RI1) assessed in environments where high temperature and water deficit occurred greatly help to refine the genetic interval of the *qYDH.3BL* and provided strong evidence for a role of *qYDH.3BL* in drought tolerance. The use of four populations has been extremely useful to saturate the genetic region with molecular markers and physical contigs. Using different bi-parental populations increases the chance of detecting polymorphic markers. The pedigree of each parental line is available at http://www.bioplatforms.com.au/, where it can be seen that Gladius contains large proportion of Excalibur, Kukri and RAC875. However the cross with Drysdale increases the genetic variability given its different pedigree. The benefit of the consensus map combined with the three MEIM-QTL analyses allowed definition of the targeted region to three contigs ctg3169, ctg660 and ctg61 that showed high statistical confidence. As an example Quraishi et al. (2011) reported the usefulness of a consensus genetic map to find consensus QTL important for positional cloning of genes involved in grain fibre content in wheat.

2. Exploiting the 3B physical map to refine the localisation of the *qYDH.3BL* QTL

Two physical contigs ctg660 and ctg61 representing about 1.5 Mb of sequence in total were identified under the *qYDH.3BL QTL* peak. Gene annotation revealed the presence of 30 predicted genes on these contigs. This represents a density of one gene every 50 kb which is very similar to the density observed (1 gene/ 86 kb) by Choulet et al. (2010) in the distal regions of chromosome 3B. Our comparative analyses with rice and *Brachypodium* also confirmed the previous observation that synteny is decreased in the distal part of the wheat chromosomes (Choulet et al. 2010).

We have defined two groups of contigs (high and low confidence) based on their physical location (directly under the QTL peak or on either side) at the *qYDH.3BL* QTL. To further investigate these two groups, we will need to improve the physical map at the *qYDH.3BL* locus. This can be done by identifying additional physical contigs in the targeted region. A pseudomolecule is currently under construction at INRA GDEC and physical contigs are being merged. The recently published barley genome sequence information (IBSC 2012) can also be used to guide contig merging based on the information of gene content in the wheat and barley contigs. Physical contigs of barley that overlap with one or two 3B contigs could be investigated and barley genes located on these contigs could be used to search for additional 3B contigs and sequences. Fine mapping of *qYDH.3BL* could also be improved by screening a larger population with molecular markers to detect recombinant events in either of these populations and phenotype those individuals in new field experiments. About 10 000 lines are available for these population (Fleury et al. 2010). Lines would also have to be selected on phenology genes due to the major genes that segregate in these populations (*Ppd-D1, Ppd-B1, VrnA1* and *VrnD1*). Reducing the range of flowering time will allow plants to have a similar development along the crop cycle where unfavourable environmental conditions occur (heat and drought), facilitating the interpretation of the results (Reynolds et al. 2009).

Polymorphism in non-coding regions of *qYDH.3BL* can also be associated with phenotypic variations. Several studies showed major candidate QTL identified in regulatory elements region. In maize, an apical dominance QTL was detected 11 kb upstream *tb1* ORF gene. The phenotypic variation was caused by a transposable element inserted upstream the gene *tb1* (Studer et al. 2011). In Arabidopsis, a similar study demonstrated a *cis*-regulatory

polymorphism (SNP) upstream the gene *AtHKT1;1* involved in salinity tolerance (Baxter et al. 2011) where the candidate gene was originally detected by QTL analysis. In barley, a candidate gene underlying a major QTL for fungal resistance was ignored due to the presence of polymorphism in the coding region. Later, it was proven that the polymorphism was not related to the coding region but to expression level, indicating a possible polymorphism in the promoter region (Moscou et al. 2011). Finally in rice, the QTL (*GS5*) involved in grain characteristics presented no variability in coding region but in non-coding regions where 3 haplotypes were identified (Li et al. 2011).

3. Genes located on a region showing presence/absence variation are good candidates for *qYDH.3BL*

Evidence that a segment of the chromosome 3BL is deleted or introgressed in the three favourable cultivars (RAC875, Excalibur, and Drysdale) compared to Kukri and Gladius suggests a putative role in drought tolerance for the genes present in this region. The use of cv. Chinese Spring as a genomic reference allowed the identification of the deletion or replacement segment. The segment lacking in the RAC875, Excalibur, and Drysdale lines contains putative Glutathione S-transferases (GSTs), disease resistance genes and a protein containing F-box domain. The GSTs and F-box domain containing genes are good candidates for being involved in abiotic stress tolerance. Seven gene sub-families of GSTs have been found in plants and often seen in clusters (Dixon et al. 2010). A total of three GSTs were annotated on ctg660 (1) and ctg61 (2). These three GST correspond to the TAU sub-family which seems to be related to auxin-response. TAU GSTs play important roles in intracellular signalling, biosynthesis of anthocyanin, responses to soil stresses and responses to auxin and cytokinin hormones. Three of Early-responsive to dehydration protein have been identified as GSTs, ERD9 (Alves et al. 2011), ERD11 and ERD13 discovered by Kiyosue et al. (1993). More specifically, ERD9 corresponds to AtGSTU17 (TAU sub-family) which negatively responds to drought stress in Arabidopsis, indicating that the absence of the gene function could contribute to drought tolerance by increasing the rate of root and shoot development (Chen et al. 2011). The role of the genes located in the missing or replaced segments in drought tolerance needs further validation. Two methods could be used to identify a role for the missing genes either by knocking down the GSTs in Gladius and Kukri or by complementing the parental lines with the GSTs (RAC875, Excalibur and Drysdale). If a new segment was introgressed in the three

cultivars compare to Chinese Spring, chromosome walking could be performed using a new BAC library from RAC875.

In addition to the GSTs, a gene encoding for a protein containing an F-box domain was found in this segment. F-boxes were reported to be part of the SCF complex involved in protein degradation through the unbiquitination pathway which is involved in abiotic stress responses (Sadanandom et al. 2012). Ubiquitination is a post-translational protein modification enzymatic process carried by the proteasome complex. It consists in three steps (1) the ubiquitin is activated by E1 enzyme, (2) E2 conjugates and activates ubiquitin and (3) ligates it to substrate recruited by E3 (HECT or RING) (scheme done by Roger B. Dodd, at http://en.wikipedia.org/wiki/File:Ubiquitylation.svg). The degradation process can promote or block biological pathways favourable or unfavourable for the whole plant depending on the role of the target protein. Ubiquitination mediates growth, development, hormone perception, circadian clock, biotic and abiotic responses (Sadanandom et al. 2012). The Ubiquitin-Proteasome System (UPS) modulates damaged proteins after abiotic stress occurrence. It also suppresses stress signalling and eliminates negative regulators when conditions are favourable; UPS can also attenuate signalling pathways and allows growth under moderate abiotic stresses (Lyzenga and Stone 2012). The role of ubiquitination for regulating grain development in wheat was studied recently (Capron et al. 2012). Knowing that *qYDH.3BL* is associated with thousand grain weight, roundness and thickness, would support a role for this kind of gene family. Some E3 ligase and UPS were shown to be up or down regulated in the early and late stages of grain development (Capron et al. 2012).

RING (Really Interesting New Gene) E3 ligases have also be shown to be involved in the degradation of transcription factors which, in turn, regulate gene expression in response to abiotic stresses. There are many types of RING E3 ligases. For example, *COP1* in Arabidopsis (constitutive photomorphogenesis 1) was identified as a negative regulator of responses to light (Seo et al. 2003). *DRIP1* and *DRIP2* (BREB2A-interacting proteins 1 and 2) regulate negatively drought-responsive gene *DREB2A* expression in Arabidopsis (Qin et al. 2008). Another E3 ligase, SINAT5 is involved in degradation of NAC1 reducing auxin signals in Arabidopsis (Xie et al. 2002). SINAT5 was recently reported to have high sequence similarity with *OsDIS1* (Ning et al. 2011), a drought responsive gene in rice. Finally *AIP2* is involved in degradation of *ABI3* in Arabidopsis (B3 transcription factor) (Zhang et al. 2005). *TaGW2* has been recently described as a RING-type protein

with E3 ubiquitin ligase activity regulating grain width and weight in wheat (Su et al. 2011). Down-regulation of *TaGW2* reduces grain weight and size (Bednarek et al. 2012). One gene located under *qYDH.3BL* on contig ctg660 has homology to a *Brachypodium* gene coding for a RING-finger protein containing a C3HC4-type domain. Many RING-finger proteins, such as the RING-type E3 ubiquitin ligase, play a key role in the ubiquitination pathway. RING-type E3 ligases are extremely substrate specific indicating that changes to promoter region and/or coding region could affect its performance. Interestingly, the same polymorphic SNPs were found in this gene in the three parental lines carrying the favourable allele suggesting that gene ER_07_CAT04_15 is also a good candidate for *qYDH.3BL*. The transcription of this gene may be affected by a SNP found in the promoter region. Moreover, the protein function might also be affected by a SNP found in the third exon which results in an amino acid change from a histidine (positive 10%, neutral 90%) into an arginine (positive). The slight change of charge could affect the function of the protein. The *qYDH.3BL* was found to be associated with thousand grain weight in RK_DH and RK_RI but also with grain characteristic traits such as roundness and thickness in GD_RIPop1. If the gene is an E3-ligase then it may be also a good candidate gene to explain the effect of *qYDH.3BL* on grain characteristic traits responding differently under abiotic stresses.

Finally, two genes encoding proteins containing a F-box domain were located on ctg660 (Table 4.4). F-box protein is a subunit giving the specificity to the SCF complex playing the role of E3 ligase for protein degradation. The SCF complex includes four subunits, F-box, Skp1, Cullin (Cul1) and Rbx1 as described by Sadanandom et al. (2012). About 700 F-boxes are identified in Arabidopsis (Gagne et al. 2004; Schumann et al. 2011). Four F-box genes were found in the *qYDH.3BL* region (Appendix 4.3). A F-box protein DOR increases the susceptibility to drought in Arabidopsis; mutations of the gene give plants more tolerant to water deficit (Zhang et al. 2008). Thus, proteins containing F-box domain could also be considered as potential candidates. These two genes on ctg660 coding for proteins containing a F-box domain potentially involved in protein degradation have been identified in the same region under the QTL peak.

4. Differential expression of candidate genes under cyclic drought

Two genes located on contigs ctg647 showed a significant statistical difference between RAC875 and Kukri during cyclic drought. Ctg647 has been anchored by the *wmc236* and *cfb539* markers. The marker *cfb539* co-located with *wPt-8021* assigned on ctg660, which is of high confidence in the Excalibur/Kukri genetic map whereas *wmc236* was mapped in RK_RI, RK_DH and GD_RI1 in the distal genetic region of *qYDH.3BL*. Therefore these genes might not be the best candidates but can still be considered for further investigation. These two genes encode a protein phosphatase 2C, and a protein that contains a basic helix-loop-helix DNA-binding domain. The proximity of these genes to the QTL peak and their putative functions make them good candidates for *qYDH.3BL*.

The Protein Phosphatase 2C (PP2C) is one of the major players in ABA signalling There are two PP2C named *ABI1* and *ABI2* that have been shown to repress the ABA responses in Arabidopsis (Merlot et al. 2001) and it is well known that ABA is involved in responses to biotic and abiotic stresses. The *PP2C* annotated on contig ctg647 is highly similar to *ABI2* in Arabidopsis which reinforces this gene as a good candidate. Interestingly, the gene is differentially expressed in the two parental lines RAC875 and Kukri during the second cyclic drought. In Kukri, the gene is down-regulated whereas in RAC875 its expression is maintained. The continuous expression of *PP2C* in RAC875 could indicate less sensitivity to stress, therefore allowing development to continue under drought stress. The gene containing a basic helix-loop-helix DNA-binding domain was found to be homologous to a protein bHLH transcription factor in *Brachypodium*. Feller et al. (2011) reported more than 162 bHLH in Arabidopsis and 111 in rice and an even larger number could be expected in wheat. Several bHLH transcription factors have been reported to be induced by environmental stimuli. *AtICE1* (inducer of CBF expression 1) is induced by low temperature or water deficit (Zhu et al. 2007) and *AtMYB2* is induced by drought and ABA treatment (Abe et al. 2003). Three basic-helix-loop-helix transcription factors called SPEECHLESS, MUTE and FAMA play an important role in stomatal development (Hamanishi et al. 2012). *qYDH.3BL* was associated with canopy temperature in RAC875/Kukri populations which reflects a role in controlling the opening of the stomatal aperture. Further studies will be required to test whether there is a correlation between this bHLH TF expression and stomata measurement or its proxy, canopy temperature.

V. Conclusion

In this study, fine mapping and access to the physical map and sequence of chromosome 3B enabled us to identify a number of potential candidate genes for *qYDH.3BL*. Based on combined lines of evidence, four genes that are involved in ubiquitination (probable RING-type E3-ligase and F-box), ABA signalling (PP2C) and transcription factors (bHLH) should be analysed in more detail. In addition, a presence/absence polymorphism was observed which includes genes with functions related to responses to abiotic stresses. Further functional studies will be required to confirm the importance of the PAV (presence/absence variation) and further analysis is also required on the role of all candidate genes in maintaining yield under drought. The sequence assembly of the pseudomolecule of chromosome 3B and the newly released barley genome resources (Consortium 2012) will help to fill the gaps between physical contigs and confirm the overlap between the two contigs that contain interesting candidate genes (ctg660 and ctg61) located under the QTL peak.

Chapter 5

Chapter 5: General Discussion

I. Introduction

The aim of the project was to define the genetic region and environmental responses associated with a QTL for grain yield located on chromosome 3BL in bread wheat (*Triticum aestivum* L.). The QTL named *qYDH.3BL* was expressed differently across a range of environments including well-watered, moderate to severe drought and high temperature. The locus was expressed in three bi-parental crosses (RAC875/Kukri, Excalibur/Kukri and Gladius/Drysdale). The analysis framework and new resources generated through this project will benefit wheat improvement at several levels: (1) the information will help guide breeders in the use of molecular markers (MAS) to develop new cultivars with improved tolerance to drought and heat stress (2) the detailed analysis of this regions will help biologists and physiologists understand the genetic control of this QTL/gene(s) for increasing grain yield under heat and drought stress (3) the definition of this region opens opportunities to seek novel variation for wheat improvement and (4) through fine mapping and positional cloning the option to genetically engineer wheat to increase stress tolerance. Molecular markers are available (*cfb560*, *cfb43*, *cfs6009*, *cfp6047*, *cfb539*, *cfb511*) and some are already in use for MAS and a preliminary list of candidate genes underlying this QTL has been prepared. The candidate genes will form the basis for further investigation. The use of biotechnology through transgenesis or complementation with these candidate genes is an important approach to improve heat and drought tolerance.

The genetic analysis of a region associated with grain yield under heat and drought in wheat was ambitious. However, substantial progress was made in sequencing the wheat genome (especially chromosome 3B) and this has proved critical in making progress in this project. Figure 5.1 shows a summary of the outcome. The analysis has delivered a list of markers available for MAS, two candidate genes selected based on putative function and polymorphism, three candidate genes selected for their significant difference in expression between the two parental lines RAC875 and Kukri, and finally an interesting insertion or deletion of a segment of chromosome 3B (Figure 5.1).

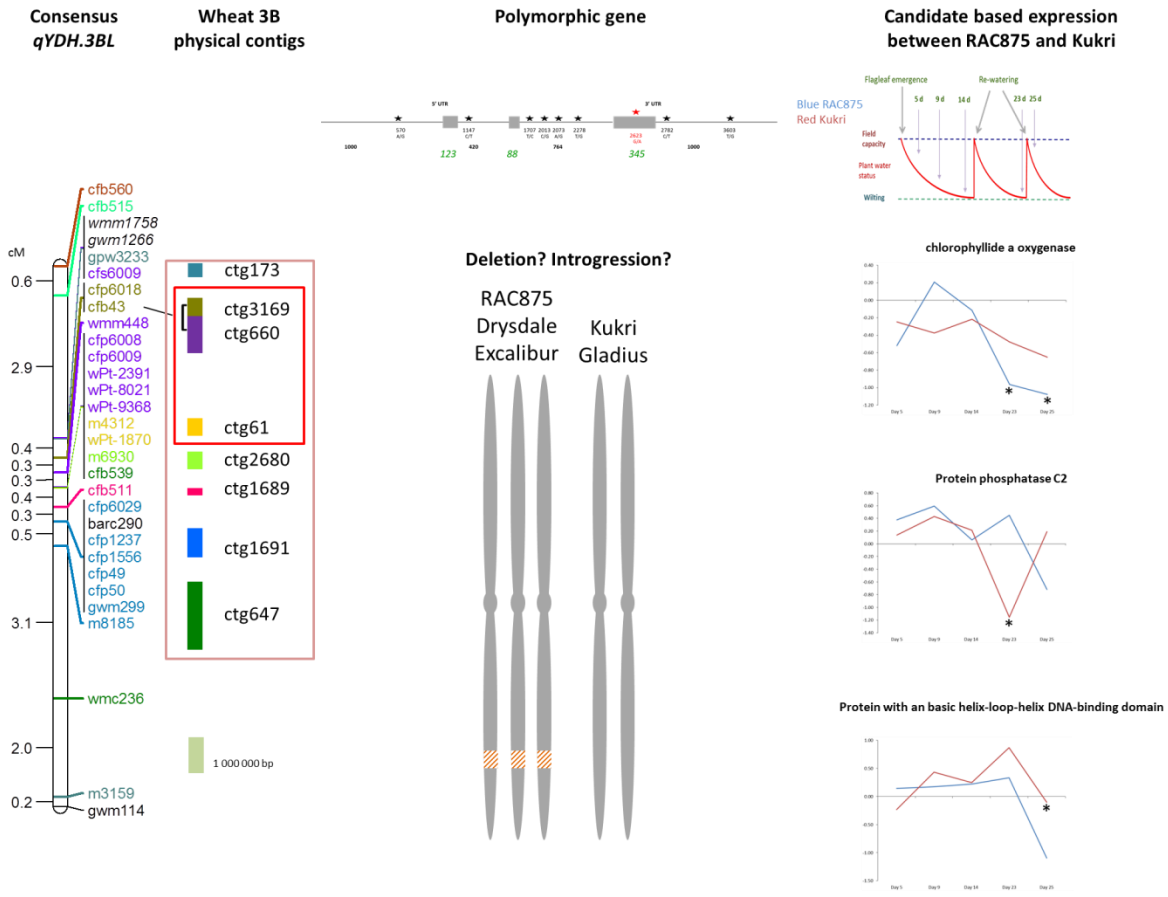


Figure 5.1 Summary of the outcomes of the research. The scheme represents the consensus genetic map of *qYDH.3BL*, the contig assignment with the annotated genes. The two genes highlighted for their SNP polymorphism, the probable deletion (orange hashed) or introgression and finally the three genes differentially expressed.

1. Breeding programs and *qYDH.3BL*

The QTL *qYDH.3BL* was reported in several previous studies and appears to contribute to yield improvement under various climatic conditions including heat and/or drought. Despite recent progress most genes underlying QTL associated with grain yield improvement under biotic or abiotic stresses are unknown but breeders have been using MAS to introgress these QTLs in elite varieties (see reviews for biotic stresses (Collard and Mackill 2008) and abiotic stresses examples (Ashraf and Foolad 2012)). Markers flanking the region of *qYDH.3BL* are already available to breeding programs and, in Australia, the introgression of *qYDH.3BL* has started (Melissa Garcia, University of Adelaide). The benefit of the favourable allele from RAC875 breeding line in RAC875/Kukri DH population was found to increase grain yield up to 12.5% in late sowing under high temperature in Mexico. The same allelic effect was not found in many of the Australian trials but the effect was detected in climatic conditions which included

extreme drought and high temperatures (Chapter 2). In order to understand the opposite effect, a new analysis of the seed number per m^2 could be performed to test the QTL linkage to reduced seed number per m^2 which could potentially be negative in favourable environments and positive elsewhere. A complementary study of the effects of *qYDH.3BL* in another population (Gladius/Drysdale) showed that the contribution of Drysdale allele increased yield from 4 to 8% in diverse environments (Chapter 3). The QTL was detected in environments with water deficit (Mexico) and high temperature in Australia (New South Wales). The detection of apparently the same QTL in more than one population indicates the importance of the QTL and its potential for grain yield improvement. The pedigrees of RAC875 and Drysdale are very different (RAC655/3/Sr21/4*Lance//4*Bayonet and Hartog*3/Quarrion) indicating that the allele was sourced from a distant ancestor or different haplotypes (alleles) resulted in a similar phenotype. Gladius pedigree includes Kukri, indicating that Gladius might carry the Kukri allele for stress sensitivity and lower yield; this could be supported by the same insertion/deletion in both cultivars.

Both RAC875 and Gladius show good performance in South Australia (Mediterranean climate with low rainfall, hot and dry summer and shallow soil). Drysdale yields well under similar conditions in New South Wales (warm-temperate climate where heat waves are common but the soil profile is deeper than in southern Australia). Drysdale was bred specifically for transpiration efficiency based on carbon isotope discrimination (http://www.ipaustralia.gov.au/). The soil differences between the two regions could reflect differences in the most appropriate root architecture to ensure stress tolerance, a trait of high interest. The introgression of *qYDH.3BL* from RAC875 and Drysdale into elite varieties in both SA and NSW could support breeding programs and improve cultivars for broad environmental conditions. In South Australia, either the Drysdale or RAC875 allele should be beneficial given the prediction for seasons to become hotter and drier than at present (Zheng et al. 2012). In Mexico, several genotypes have been selected for their high yield under drought and heat conditions from both populations (RAC875/Kukri and Gladius/Drysdale) and are now in use in breeding programs aimed to improve grain yield in the CIMMYT program.

2. Positional cloning of *qYDH.3BL*

Positional cloning of QTL associated with yield under abiotic stress, especially heat and drought, has not been reported in wheat. The studies reported here provide an example of

how new genomic resources, such as wheat genomic sequences, greatly facilitate the identification of candidate genes under a target QTL. The preliminary list of candidate genes was based on the available information (contig sequences and gene annotation) but there are still gaps under *qYDH.3BL*. Therefore further work and validation is required. Five approaches could be considered to validate the gene position and responsiveness:

1. generate near isogenic lines to support fine mapping,
2. study a larger recombinant population,
3. generate transgenic or mutant lines with gene complementation,
4. comparison of yield QTL with QTL associated with gene expression (eQTL) or metabolite levels (mQTL)
5. develop easier and more direct assays for the trait

a. Near isogenic lines from recombinant inbred lines

A cultivar presenting the Gladius genetic background with the *qYDH.3BL* allele from Drysdale could potentially improve the performance of South Australian cultivars. However this will require the generation of near isogenic lines (NIL). NIL will also help understand the QTL effect and, in breeding programs the NIL could potentially show better performance that the parent genotypes. Creation of NILs takes time, but the availability of RILs for this population (Gladius/Drysdale) could be used to accelerate NILs development. For example, two RILs with the Drysdale allele at *qYDH.3BL* region but the majority of Gladius genetic background for the rest of the genome could be used to fix the locus (from Drysdale). In a second step flanking molecular markers (*cfb515* and *cfb511*) can be used to track the lines containing the desirable region. For a more specific region markers *cfb43*, *cfs6009* and *cfp6047* could be used. Finally the lines can be backcrossed to Gladius to create the NILs. The method is illustrated in Figure 5.2. The ideal environments to evaluate the NILs would be in Obregon (CIMMYT experimental station in Mexico) or at Leeton (experimental station in NSW) where *qYDH.3BL* was detected. The advantage of the NIL is that the only variation between genotypes will be the *qYDH.3BL* locus, limiting the effect of the genetic background and permitting clear evaluation of the effects of the different *qYDH.3BL* alleles.

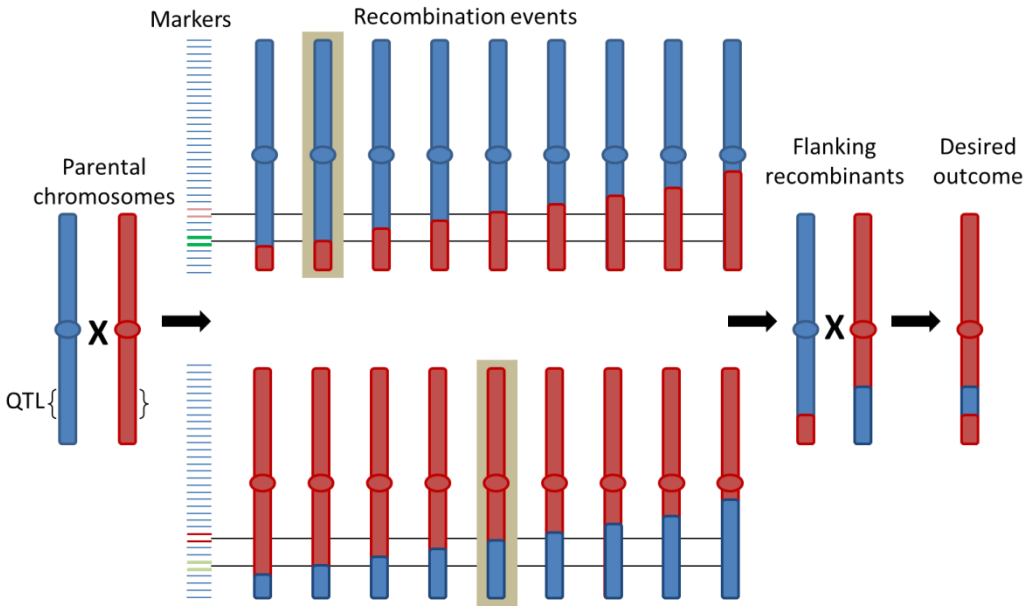


Figure 5.2 Illustration of the NIL production for RIL genotypes to generate a desirable outcome

b. The use of large recombinant inbred line populations

Using the available markers and gene information, a large screening program could be initiated across the three RIL populations which in total represent 10 000 lines. Phenotypic screening of a set of 67 RIL from RAC875/Kukri recombinant between *cfb560* and *wmc236* is underway at the Waite Campus (University of Adelaide). Around 2000 DNAs are available for further screening in the population of RAC875/Kukri. Markers designed along the contigs on each side of the annotated genes and even within the gene, could enhance selection of recombinant lines that could then be assayed under field conditions in well characterized environments to define the phenotype. A total of 102 ISBP (Insertion site-based polymorphism) markers using ISBP*finder* (Paux et al. 2010) have already been designed along the two most likely contigs (ctg660 and ctg61) that contains the annotated genes (Chapter 4).

c. Gene complementation: between genetic and biological validation

Candidate genes for grain yield increase in these three populations can be used directly in gene complementation assays using the alternative alleles from RAC875, Drysdale and Excalibur versus Kukri and Gladius. All varieties can be transformed by biolistics (Dr Eliby Pers. Comm.) and the transgenic can be compared to the original parental lines where early vigour, grain characteristic and grain yield could be measured. The analysis of

the phenotypes would help support, or otherwise, the significance of each candidate gene and increase our understanding of the gene effect.

d. The use of other types of QTL analysis

QTL analysis has been widely used for genetic dissection of agronomical and physiological traits, and recently QTL analysis has been used to study the genetic control of gene expression and metabolite(s) levels which can help identify gene candidates underlying plant responses to stress. eQTL (expression QTL) were used by Jordan et al. (2007) to study seed development in wheat. While mQTL (metabolite QTL) were illustrated by Feng et al. (2012) who studied metabolic networks controlling glucosinolate levels in *Brassica napus* L. Recently, mQTL were mapped in the DH population of Excalibur/Kukri, to detect QTL associated with metabolite levels in plants grown under drought stress (Camilla Hill, submitted for publication). The ideal outcomes from an mQTL study would be to identify common QTL with agronomical traits. These QTL analyses (eQTL and mQTL) could be performed with new resources on the RAC875/Kukri population and used for meta-analysis to identify overlapping QTL. This could validate and support the findings of this thesis.

e. Easier phenotyping to facilitate genetic validation

Grain yield is a multigenic trait, meaning that many QTL/genes are associated with the variation for this trait, however in this thesis the focus was only on *qYDH.3BL*. Additionally, grain yield is determined by the summation of events occurring throughout the growth cycle. An easily assessed trait that is independent of the environment but highly correlated to grain yield could facilitate fine mapping. Traits such as early vigour detected in RAC875/Kukri could provide such an easy measurement. In Chapter 2, early vigour was shown to be associated with *qYDH.3BL*. A scale for early vigour from 1 to 14 has been generated to test the hypothesis.

II. Difficulties remain but solutions are available

1. Unknown environmental interactions

The opposite allelic effects of *qYDH.3BL* observed in different environments where the RAC875/Kukri DH population was tested indicate the complexity of plant responses to the

environment. The factors describing the environment such as timing and period of the occurrence of the stress can result in major differences in response (Chapter 2). Consequently, in breeding programs it would be necessary to assess allelic effects across diverse environments to allow selection of the allele most suited to the target environment. To understand the close relationships between the environment and the plant responses, a meticulous description of the environment, often characterized by multiple factors, would be required; for example soil composition (toxic elements), texture and soil structure for root development, moisture, temperature, light intensity, CO_2 concentration, pathogen load *et cetera*). In many cases these characteristics are not known or are ignored. However, if available, this information can be integrated in programs such as APSIM (http://www.apsim.info/wiki) to simulate possible effects of environmental parameters on plant performance.

2. Biological function and validation of the candidate gene

Three main approaches were proposed for the delivery of outcomes for this project once the biological function of the gene is determined: the use of transgenesis (silencing or over-expression of the gene), the identification of mutations in the target gene using a wheat TILLING population or the identification of novel variation through germplasm screens.

The use of biotechnologies (trangenesis and complementation) could help to identify the gene underlying the QTL and explain the function by over-expressing or silencing the gene. One example of success of gene complementation in wheat has been described by Martin et al. (2006) for the softness of the grain due to the two genes *Pina* and *Pinb*. The complementation of the Drysdale or RAC875 candidate gene could be performed in Gladius and/or Kukri. Studying the phenotype of control and complemented plants could confirm and validate the gene as the basis for the QTL. The other possibility would be to knockdown the candidate gene and compare the transformed plant to the control and study the effect of candidate gene silencing.

Screening a TILLING population could help identifying new haplotypes of the genes to study gene function and expression under abiotic stresses. Searching for mutants of the preliminary candidate gene list could help identify genetic variation and understand the role of the gene by testing the mutants and cultivars in controlled environments including heat and/or drought. The parameters for drought and heat would be similar to field

conditions if tested in glasshouses or could be evaluated under environmental field conditions applied at the managed facility in Ciudad de Obregon (CIMMYT, Mexico). A TILLING population for Gladius is available and there are extensive germplasm collections that can be used to seek alternative alleles for the candidate genes.

III. The wheat genome sequence: resource for answers

This research demonstrated the value of two international and national projects (1) the International Wheat Genome Sequencing Consortium (http://www.wheatgenome.org/), in particular the advance research on chromosome 3B (Catherine Feuillet, Pers. Comm.) and (2) the Australian project for profiling genetic diversity of 15 genotypes (Edwards et al. 2012) and the available data at http://www.bioplatforms.com.au/special-initiatives/agriculture/wheat-datasets. The preliminary annotation of physical contigs was used to identify genes located under the *qYDH.3BL* QTL allowing pre-selection of candidates based on their expression in *Triticum* followed by selection based on gene polymorphisms and gene expression with likelihood to respond to high temperature or water deficit. By combining this annotation and the cultivar sequences (RAC875, Kukri, Gladius, Drysdale and Excalibur), it was possible to identify one gene (a RING finger protein containing a C3HC4 domain) with the same allele in the three parental lines that contribute to grain yield (RAC875, Drysdale and Excalibur). This approach also contributed to SNP discovery in gene sequences annotated in the region of *qYDH.3BL*. As an example, the SNP designed on a particular gene coding for a RING-finger protein containing a C3HC4 domain was mapped genetically in the three populations. Further progress on the analysis of this gene structure and protein function will require additional work including protein modelling to identify changes in conformation or functionality due to the amino acid differences (Chapter 5).

There is still work needed to develop overlapping physical contigs to ensure that there are no missing sequences that may contain candidate genes. Consequently, the preliminary candidate gene list could change. However, the chromosome 3B pseudomolecule construction has started and should help resolve these issues (C Feuillet, Pers. Comm.). The synteny with barley (*Hordeum vulgare* L.) will also help fill the physical gaps and identify missing genes in the target region of 3BL. Although not fully sequenced, a

comprehensive barley genome assembly was recently published (The International Barley Sequencing Consortium, 2012).

Appendix

Appendix

Appendix 1.1 Main studies investigating QTLs for heat and/or drought tolerance in *Triticum aestivum* and *Triticum turgidum* subsp. *durum*

Plant material	**stress applied**	**Data analysis**	**Main traits**	**References**
RIL population	Irrigated and rain fed – field conditions	SMA and CIM	CT, chlorophyll content	(Diab et al. 2008)
DH population	Rain fed and irrigated – field conditions	CIM	Yield and yield components, phenology	(Yang et al. 2007)
DH population	Drought, Heat irrigated, well-water condiction – field conditions	CIM	CT, yield	(Bennett et al. 2012c)
RIL population	Drought environment – field condition	SIM	Yield, Anthesis Height	(Mathews et al. 2008)
DH population	Terminal drought – heat during the growth cycle – field conditions	CIM	Yield and yield components	(Bennett et al. 2012b)
RILs mapping population	Heat and drought in the field temperature >30°C during grain-filling	CIM	CT and grain yield	(Pinto et al. 2010)
RILs mapping population	Heat stress (35/30°C 14/10h day/night) 3 days GC	CIM	Stress susceptibility index	(Mohammadi et al. 2008)
RILs mapping population	Heat stress (38/25°C 14/10h day/night) for 2 days GC	SMA and CIM	Yield and yield components	(Hays et al. 2007)
F5 population	(38/18°C) for 3 days GC	CIM	Yield and yield components	(Mason et al. 2010)
F3 and F4 population	Terminal drought – field condition	SMA and CIM	Yield and yield components	(Golabadi et al. 2011)
DH population	Multiple rain fed – field experiment	CIM	Yield and yield components	(Wu et al. 2012)
RIL	Moderate drought – field conditions	CIM	Yield, Anthesis Height	(Maccaferri et al. 2008)
RIL	Irrigated and drought – field	CIM	Phenology, yield and yield	(McIntyre et al. 2010)

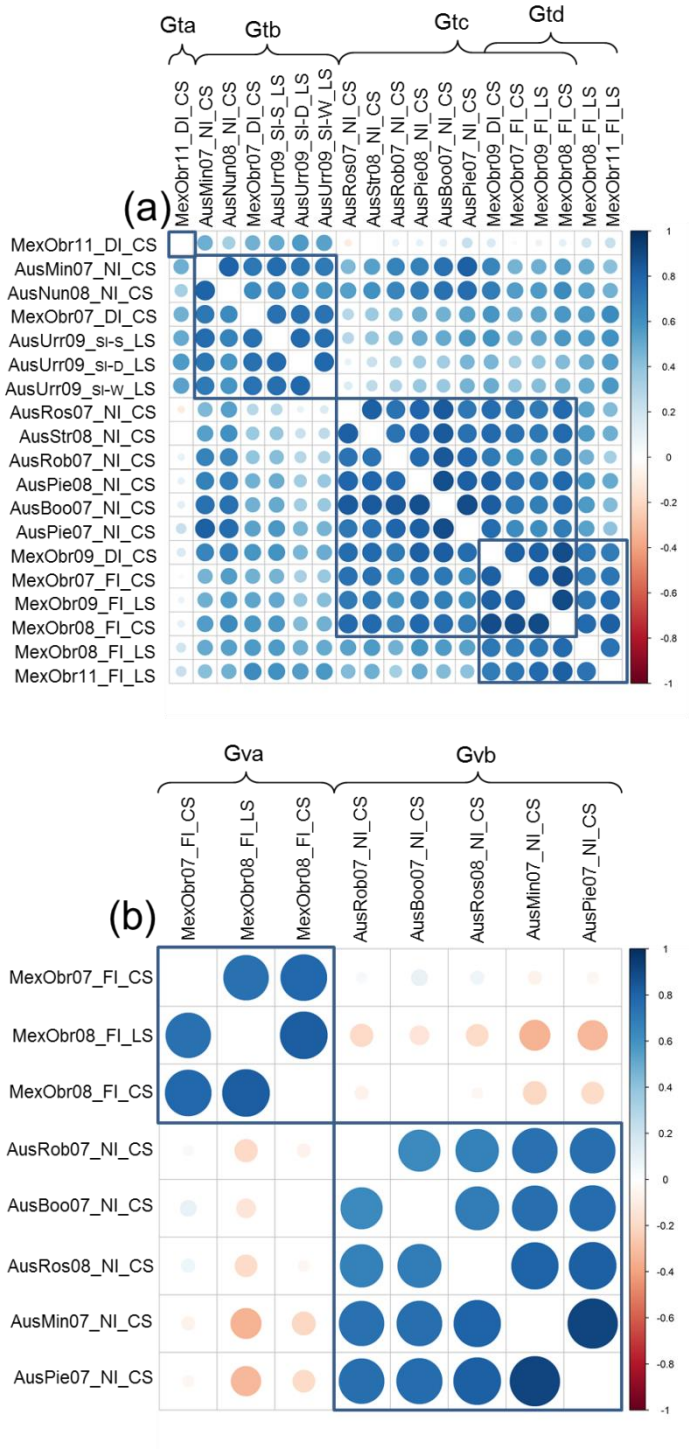
	condition		components	
RIL	Heat – field condition	CIM	CT, yield and yield components	(Paliwal et al. 2012)
RIL	Drought – semi field condition	CIM	Yield and yield components, root morphology	(Kadam et al. 2012)
RIL	Drought and control condition – field condition	CIM	Yield and yield components	(Kirigwi et al. 2007)
RIL	Drought and control condition – field condition	Multiple interval mapping	Yield and yield components	(Alexander et al. 2012)

GC: growth chamber, CIM: Composite Interval Mapping, SMA: Single marker analysis, CT: Canopy Temperature

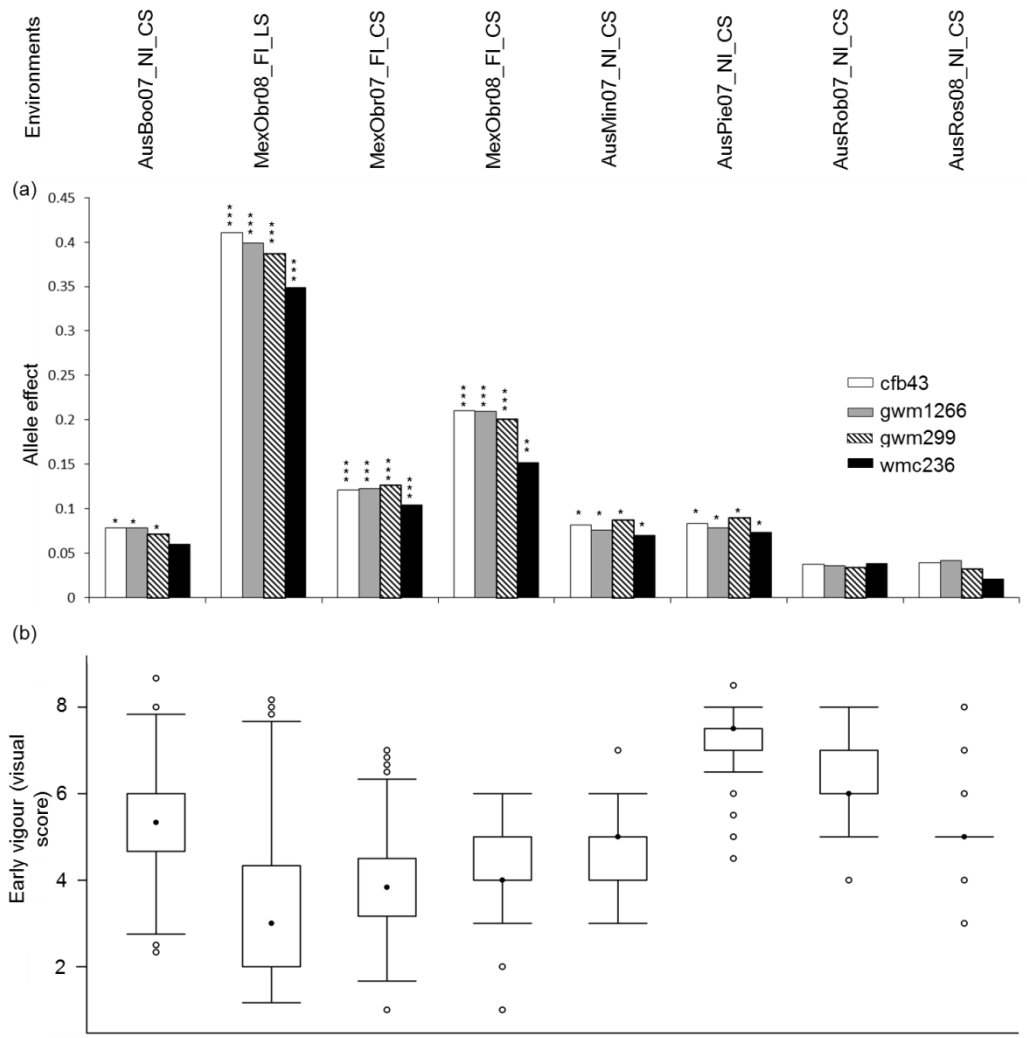
Appendix 2.1 Primer sequences of molecular markers tested for polymorphism between RAC875 and Kukri

Marker name	Forward primer sequence	Reverse primer sequence
barc1124	GCGGGATGTTTCTAATTTTTGTGG	GCGAAAAGGCTAAGATATTGACTATGTA
barc206	GCTTTGCCAGGTGAGCACTCT	TGGCCGGGTATTTGAGTTGGAGTTT
barc290	GCGACCAATTGATCCTAAAAGA	GCGCGATAGCTAGCAAAGAAAATG
barc84	CGCATAACCGTTGGGAAGACATCTG	GGTGCAACTAGAACGTACTTCCAGTC
cfb22	CGAGAACCACCTAGCGGCTAC	GGACGACTGGCACGGGAC
cfb3200	GTATTCTGTTCCTGATCTTGCC	GACTGATGAGCCACGCTG
cfb3366	TGGGAGAGATAGTGAAAGTGG	GCATGTAGCAACTCGATAGG
cfb3375	CCATCATGCAGATTCATAGACC	CTCACGCAGACCTGCAAG
cfb43	AGCTTCCTCAAGAGCCATC	CCAAGTAAGCAAGAGGATGAG
cfd9	TTGCACGCACCTAAACTCTG	CAAGTGTGAGCGTCGG
cfp1237	GCCCTTTTCCTAGTAGTGGCATC	CTCTGTGTAACCCTAGCCCTCTC
cfp1263	CACTGTGGGTTATCACCAGGC	ATCGGCGTTCTACTCTGCAAAG
cfp1556	TCAAGGTCGAGCGCGAGCAG	TCCATGTCAGAATCACGGGGCAG
cfp1774	TGGTCCTTAGAAGGCCGATCAC	TGTCTCTCTCACAGAGCCCAC
cfp1822	CACCTCCCATCCCCTCTTATCAC	TCTCCCTGCCTAGCCCCC
cfp2012	GGAAGAGAGGAGGAAGAAGAGGG	GCGTCAAACCACTAATGTCGCTG
cfp2101	ATGGTAGTCGGGTTCAGGAGCG	AAGCGGTGGGCACATAGGG
cfp24	GGCCATGGTTGACGGAGAG	AACCGACTTTGCGGCCAAG
cfp3019	CCGGGACTAATGGGCTGGAC	GGAGGAGGAGGTGGTTGTAGG
cfp3080	CGGTAAACCGACGGAGGTAAC	GGTGAAGAAACCTGCCCAGAAG
cfp3338	CGTTCCGTTTTGACTCCGTCTG	GGAGGTTCACATGCCTATGTCTG
cfp3353	GCAACACCTCATCCCTCCTTG	TGCCTAGATCTTTCCTCACCACG
cfp3382	CGCCAGTTCTTGCTGCTCC	CCGACTCTATGTAACCCTAGCCC
cfp3427	TGGAGGTAGGGGATGGATCT	CCCGAACCTGGGTAAACATT
cfp49	ATGGCGTATCACAAACTGGTG	GGACCCCCTAAACATTGGTG
cfp50	CGCATCAAACAAGGGTTC	AGAATCACGGGGCAGGAG
cfp7	GGCAAAGAGAATAGCCAGCC	TCTGGGTCGTTGATGGTCG
cfp8	TGTCCCTTGTCCAGTTTAAGC	GGCTTGTTAGTGATATATGGCC
gpw4044	AAGTGCAGTAGGCGACGG	CGGAAATCCTTTAGCATCCA
gpw5007	AAAGTTGGTATGTGGCTCTGG	CAGTAAGCTGGGCTCGGTAG
gwm181	TCATTGGTAATGAGGAGAGA	GAACCATTCATGTGCATGTC
gwm247	GCAATCTTTTTTCTGACCACG	ATGTGCATGTCGGACGC
gwm299	ACTACTTAGGCCTCCCGCC	TGACCCACTTGCAATTCATC
gwm340	GCAATCTTTTTTCTGACCACG	ACGAGGCAAGAACACACATG
gwm4	GCTGATGCATATAATGCTGT	CACTGTCTGTATCACTCTGCT
gwm705	ACCATAAAATATGAGCTAAGG	TCCTACAAGGTGAAGTAAAA
psp3001	GCAGAGAGATGAGGGCACC	CTCTGCTCCCTTAACTTCTG
psp3065	TGCTCCACGCCGCGCCAC	ACCACCTCGTACGGCCCATC
wmc169	TACCCGAATCTGGAAAATCAAT	TGGAAGCTTGCTAACTTTGGAG
wmc206	TTGTGCTCGTGAATTGCATACC	GCCAAAATGGCAGCTTCTCTTA
wmc236	TGGTCACTATGGTAACCGAGGA	CCCTGGGTGATGAATAGACTTT
wmc261	GATGTGCATGTGAATCTCAAAAGTA	AAAGAGGGTCACAGAATAACCTAAA
wmc274	GCAAGCAAGCAGCAAAACTATCAA	AATGAATGAATGAATGAATCGAGGC
wmc322	CGCCCCACTATGCTTTG	CCCAGTCCAGCTAGCCTCC
wmc632	GTTTGATTGGTCGTTCCTGGTC	AACAGCGAATGGAGGGCTTTAG
wmc687	AGGACGCCTGAATCCGAG	GGGAGCGTAGGAGGACTAACA

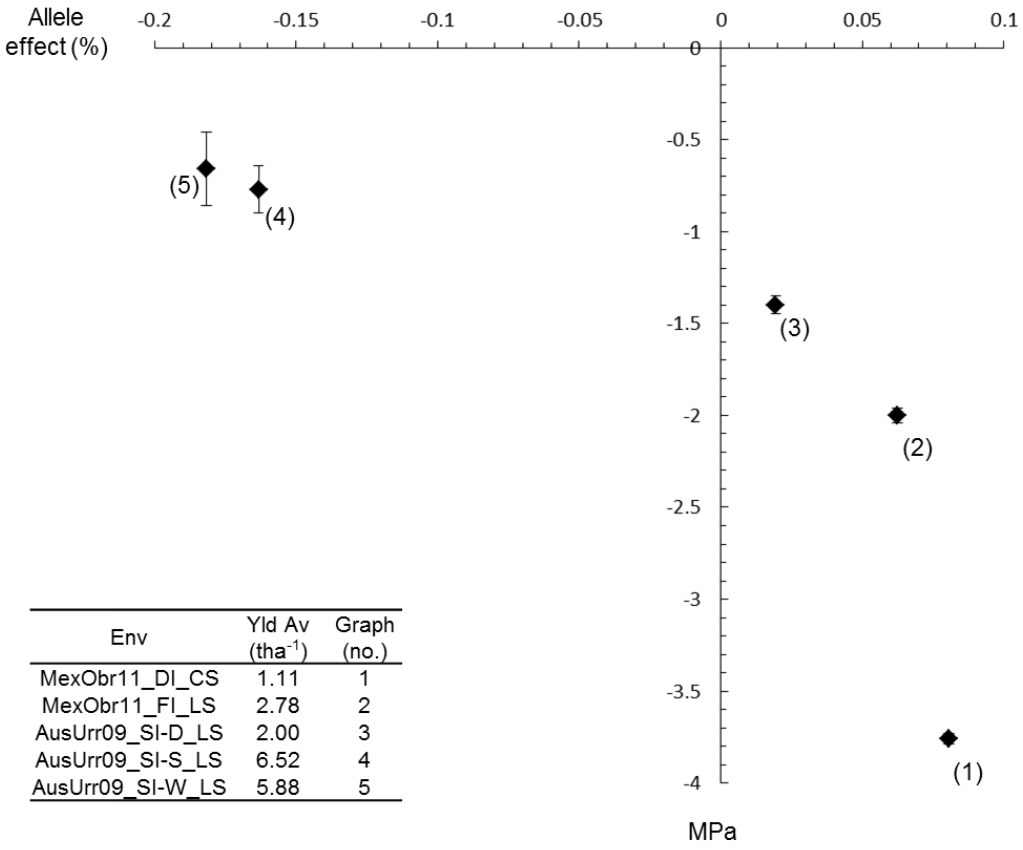
In addition, the following markers obtained under material transfer agreements were also screened: gpw1027, gpw3233, gpw4044, gpw4143, and gpw7335 from INRA (Dr. Pierre Sourdille); gwm1266, gwm1311, gwm1564 and gwm4704 from TraitGenetics and finally gwm655 from IPK (Prof. Dr. A. Graner).



Appendix 2.2 Multi-environment genetic correlation for thousand grain weight (a) extracted from the associated FA3 model and vigour (b) extracted from the associated FA2 model. Each circle represents a pairwise correlation coefficient between environments of 0.4 or more, with the diameter of the circle proportional to the absolute value of the correlation coefficient and the colour of the circle indicating whether the correlation is positive (blue) or negative (red). Groups of genetically correlated environments are outlined by squares and labelled at the top of the figure.



Appendix 2.3 Allele effects (**a**) of four loci (*cfb43*, *gwm1266*, *gwm299* and *wmc236*) on chromosome 3B and trait means (**b**) for early vigour in each environment. Allele effects are expressed as percentage relative to the trait mean. A positive effect indicates that the RAC875 allele increased the trait value while a negative effect indicates that the Kukri allele increased the trait value. Levels of significance are represented by: * p <0.05, ** p <0.01, *** p <0.001. In the boxplots (**b**), the black dot indicates the median value, the box encloses the second and third quartiles, the whiskers extend to ±1.5 times the inter-quartile range, and the empty dots indicate outliers.



Env	Yld Av (tha^{-1})	Graph (no.)
MexObr11_DI_CS	1.11	1
MexObr11_FI_LS	2.78	2
AusUrr09_SI-D_LS	2.00	3
AusUrr09_SI-S_LS	6.52	4
AusUrr09_SI-W_LS	5.88	5

Appendix 2.4 Allele effect plotted against midday leaf water potential (MPa) measured in five environments (n>10) using a Scholander pressure chamber (Scholander et al. 1964). A positive effect indicated that RAC875 allele increase the value of the trait (grain yield) while a negative effect showed that Kukri allele increase its value. The table indicates the mean grain yield for each of these environments.

Appendix 2.5 Summary of recent research in QTL mapping in wheat in the region of interest on 3BL associated with biotic and abiotic stresses.

References – plant material	Trait associated	Environmental conditions (stress – condition – location)	Common locus or distance (cM) from common locus
Biotic stress			
Diab et al. 2008 - *Triticum durum*, RIL	- Canopy temperature - Chlorophyll content - Photosynthesis active radiation - Carbon isotope discrimination - Photosynthesis efficiency	Irrigated and rain fed - field- Syria	*gwm299*
Pinto et al. 2010 - *Triticum aestivum*, RIL	- Grain yield - Grain per square meter - Thousand grain weight - NDVI - Canopy temperature - Water soluble carbohydrate	drought, heat and irrigated - field - North-western Mexico	*wPt8021*
Huang et al. 2004 ***Triticum aestivum*, BC_2F_3**	- Grain weight per ear - Thousand grain weight	Unstressed- field - Germany	*gwm299*
Golabadi et al. 2011 - *Triticum durum*, F_3 and F_4	- Thousand grain weight	Terminal drought - field - Iran	*gwm299*
Narasimhamoorthy et al. 2006 - *Triticum aestivum*, $BC_2F_{2:3}$	- Tiller number per square meter - kernel hardness	Unstressed - field - Kansas (US)	6 cM to *gwm114*
Bennett et al. 2012a - *Triticum aestivum*, DH	- Canopy temperature - Kernel per square meter - Water soluble carbohydrate - Flag leaf width	drought, heat and irrigated - field - North-western Mexico	*wPt8021, wPt4401, gwm114*

Bennett et al. 2012b - *Triticum aestivum*, DH	- Ratio of small grains	Warm and water-limited - field- South Australia	*wPt8021, wPt4401, gwm114*
Wu et al. 2012 - *Triticum aestivum*, DH	- Total number of spikelets per spike	Multiple rain-fed - field - Northern of China	4.6 cM from *wmc236 and gwm299*
Quarrie et al. 2005 - *Triticum aestivum*, DH	- Grain yield	Unstressed - field - Spain	0.60 cM from *barc77* (Paux et al. 2008)
Maccaferri et al. 2008 - *Triticum durum*, RIL	- Grain yield	Moderate drought - field - Tel Amara (Lebanon)	*wmc236*
Mason et al. 2010 - *Triticum aestivum*, F_5 lines	- Heat stress index for kernel weight	Heat stress - glasshouse	Two loci *barc77* and *wmc236*
Börner et al. 2002 - *Triticum aestivum*, RIL	- Thousand grain weight	Unstressed - field - Germany	less than 2cM from *gwm299* in (Paux et al. 2008)
McIntyre et al. 2010 - *Triticum aestivum*, RIL	- Harvest index - Ratio of small seeds - water soluble carbohydrate	Irrigated and drought - field - North-western Mexico	*wPt8021*
Abiotic stress			
Houshmand et al. 2007 - *Triticum durum*, DH	- Stem solidness	*Cephus cinctus* N – field - (Canada)	*gwm114*
Bovill et al. 2010 - *Triticum aestivum*, DH	- Whole plant symptoms	*Fusarium pseudograminearum* - glasshouse	*gwm299*
Schmidt et al. 2005 - *Triticum aestivum*, DH	- Number of *P. thornei* per kg soil plus roots	*Prachylenchus thornei* - glasshouse	*gwm299*
Xu et al. 2010 – *Triticum aestivum*, F_2	- Leaf symptoms	*Blumeria graminis* - glasshouse	5.3 cM from *gwm299*.

Appendix 2.6: Allele effect detected on chromosome 3BL of other agronomical and physiological traits.

I. Introduction

A set of lines (34 doubled haploid and 109 recombinant inbred lines) from the bi-parental cross between RAC875 and Kukri was tested in four experiments conducted in Mexico in 2011 and 2012 (Table 1). Results of the analysis of the data for yield and thousand grain weight for the two experiments conducted in 2011 were reported in Chapter 2 of this thesis. Other agronomic traits were also measured in these experiments, including water status indicators (canopy temperature, leaf rolling), biomass (normalized difference vegetative index, NDVI), yield components (grains/m^2, number of tillers, number of spikelets, spikelet fertility) and phenology (including heading and anthesis date). In 2012, a subset of the lines tested in 2011 were grown under similar environmental conditions (drought and heat) and grain yield, grains/m^2, thousand grain weight, canopy temperature, NDVI and phenology were measured. This chapter reports on the effects of the QTL on 3BL on these aforementioned traits. It comments also on direct and indirect links between traits sometimes found to be associated with the genetic region on chromosome 3B or not.

1. Plant Materials

The materials used in this appendix correspond to a set of genotypes including 34 doubled haploids and 109 recombinants lines from two parental lines with RAC875 (RAC655/3/Sr21/4*Lance//4*Bayonet) drought and heat tolerant and Kukri (76ECN44/76ECN36//Madden/6*RAC177) sensitive. The description of the environments is detailed in Appendix 2.6 Table 1 following the same setting as the Chapter 2.

Canopy temperature (CT) was measured with an infrared thermometer (Mikron M90 series) on erach plot when the weather conditions where optimal (no wind, no clouds) (Pinto et al. 2010). The leaf rolling score was performed based on a three level scale with 1 the leaf was not rolled, 2 half of the leaf was rolled and 3 the entire leaf was rolled. The NDVI (Normalized difference vegetative index) was measured at different time of the growth cycle using the method described by Pinto et al. (2010).

The number of tillers was measured twice per plot on 25 cm long in each bed row. Based on six spikes from different plant representative of the plot the number of spikelet was

measured manually. The biomass was measured on 4-5 “hand full” samplings in the plot to reach approximately 25 well developed spikes as well as tiller biomass.

The phenology traits (heading date, flowering date and maturation date) were all measured when 50% of the plot had reached the stage. The heading corresponded to 50% of head visible across the plot. The flowering date corresponded to visible yellow anthers out of the awn. Finally, the maturation date was measured when the peduncle from the spike was yellowish.

The measurements along the two experiments conducted in 2011 were dated along the growth cycle and visualized in Figure 1 and Figure 2.

2. Statistical analysis

Each set of data for each trait was analysed by using a mixed linear model to reduce the spatial effect using ASReml package (Glimour et al. 1995). Additionally, the genotyping information of *Ppd-D1* and *Ppd-B1* was added to the model to reduce confounding effect of phenology. An extension of this model was performed by adding one by one of the marker information for testing the allele effect along the region of interest on chromosome 3B.

II. Results and discussion

Overall the QTL on 3BL was associated with grain yield in the four environments, with RAC875 carrying the favourable allele increasing grain yield from 100 to 250 kg/ha. This result confirmed the presence of the QTL in a different set of progeny lines (DHs and RILs) from RAC875/Kukri cross (Bennett et al. 2012a; Bennett et al. 2012b) and identified the QTL as heat and drought responsive. Meanwhile, through the analysis of allele effect, the QTL was sometimes associated with other agronomical, phenological and physiological traits such as biomass, thousand grain weight, height, grain/m^2, spikelet number, tiller number (tiller/m^2), heading date, and water status indicators such as canopy temperature, leaf rolling, and finally with NDVI. These results enhanced the importance of understanding the genetic control of this QTL. These results are presented in Table 2 to Table 5.

Grain yield is the final trait measured and it corresponds to the summation of the growth cycle for each genotype that was tested in specific environments that originally appeared to be similar (drought 2011 versus drought 2012 or heat 2011 versus heat 2012). However the

result of allele effect across environments and traits showed variable results. The approach of these results was divided in two types of trait (physiological and agronomic) that have been measured in two to four environments but showed presence or absence of the allele effect. Canopy temperature was measured many times in the four environments. Its measurement depends on the person performing the measure (inclination of the thermometer, shadow of the human body), on weather conditions (wind, clouds which would affect stomata aperture), on the plot itself including density of plants (good coverage), height of the plant and biomass (Giunta et al. 2008). It has been showed in 'hot and dry' environments that canopy temperature was negatively correlated with grain yield (Bennett et al. 2012b; Pinto et al. 2010) indicating that plants maintain their development even with water deficit and/or high temperature occurance. However a positive correlation with yield was found and studied in wheat (Amani et al. 1996) suggesting that cooler canopy temperature can be also deleterious for grain yield in extreme drought condition (it could be assigned to a survival mechanism for a certain time during the day or the growth cycle). In the results presented here for canopy temperature measured at two different stages (vegetative and flowering), were opposite, with the allele of RAC875 contributing to cooler temperature at the vegetative stage and the allele of Kukri contributing to cooler canopy temperature at flowering stage (Table 4b). Out of twenty measurements of canopy temperature during the experiment conducted in 2011 under heat only six showed a significant allelic effect. QTL analyses for canopy temperature were already undertaken on the DH population (RAC875/Kukri) and published by Bennett et al. (2012b). In this experiment in 2011 the results seemed to be inconsistent. Canopy temperature is an easily measurable variable that provides an empirical indication of the physiological water status of the crop, but it is subject to environmental variation and therefore not very reliable for QTL analysis. With infrared photography of an entire experiment, some of the variation could be eliminated, because the temperatures of all plots would be measured simultaneously. With sequential photographs throughout a day, it would be possible to investigate the kinetics of plant transpiration under water deficit and/or high temperature. New technologies are starting to be developed for field evaluation and large scale phenotyping such as Unmanned Aerial Vehicles (UAV) (http://www.regional.org.au/au/asa/2012/precision-agriculture/7933_perrym.htm).

Grain yield components such as thousand grain weight, tiller number and seeds per spike were measured in the two experiments in 2011. The results were also not consistent; tiller/m^2 seemed to be associated with the genetic region under drought but not under heat.

RAC875 was contributing to higher number of tillers. Again, the same observations were observed for thousand grain weight, where the allele effect could be significant or not between these two experiments.

Leaf rolling seemed to be more consistent between measures but unfortunately it was measured only in one environment with a scale that was not fully developed. The scale used for this measurement included only three levels. However if new experiments were conducted in similar environmental conditions a better scale (including 5 levels) could be used such as the one described by O'Toole et al. (1979). NDVI measurements were not consistent along the crop cycle and between experiments. Regarding the result for the allelic effect in two environments for heading and anthesis date was surprising however the effect itself was less than a day. The measurement of the heading and anthesis date is not really accurate; indeed the measure was collected every two to three days with an estimation of the day. The presence of significant effect of this genetic region associated with heading and anthesis date were not previously detected in these bi-parental populations (DHs and RILs) in any other environments studied in Chapter 2. It would not be appropriate to base any conclusion on a QTL where the difference is less than a day with an approximated value ± one day.

Table 1 Descriptions of the four environments were experiments were conducted on the wheat populations, showing locations, water supply from rainfall and/or irrigation, criteria used to select (RAC875/Kukri DH and RI, lines for inclusion, numbers and types included, sowing densities, mean temperatures around flowering time.

Environment [a]	Location	Latitude	Longitude	Altitude (M)	Selection criterion [b]	Lines tested [c]	Sowing density seed m^{-2}	Rainfall and/or irrigation mm	Mean temperature around flowering time[d]	
									Sept	**Oct**
MexObr11_DI_CS	Ciudad de Obregon	27°28' N	109°56' W	38	Marker genotypes	34 DH/77 RI	200	150	24.5	29.3
MexObr12_DI_CS	Ciudad de Obregon	27°28' N	109°56' W	38	Marker genotypes	27 DH/40 RI	200	100	24.7	26.6
									April	**May**
MexObr11_FI_LS	Ciudad de Obregon	27°28' N	109°56' W	38	Marker genotypes	34 DH/109 RI	200	1050	30.7	34.8
MexObr12_FI_LS	Ciudad de Obregon	27°28' N	109°56' W	38	Marker genotypes	27 DH/40 RI	200	900	30.5	37.8

a Aus Australia, Mex Mexico, 07-11 2007 to 2011, DI drip irrigation, FI for flooding irrigation, LS late sowing, CS conventional sowing.
b Marker genotypes: recombinant lines between the two loci *barc77* and *gwm114* and genotyped for *PPd-D1* and *barc13*
c DH doubled haploid lines, RI recombinant inbred lines.
d The temperature data for Mexico were collected at www.agroson.com.mx , whereas the temperature data for Australia were collected at http://www.bom.gov.au/.

Table 2 Allele effect calculated for individual traits (grain yield, thousand grain weight, emergence NDVI, phenology, height and canopy temperature) measured in a 'drought' environment in Mexico 2012 where 27 DH and 40 RI form RAC875/Kukri DH and RI populations.

RAC875/Kukri experiment MedObr12_DI_CS																				
TRAITS	Grain Yield (kg/m^2)		TGW[a] grams		Grain/m^2		NDVI[b] emergence		Anthesis date		Maturation date		Height (cm)		CTv average		CTg average		NDVI average	
Marker	Effect	pv	Effect	pv	Effect	pv	Effect	pv	Effect	pv	Effect	pv	Effect	pv	Effect	pv	Effect	pv	Effect	pv
barc77	-4.415		-0.459		38.719		0.000		-0.016		-0.155		-0.439		0.003		0.080		-0.005	
gpw7108	3.273		0.644		-132.928		0.004		-0.419		-0.340		0.091		-0.024		-0.027		-0.003	
wmm1966	3.273		0.644		-132.928		0.004		-0.419		-0.340		0.091		-0.024		-0.027		-0.003	
cfp6016	2.156		0.537		-135.796		0.003		-0.351		-0.330		-0.006		-0.021		-0.015		-0.004	
wmm1420	7.349		0.335		94.215		0.005	*	-0.412		-0.621		0.370		-0.039		0.017		0.002	
cfb503	7.474		0.431		70.200		0.006	*	-0.495		-0.701	*	0.468		-0.032		0.027		0.001	
wmm454	12.888	***	0.658		197.931		0.006	*	-0.332		-0.187		-0.173		-0.052		-0.035		0.007	
wmm480	12.899	***	0.599		224.254		0.005	*	-0.448		-0.306		-0.171		-0.055		-0.027		0.006	
wmm517	13.538	***	0.653		221.975		0.006	*	-0.518		-0.355		-0.244		-0.070		-0.038		0.008	
wmm408	13.274	***	0.637		217.191		0.006	*	-0.250		-0.143		-0.173		-0.066		-0.042		0.007	
cfb528	13.459	***	0.697		203.240		0.006	**	-0.270		-0.139		-0.087		-0.076	*	-0.050		0.008	
cfb515	14.975	***	0.667		253.861	*	0.006	**	-0.570		-0.473		-0.038		-0.077	*	0.031		0.009	*
wmm1758	17.064	***	0.861	*	264.030	*	0.007	***	-0.672	*	-0.413		0.134		-0.092	*	0.066		0.012	**
gwm1266	17.064	***	0.861	*	264.030	*	0.007	***	-0.672	*	-0.413		0.134		-0.092	*	0.066		0.012	**
cfs6009	17.208	***	0.869	*	265.426	*	0.007	***	-0.631		-0.399		0.221		-0.094	*	0.064		0.012	**
cfp6018	16.124	***	0.733	*	279.737	*	0.006	**	-0.650		-0.249		-0.036		-0.075	*	0.146		0.011	**
cfb43	17.064	***	0.861	*	264.030	*	0.007	***	-0.672	*	-0.413		0.134		-0.092	*	0.066		0.012	**
wmm448	17.064	***	0.861	*	264.030	*	0.007	***	-0.672	*	-0.413		0.134		-0.092	*	0.066		0.012	**
cfp6008	16.861	***	0.826	*	270.392	*	0.007	**	-0.641		-0.403		0.100		-0.092	*	0.078		0.011	**
cfp6009	16.896	***	0.797	*	277.305	*	0.007	***	-0.527		-0.347		0.121		-0.091	*	0.079		0.012	**
cfb511	15.680	***	0.860	*	213.661		0.005	*	-0.634		-0.416		0.029		-0.082	*	0.065		0.010	**
cfp1556	15.590	***	0.874	*	201.107		0.006	*	-0.828	*	-0.584		0.390		-0.094	*	0.072		0.011	*
cfp49	15.680	***	0.860	*	213.661		0.005	*	-0.634		-0.416		0.029		-0.082	*	0.065		0.010	**
gwm299	15.552	***	0.815	*	232.444		0.004		-0.680		-0.511		0.057		-0.079	*	0.066		0.009	*
cfp6029	16.432	***	0.874	*	229.172		0.005	*	-0.708	*	-0.426		0.029		-0.081	*	0.075		0.009	*
cfp1237	15.680	***	0.860	*	213.661		0.005	*	-0.634		-0.416		0.029		-0.082	*	0.065		0.010	**
cfp50	15.567	***	0.843	*	214.158		0.005	*	-0.657		-0.434		-0.178		-0.083	*	0.039		0.010	*
barc290	15.680	***	0.860	*	213.661		0.005	*	-0.634		-0.416		0.029		-0.082	*	0.065		0.010	**
wmc236	11.479	***	0.609		175.084		0.002		-0.359		-0.199		0.000		-0.051		-0.043		0.008	*
gwm114	4.286		0.209		41.587		0.000		0.357		0.560		0.497		0.011		0.047		0.007	

a TGW thousand grain weight (grams)
b NDVI Normalized difference vegetative index

Table 3 Allele effect calculated for individual traits (grain yield, thousand grain weight, emergence NDVI, phenology, height and canopy temperature) measured in a 'heat' environment in Mexico 2012 where 27 DH and 40 RI form RAC875/Kukri DH and RI populations.

RAC875/Kukri experiment MedObr12_FI_LS																						
TRAITS	Grain yield (kg/m^2)		TGW[a] grams		Grain/m^2		NDVI[b] emergence		Heading date		Maturation date		Height (cm)		CTv Average AM		CTg Average AM		CTg Average PM		NDVI Vegetative		NDVI Grain Filling	
Marker	Effect	pv	Effect	pv	Effect	pv	Effect	pv	Effect	pv	Effect	pv	Effect	pv	Effect	pv	Effect	pv	Effect	pv	Effect	pv	Effect	pv
barc77	-10.754	*	-0.142		-332.552		-0.003		0.661	*	-0.323		-0.762		0.047		-0.053		0.089		-0.011	*	-0.007	
gpw7108	3.273		-0.050		107.053		0.000		0.058		-0.587		0.389		-0.030		0.099		0.032		-0.003		-0.003	
wmm1966	3.273		-0.050		107.053		0.000		0.058		-0.587		0.389		-0.030		0.099		0.032		-0.003		-0.003	
cfp6016	2.967		-0.147		111.296		0.000		0.087		-0.603		0.219		-0.004		0.110		0.039		-0.004		-0.003	
wmm1420	0.818		-0.002		40.511		0.002		-0.032		-0.408		0.695		-0.020		0.146		0.033		0.001		-0.002	
cfb503	0.934		0.089		26.816		0.002		-0.102		-0.379		0.696		-0.048		0.141		0.025		0.001		-0.002	
wmm454	13.870	**	0.418		348.341		0.003		-0.369		0.041		1.023		-0.136		-0.031		-0.154	*	0.012	**	0.009	
wmm480	14.952	**	0.157		432.243	*	0.002		-0.415		0.052		1.014		-0.129		-0.006		-0.148		0.011	*	0.007	
wmm517	15.870	**	0.193		452.963	*	0.002		-0.435		0.016		0.918		-0.110		0.045		-0.143		0.011	*	0.005	
wmm408	12.739	**	0.514		314.960		0.003		-0.301		0.043		0.962		-0.138		-0.019		-0.143		0.013	**	0.010	
cfb528	14.748	**	0.710		342.276		0.004		-0.333		0.031		1.116	*	-0.160		-0.022		-0.160	*	0.014	**	0.011	*
cfb515	19.456	***	0.376		542.763	**	0.004		-0.650	*	-0.016		1.061	*	-0.113		-0.040		-0.166	*	0.015	***	0.010	
wmm1758	22.122	***	0.626		590.330	***	0.004		-0.770	**	-0.102		1.544	**	-0.131		-0.039		-0.184	*	0.019	***	0.013	*
cfs6009	21.557	***	0.651		568.525	***	0.004		-0.752	**	-0.125		1.565	**	-0.125		-0.039		-0.171	*	0.019	***	0.012	*
gwm1266	22.122	***	0.626		590.330	***	0.004		-0.770	**	-0.102		1.544	**	-0.131		-0.039		-0.184	*	0.019	***	0.013	*
cfp6018	21.367	***	0.730		522.116	**	0.004		-0.728	**	-0.090		1.360	**	-0.106		-0.046		-0.194	**	0.018	***	0.014	**
cfb43	22.122	***	0.626		590.330	***	0.004		-0.770	**	-0.102		1.544	**	-0.131		-0.039		-0.184	*	0.019	***	0.013	*
wmm448	22.122	***	0.626		590.330	***	0.004		-0.770	**	-0.102		1.544	**	-0.131		-0.039		-0.184	*	0.019	***	0.013	*
cfp6008	22.527	***	0.622		598.921	***	0.004		-0.753	**	-0.085		1.491	**	-0.125		-0.037		-0.185	*	0.019	***	0.014	**
cfp6009	22.594	***	0.523		622.096	***	0.005		-0.708	*	-0.124		1.625	**	-0.158		-0.041		-0.196	*	0.020	***	0.015	**
cfb511	21.832	***	0.377		626.419	***	0.003		-0.848	**	-0.133		1.553	**	-0.103		-0.048		-0.140		0.016	***	0.014	**
cfp1556	23.248	***	0.454		663.047	***	0.003		-0.963	***	-0.108		1.885	***	-0.077		-0.001		-0.150		0.017	***	0.013	*
cfp49	21.832	***	0.377		626.419	***	0.003		-0.848	**	-0.133		1.553	**	-0.103		-0.048		-0.140		0.016	***	0.014	**
gwm299	23.475	***	0.166		710.839	***	0.002		-0.925	***	-0.139		1.642	**	-0.106		-0.041		-0.155	*	0.015	***	0.012	*
cfp6029	21.876	***	0.344		631.886	***	0.004		-0.918	***	-0.097		1.411	**	-0.104		-0.020		-0.166	*	0.017	***	0.014	*
cfp1237	21.832	***	0.377		626.419	***	0.003		-0.848	**	-0.133		1.553	**	-0.103		-0.048		-0.140		0.016	***	0.014	**
cfp50	21.394	***	0.333		620.276	***	0.003		-0.925	***	-0.151		1.413	**	-0.119		-0.063		-0.182	*	0.016	***	0.013	*
barc290	21.832	***	0.377		626.419	***	0.003		-0.848	**	-0.133		1.553	**	-0.103		-0.048		-0.140		0.016	***	0.014	**
wmc236	14.424	**	0.309		403.862	*	0.003		-0.740	**	-0.092		0.759		-0.177	*	-0.052		-0.093		0.016	***	0.011	*
gwm114	11.794	*	0.448		288.848		0.004		-0.393		0.683	*	0.636		-0.087		0.050		-0.055		0.016	***	0.013	*

a TGW thousand grain weight (grams)
b NDVI Normalized difference vegetative index

Table 4 (a, b, and c) Allele effect calculated for individual traits (grain yield, thousand grain weight, emergence NDVI, phenology, height and canopy temperature, harvest index, biomass, and several yield components) measured in a 'drought' environment in Mexico 2011 where 34 DH and 77 RI form RAC875/Kukri DH and RI populations.

RAC875/Kukri experiment MedObr11_DI_CS (a)

TRAITS	Grain yield (kg/m^2)		Anthesis date		Maturity date		Height (cm)		TGW[a]		Grain/m^2		Tiller[b] Biomass (g)		Spike weight (g)		Biomass (g)		Harvest index		Seed per Spike	
marker	Effect	pv	Effect	pv	Effect	pv	Effect	pv	Effect	pv	Effect	pv	Effect	pv	Effect	pv	Effect	pv	Effect	pv	Effect	pv
barc77	-4.039		-0.004		-0.060		-0.786		0.207		-170.081		0.633		-0.942		-0.318		-0.011		-1.532	*
gpw7108	4.623		-0.600		-0.596		-0.169		0.349		93.730		1.130		-0.400		0.653		-0.006		-1.017	
wmm1966	4.623		-0.600		-0.596		-0.169		0.349		93.730		1.130		-0.400		0.653		-0.006		-1.017	
cfp6016	3.754		-0.538		-0.547		-0.320		0.400		62.760		1.189		-0.518		0.617		-0.007		-1.185	
wmm1420	4.712		-0.508		-0.500		-0.351		0.499		106.264		2.015		-0.712		1.180		-0.013		-1.426	*
cfb503	4.335		-0.488		-0.516		-0.254		0.533		83.250		2.253	*	-0.675		1.435		-0.014		-1.438	*
wmm454	4.328		-0.519		-0.299		-0.942		1.511	***	21.869		3.891	***	-1.336	*	2.443	*	-0.030	***	-2.528	***
wmm480	6.335		-0.646		-0.528		-0.873		1.306	**	107.515		3.693	***	-1.127		2.450	*	-0.028	***	-2.244	***
wmm517	7.311	*	-0.737	*	-0.517		-0.975		1.383	**	124.270		3.764	***	-0.969		2.626	**	-0.027	***	-2.146	***
wmm408	4.250		-0.472		-0.218		-1.022		1.530	***	25.614		3.901	***	-1.391	*	2.390	*	-0.030	***	-2.564	***
cfb528	3.875		-0.453		-0.213		-0.994		1.571	***	9.470		4.129	***	-1.492	**	2.591	**	-0.031	***	-2.653	***
cfb515	9.286	**	-0.660		-0.464		-0.964		1.315	**	210.602		4.168	***	-0.949		2.953	**	-0.027	***	-2.163	***
wmm1758	8.297	*	-0.676	*	-0.302		-0.814		1.590	***	145.941		4.768	***	-0.944		3.535	***	-0.031	***	-2.326	***
cfs6009	8.725	*	-0.733	*	-0.311		-0.811		1.289	**	187.219		4.023	***	-0.755		3.015	**	-0.029	***	-1.939	**
gwm1266	8.620	**	-0.736	*	-0.399		-0.797		1.528	***	169.349		4.660	***	-0.894		3.487	***	-0.030	***	-2.218	***
cfp6018	9.058	**	-0.814	*	-0.522		-0.879		1.495	***	175.485		4.345	***	-0.690		3.457	***	-0.026	***	-2.039	***
cfb43	9.671	**	-0.912	**	-0.602		-0.696		1.748	***	177.315		4.815	***	-0.757		3.792	***	-0.029	***	-2.264	***
wmm448	9.378	**	-0.859	*	-0.518		-0.703		1.707	***	173.222		4.917	***	-0.790		3.855	***	-0.030	***	-2.254	***
cfp6008	10.213	**	-0.935	**	-0.573		-0.569		1.308	**	228.533	*	4.592	***	-0.430		3.904	***	-0.024	***	-1.734	**
cfp6009	10.798	***	-0.943	**	-0.697	*	-0.334		1.287	**	264.347	*	4.676	***	-0.388		4.064	***	-0.024	***	-1.598	*
cfb511	9.454	**	-0.861	*	-0.414		-0.235		1.588	***	167.200		4.024	***	-0.409		3.224	***	-0.021	**	-1.629	*
cfp1556	8.978	*	-0.989	**	-0.581		0.095		1.960	***	112.195		4.418	***	-0.355		3.752	***	-0.022	**	-1.724	*
cfp49	10.457	**	-0.991	**	-0.603		-0.277		1.349	**	217.555		3.616	***	-0.211		3.022	**	-0.018	**	-1.362	*
gwm299	10.055	**	-0.955	**	-0.630		-0.195		1.508	***	195.671		3.741	***	-0.245		3.113	***	-0.019	**	-1.452	*
cfp6029	10.459	**	-0.968	**	-0.494		-0.326		1.434	***	211.061		3.733	***	-0.299		3.067	**	-0.019	**	-1.457	*
cfp1237	9.726	**	-0.899	**	-0.528		-0.204		1.464	***	183.786		3.774	***	-0.272		3.124	***	-0.019	**	-1.488	*
cfp50	9.001	**	-1.067	**	-0.589		-0.472		1.827	***	125.380		3.809	***	-0.511		2.933	**	-0.022	**	-1.846	**
barc290	9.424	**	-0.934	**	-0.551		-0.304		1.479	***	177.153		3.829	***	-0.335		3.120	***	-0.020	**	-1.562	*
wmc236	4.770		-0.847	*	-0.335		-0.917		1.536	***	38.249		2.736	**	-0.535		1.923	*	-0.016	*	-1.389	*
gwm114	3.046		-0.179		0.200		-0.534		0.814		72.052		2.116	*	0.089		1.941	*	-0.006		-0.161	

a Thousand grain weight
b Tiller biomass was measured on average for 25 tillers
c Seed per spike was measured on 25 spike on average

RAC875/Kukri experiment MedObr11_DI_CS (b)

TRAITS	CT[a]-1		CT-2		CT-3		NDVI[b] 1		NDVI 1		LR[c]-1		LR-2		LR-3	
marker	Effect	pv	Effect	pv	Effect	pv	Effect	pv	Effect	pv	Effect	pv	Effect	pv	Effect	pv
barc77	0.039		0.067		0.020		-0.004		0.005		-0.033		-0.026		-0.024	
gpw7108	-0.049		0.067		-0.029		-0.006		0.007		-0.066		-0.049		-0.083	
wmm1966	-0.049		0.067		-0.029		-0.006		0.007		-0.066		-0.049		-0.083	
cfp6016	-0.035		0.089		-0.016		-0.006		0.007		-0.061		-0.048		-0.088	
wmm1420	-0.102		0.084		0.009		-0.004		0.004		-0.127	*	-0.058		-0.157	***
cfb503	-0.107		0.080		0.009		-0.004		0.004		-0.122	*	-0.058		-0.154	**
wmm454	-0.125	*	0.263	**	0.083		0.002		-0.003		-0.242	***	-0.105	**	-0.185	***
wmm480	-0.139	*	0.278	***	0.068		0.001		-0.002		-0.235	***	-0.104	**	-0.185	***
wmm517	-0.147	*	0.292	***	0.061		0.001		-0.002		-0.224	***	-0.102	**	-0.192	***
wmm408	-0.128	*	0.261	**	0.091		0.003		-0.004		-0.249	***	-0.107	***	-0.187	***
cfb528	-0.135	*	0.236	**	0.088		0.002		-0.003		-0.256	***	-0.116	***	-0.182	***
cfb515	-0.233	***	0.248	**	0.046		0.002		-0.004		-0.277	***	-0.115	***	-0.207	***
wmm1758	-0.251	***	0.268	**	0.090		0.005		-0.007		-0.282	***	-0.120	***	-0.231	***
cfs6009	-0.212	***	0.250	**	0.107		0.002		-0.004		-0.270	***	-0.121	***	-0.224	***
gwm1266	-0.245	***	0.258	**	0.091		0.004		-0.006		-0.279	***	-0.125	***	-0.233	***
cfp6018	-0.225	***	0.222	**	0.074		0.004		-0.005		-0.246	***	-0.123	***	-0.230	***
cfb43	-0.271	***	0.242	**	0.090		0.004		-0.006		-0.281	***	-0.136	***	-0.245	***
wmm448	-0.267	***	0.238	**	0.095		0.005		-0.007		-0.282	***	-0.138	***	-0.246	***
cfp6008	-0.229	***	0.184	*	0.060		0.003		-0.004		-0.256	***	-0.129	***	-0.231	***
cfp6009	-0.244	***	0.180	*	0.068		0.002		-0.003		-0.240	***	-0.137	***	-0.239	***
cfb511	-0.256	***	0.163		0.028		0.005		-0.006		-0.251	***	-0.092	**	-0.221	***
cfp1556	-0.281	***	0.152		0.017		0.004		-0.006		-0.247	***	-0.079	*	-0.224	***
cfp49	-0.247	***	0.142		0.021		0.004		-0.005		-0.237	***	-0.095	**	-0.216	***
gwm299	-0.249	***	0.160		0.018		0.004		-0.006		-0.241	***	-0.095	**	-0.222	***
cfp6029	-0.251	***	0.158		0.002		0.004		-0.005		-0.251	***	-0.093	**	-0.228	***
cfp1237	-0.251	***	0.153		0.018		0.003		-0.005		-0.234	***	-0.090	**	-0.212	***
cfp50	-0.267	***	0.183	*	0.019		0.003		-0.005		-0.284	***	-0.100	**	-0.244	***
barc290	-0.244	***	0.163	*	0.031		0.003		-0.005		-0.240	***	-0.098	**	-0.217	***
wmc236	-0.141	**	0.174	*	0.055		0.006		-0.008		-0.236	***	-0.070	*	-0.186	***
gwm114	-0.108	*	0.046		0.021		0.011	*	-0.013	**	-0.138	**	-0.029		-0.092	*

a CT canopy temperature CT-1 (vegetative stage), CT-2 (During flowering stage) and CT-3 (after flowering stage) measurements were performed at the dates indicated in the Figure 1
b Normalized difference vegetative index was measured at the date sowed in Figure 1
c LR leaf rolling score during flowering time LR-1 (Afternoon) LR-2 and LR-3 (next morning and afternoon)

RAC875/Kukri experiment MedObr11_DI_CS (c)

TRAITS	Tiller/m^2		Spike/m^2		Fertile spikelet		Total spikelet Per spike		Spikelet abortion of total spikelet	
marker	Effect	pv	Effect	pv	Effect	pv	Effect	pv	Effect	pv
barc77	5.315		2.270		-0.195		-0.121		0.835	
gpw7108	9.238		7.798		0.129		-0.288		0.754	
wmm1966	9.238		7.798		0.129		-0.288		0.754	
cfp6016	7.736		5.427		0.042		-0.275		0.965	
wmm1420	13.449	*	6.201		-0.163		-0.155		1.725	*
cfb503	13.254	*	6.136		-0.192		-0.143		1.780	*
wmm454	21.505	***	1.465		-1.488	**	-0.079		2.498	**
wmm480	21.251	***	1.696		-1.386	**	-0.116		2.146	**
wmm517	19.869	***	1.320		-1.364	**	-0.075		2.229	**
wmm408	21.841	***	1.626		-1.491	**	-0.055		2.666	***
cfb528	22.653	***	-0.126		-1.794	***	-0.029		3.045	***
cfb515	25.346	***	3.365		-1.500	**	-0.042		2.540	***
wmm1758	24.959	***	1.040		-1.737	***	-0.020		3.093	***
cfs6009	23.384	***	0.211		-1.632	**	0.037		2.975	***
gwm1266	25.304	***	1.356		-1.715	***	-0.014		3.066	***
cfp6018	23.051	***	0.599		-1.586	***	-0.095		2.597	***
cfb43	24.839	***	0.628		-1.809	***	-0.131		2.945	***
wmm448	24.753	***	0.790		-1.775	***	-0.090		2.954	***
cfp6008	21.788	***	0.825		-1.495	**	-0.105		2.306	**
cfp6009	21.824	***	0.207		-1.527	**	-0.099		2.519	**
cfb511	17.166	**	1.099		-1.150	*	-0.089		2.177	**
cfp1556	17.468	**	-0.994		-1.469	**	-0.128		2.184	**
cfp49	16.323	**	1.374		-1.052	*	-0.096		2.051	**
gwm299	14.711	**	-0.182		-1.096	*	-0.133		1.890	*
cfp6029	18.677	***	2.244		-1.031	*	-0.148		1.801	*
cfp1237	16.050	**	0.803		-1.087	*	-0.121		2.075	**
cfp50	17.961	***	1.322		-1.231	*	-0.253		1.908	*
barc290	16.539	**	0.601		-1.152	*	-0.124		2.242	**
wmc236	13.421	*	1.247		-0.970		-0.153		1.211	
gwm114	5.516		0.292		-0.458		0.082		0.285	

Table 5 (a, b, c, d): Allele effect calculated for individual traits (grain yield, thousand grain weight, emergence NDVI, phenology, height and canopy temperature, harvest index, biomass, and several yield components) measured in a 'drought' environment in Mexico 2011 where 34 DH and 109 RI form RAC875/Kukri DH and RI populations.

RAC875/Kukri experiment MedObr11_FI_LS (a)																		
TRAITS	Grain yield (kg/m^2)		Height (cm)		Anthesis date		Maturity date		TGW		Harvest index		Biomass (g)		Spike weight (gram)			
marker	Effect	pv	Effect	pv	Effect	pv	Effect	pv	Effect	pv	Effect	pv	Effect	pv	Effect	pv		
barc77	-4.605		-0.956		0.065		0.050		-2.771		-8.179		-4.279		-17.038	**		
gpw7108	-0.267		0.591		-0.069		0.144		-0.064		-3.680		0.340		-7.928			
wmm1966	-0.267		0.591		-0.069		0.144		-0.064		-3.680		0.340		-7.928			
cfp6016	-0.449		0.404		-0.040		0.167		-1.919		-3.274		-0.605		-8.759			
wmm1420	3.463		0.942		0.035		0.311		-5.441		-5.479		3.594		-4.287			
cfb503	4.074		1.074		0.021		0.378		-4.711		-4.917		4.127		-3.015			
wmm454	0.178		0.097		0.225		0.730	*	4.210		-9.139		10.390		-0.437			
wmm480	-1.375		0.104		0.205		0.590		2.740		-6.888		7.848		-0.969			
wmm517	-0.445		0.144		0.174		0.551		2.866		-5.956		8.113		-0.593			
wmm408	-0.106		0.010		0.217		0.808	*	5.303		-9.002		11.213		0.218			
cfb528	0.287		-0.115		0.247		0.975	**	6.451		-9.528		11.379		-0.243			
cfb515	7.787		0.705		0.126		0.632		6.896		-2.262		11.941	*	11.270			
wmm1758	12.167	*	1.064		0.085		0.785	*	9.595		-0.165		15.392	**	15.240	*		
cfs6009	11.085		0.419		0.157		0.875	*	10.898		0.313		17.068	**	16.724	*		
gwm1266	13.298	*	1.012		0.064		0.659	*	9.607		2.323		14.227	*	17.619	**		
cfp6018	10.995	*	0.579		0.183		0.607		12.210		1.539		14.815	**	18.445	**		
cfb43	12.571	*	1.051		0.063		0.704	*	11.313		2.188		14.292	*	18.675	**		
wmm448	12.523	*	1.267		0.035		0.628		11.576		1.652		14.953	*	18.540	**		
cfp6008	13.694	*	1.066		-0.059		0.259		8.984		3.274		11.011		18.698	**		
cfp6009	13.883	*	1.653		0.014		0.472		10.326		3.609		13.644	*	21.686	***		
cfb511	9.642		1.621		0.090		0.472		6.186		-4.986		12.195	*	11.319			
cfp1556	8.951		2.454	*	-0.050		0.353		5.791		-6.794		11.861		12.747			
cfp49	11.063	*	1.629		0.099		0.291		6.153		-1.400		10.141		13.033	*		
gwm299	9.856		1.478		0.117		0.368		6.308		-2.424		9.656		11.645			
cfp6029	8.800		1.486		0.119		0.321		9.412		-2.554		10.254		12.045			
cfp1237	9.244		1.422		0.134		0.368		8.197		-2.889		11.316	*	13.000	*		
cfp50	10.433		1.719		0.045		0.595		7.121		-6.110		8.876		8.059			
barc290	8.086		1.453		0.209		0.529		8.803		-4.330		12.218	*	13.446	*		
wmc236	5.931		0.163		0.183		0.406		7.278		0.402		8.070		11.283			
gwm114	7.572		1.436		0.033		0.032		-2.058		5.482		4.648		15.913	*		

Spike weight was based on 25 spikes

RAC875/Kukri experiment MedObr11_FI_LS (b)

TRAITS	CT[a]-1		CT-2		CT-3		CT-4		CT-5		CT-6		CT-7		CT-8		CT-9	
marker	E	p	E	p	E	p	E	p	E	p	E	p	E	p	E	p	E	p
barc77	-0.02		-0.09		0.25		0.21		0.12		0.42		0.04		-0.68		0.08	
gpw7108	0.15		-0.08		-0.48		-0.30		-0.09		-0.19		-0.12		-0.30		-0.35	
wmm1966	0.15		-0.08		-0.48		-0.30		-0.09		-0.19		-0.12		-0.30		-0.35	
cfp6016	0.08		0.08		-0.44		-0.35		-0.09		-0.25		-0.12		-0.25		-0.13	
wmm1420	0.60		0.40		-0.09		-0.10		0.05		0.17		0.05		-0.31		-0.09	
cfb503	0.53		0.18		-0.07		-0.10		-0.01		-0.01		0.09		-0.28		-0.10	
wmm454	0.56		-0.11		-0.49		-0.46		-0.60		-0.89		-0.34		-0.79		-0.69	
wmm480	0.65		0.15		-0.33		-0.46		-0.59		-0.84		-0.26		-0.76		-0.26	
wmm517	0.62		0.01		-0.46		-0.55		-0.69		-0.81		-0.29		-0.90		-0.40	
wmm408	0.52		-0.17		-0.49		-0.48		-0.59		-0.90		-0.30		-0.71		-0.62	
cfb528	0.75		-0.19		-0.38		-0.35		-0.69		-0.65		-0.29		-0.90		-0.57	
cfb515	0.71		-0.12		-0.64		-0.49		-0.52		-1.33	*	-0.41		-0.22		-0.78	
wmm1758	0.51		-0.32		-0.61		-0.62		-0.80		-1.47	*	-0.48		-0.52		-1.12	*
cfs6009	0.36		-0.52		-0.67		-0.72		-0.85		-1.42	*	-0.80		-0.41		-1.14	*
gwm1266	0.55		-0.49		-0.66		-0.64		-0.85		-1.56	**	-0.54		-0.40		-0.93	*
cfp6018	0.31		-0.05		-0.49		-0.36		-0.58		-1.29	*	-0.33		-0.32		-0.45	
cfb43	0.56		-0.18		-0.48		-0.39		-0.63		-1.44	*	-0.46		-0.22		-0.81	
wmm448	0.59		-0.17		-0.47		-0.43		-0.62		-1.44	*	-0.45		-0.23		-0.93	*
cfp6008	0.34		-0.41		-1.11		-0.92		-0.86		-1.83	**	-0.73		0.03		-0.58	
cfp6009	0.29		-0.47		-0.93		-0.69		-0.87		-1.74	**	-0.64		0.26		-0.63	
cfb511	-0.08		-0.94		-0.91		-0.87		-0.56		-1.76	**	-0.66		-0.21		-0.75	
cfp1556	0.00		-1.26		-0.70		-1.01		-0.90		-1.68	**	-0.67		-0.05		-1.16	*
cfp49	-0.10		-0.60		-0.82		-0.73		-0.48		-1.66	**	-0.53		-0.04		-0.42	
gwm299	0.00		-0.65		-0.77		-0.74		-0.54		-1.64	**	-0.59		-0.06		-0.44	
cfp6029	-0.29		-0.80		-1.06		-1.00		-0.79		-1.86	***	-0.61		-0.05		-0.60	
cfp1237	-0.11		-0.69		-0.88		-0.81		-0.52		-1.73	**	-0.54		-0.15		-0.44	
cfp50	0.02		-0.86		-0.97		-0.97		-0.78		-1.65	**	-0.63		-0.03		-1.25	**
barc290	0.09		-0.61		-0.62		-0.58		-0.35		-1.49	**	-0.51		0.00		-0.32	
wmc236	0.49		-0.62		-0.13		0.12		0.02		-1.02		-0.35		0.28		-0.69	
gwm114	0.23		-0.19		0.12		0.44		0.52		-0.33		0.23		0.83		0.09	

a CT canopy temperature were measured during the growth cycle at different date and stages as indicated in figure 2

RAC875/Kukri experiment MedObr11_FI_LS (c)

TRAITS	CT-10		CT-11		CT-12		CT-13		CT-14		CT-15		CT-16		CT-18		CT-19		CT-20	
marker	E	p	E	p	E	p	E	p	E	p	E	p	E	p	E	p	E	p	E	p
barc77	-0.61		-0.06		-0.71		-0.05		0.10		0.74		1.08		-0.10		0.75		-0.02	
gpw7108	-0.21		0.16		-0.15		-0.35		-0.37		-0.49		-0.21		-0.10		0.72		-0.21	
wmm1966	-0.21		0.16		-0.15		-0.35		-0.37		-0.49		-0.21		-0.10		0.72		-0.21	
cfp6016	-0.28		0.19		-0.14		-0.21		-0.31		-0.33		-0.17		-0.09		0.74		-0.31	
wmm1420	0.18		0.53		0.84		0.08		-0.49		-0.45		-0.15		0.09		0.66		-0.25	
cfb503	0.20		0.47		0.70		0.07		-0.67		-0.45		-0.31		-0.10		0.50		-0.43	
wmm454	0.03		-0.17		-0.10		-0.78		-0.67		-0.99	*	-0.32		-0.56		-0.26		-0.70	
wmm480	0.12		0.01		0.04		-0.60		-0.83		-0.89		-0.08		-0.53		-0.19		-0.60	
wmm517	0.15		-0.12		0.00		-0.75		-0.87		-0.92		-0.10		-0.55		-0.25		-0.76	
wmm408	0.07		-0.19		-0.08		-0.86		-0.72		-0.99	*	-0.30		-0.49		-0.35		-0.61	
cfb528	-0.10		-0.37		-0.32		-0.96		-0.72		-0.89		-0.47		-0.72		-0.76		-0.80	
cfb515	-0.10		-0.21		-0.06		-0.99		-0.66		-0.75		-0.69		-0.66		-0.37		-0.15	
wmm1758	0.05		-0.63		-0.62		-1.16	*	-0.78		-1.06	*	-0.98		-0.86		-0.36		-0.44	
cfs6009	0.24		-0.79		-1.02		-1.30	*	-1.17		-1.05	*	-1.10		-0.68		-0.58		-0.54	
gwm1266	0.13		-0.58		-0.66		-1.13	*	-0.73		-0.99	*	-1.09		-0.89	*	-0.40		-0.41	
cfp6018	0.07		-0.53		-0.49		-0.81		-0.43		-0.66		-0.89		-0.79		-0.28		-0.15	
cfb43	0.04		-0.55		-0.60		-1.01	*	-0.72		-0.95	*	-1.24	*	-0.88	*	-0.46		-0.46	
wmm448	0.14		-0.59		-0.59		-1.10	*	-0.88		-1.06	*	-1.17	*	-0.88	*	-0.37		-0.47	
cfp6008	0.10		-0.58		-0.60		-1.18	*	-0.89		-0.93	*	-1.29	*	-0.67		-0.12		-0.12	
cfp6009	0.25		-0.44		-0.56		-0.77		-0.66		-1.14	*	-1.55	**	-0.81		-0.29		-0.41	
cfb511	-0.05		-0.35		-0.44		-1.07	*	-0.23		-1.03	*	-0.79		-0.62		0.44		0.32	
cfp1556	0.15		-0.47		-0.40		-0.97		-0.23		-1.39	**	-0.49		-0.95	*	0.59		0.53	
cfp49	0.02		-0.27		-0.46		-0.94		-0.27		-0.81		-0.88		-0.56		0.51		0.28	
gwm299	-0.04		-0.31		-0.48		-0.90		-0.15		-0.83		-0.82		-0.51		0.58		0.34	
cfp6029	-0.03		-0.40		-0.45		-0.93		-0.13		-0.74		-0.81		-0.40		0.45		0.30	
cfp1237	-0.07		-0.31		-0.48		-0.92		-0.18		-0.83		-0.85		-0.53		0.46		0.35	
cfp50	0.30		-0.51		-0.36		-1.13	*	0.06		-1.03	*	-0.73		-0.61		0.34		0.40	
barc290	0.04		-0.25		-0.34		-0.85		-0.21		-0.81		-0.63		-0.51		0.29		0.42	
wmc236	0.63		-0.11		0.34		-0.61		-0.58		-1.00	*	-0.80		-0.27		-0.25		0.67	
gwm114	0.68		0.47		1.45	**	0.62		-0.35		-0.53		-0.56		0.15		-0.51		0.74	

RAC875/Kukri experiment MedObr11_FI_LS (d)

TRAITS	Spikelet per spike		Tiller/m^2		NDVI[a]-3		NDVI-4		NDVI-7		NDVI-8		NDVI-10	
marker	Effect	pv	Effect	pv	Effect	pv	Effect	pv	Effect	pv	Effect	pv	Effect	pv
barc77	0.195		0.973		0.283		-0.169		-3.919		4.059		3.991	
gpw7108	-0.128		-0.851		0.145		0.206		-1.557		0.794		0.360	
wmm196	-0.128		-0.851		0.145		0.206		-1.557		0.794		0.360	
cfp6016	-0.013		-0.902		-0.575		0.903		-0.993		0.375		0.252	
wmm142	0.865		-1.340		3.808		-3.274		2.006		-2.926		-0.465	
cfb503	0.564		-1.351		4.212		-3.675		2.216		-2.966		-0.279	
wmm454	1.174		-0.445		-3.386		3.543		-1.508		0.492		-5.184	
wmm480	0.871		-0.845		-3.404		3.670		-1.182		0.363		-6.193	
wmm517	0.779		-0.917		-2.328		2.576		-1.355		0.515		-7.215	
wmm408	1.115		-0.525		-2.511		2.682		-1.550		0.720		-5.631	
cfb528	1.181		-0.416		-0.661		0.868		-1.164		0.365		-5.271	
cfb515	1.712	*	-0.472		3.337		-3.092		2.328		-2.766		-6.715	
wmm175	1.096		-0.335		-0.325		0.489		1.168		-1.760		-4.894	
cfs6009	1.446		-0.140		0.605		-0.479		-0.713		-0.207		-4.553	
gwm1266	1.125		-0.327		0.830		-0.657		1.590		-2.333		-5.002	
cfp6018	1.182		-0.573		-0.443		0.716		-0.386		0.253		-2.150	
cfb43	0.937		-0.674		0.178		0.020		0.558		-0.915		-4.422	
wmm448	0.802		-0.648		-1.235		1.418		0.465		-0.916		-4.689	
cfp6008	0.739		-0.707		-1.322		1.615		0.620		-0.972		-3.671	
cfp6009	1.047		-1.351		2.768		-2.425		2.935		-3.702		-3.823	
cfb511	1.265		-0.166		3.714		-3.643		5.033		-5.046		-6.595	
cfp1556	0.953		-0.510		4.487		-4.428		5.709		-5.681		-5.991	
cfp49	1.152		-0.352		2.826		-2.824		5.000		-5.017		-5.189	
gwm299	1.210		-0.366		1.415		-1.350		5.220		-5.333		-5.244	
cfp6029	1.085		0.173		3.242		-3.302		3.561		-3.894		-2.603	
cfp1237	1.248		-0.347		2.405		-2.371		5.176		-5.181		-5.228	
cfp50	0.970		0.318		2.405		-2.399		3.458		-3.388		-4.059	
barc290	1.577	*	-0.222		3.073		-2.941		3.444		-3.515		-4.128	
wmc236	0.811		-0.333		2.978		-2.936		0.881		-0.593		-4.041	
gwm114	0.405		-0.662		0.660		-0.473		3.052		-2.708		-0.591	

a Normalized difference vegetative index was measured at the date sowed in figure 2

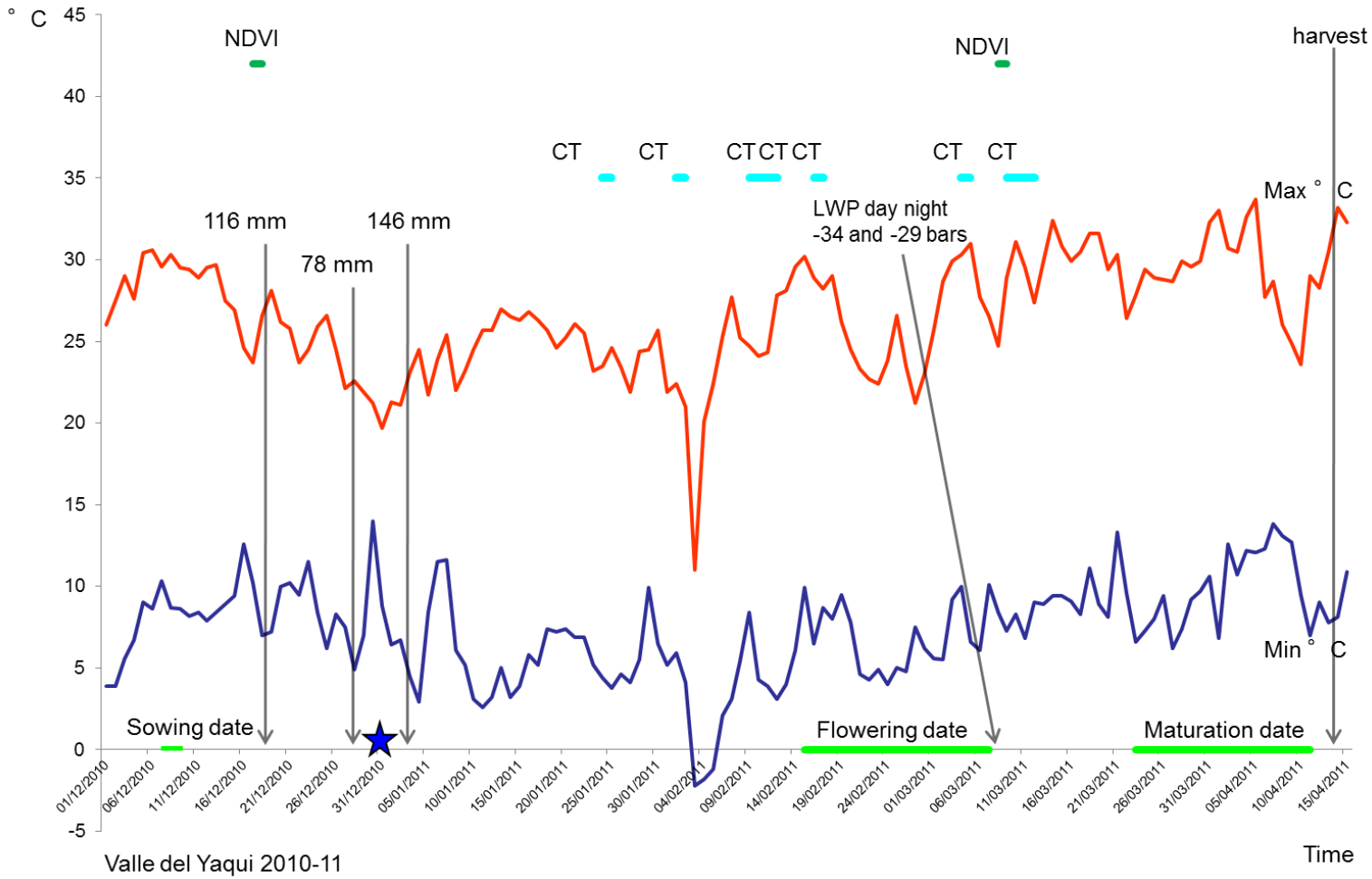


Figure 1 Representation of environmental conditions along the growth cycle in the drought experiment conducted in Mexico in 2011. CT (Canopy temperature), NDVI (normalized difference vegetative index), LWP (leaf water potential), mm (millimetres of water) the blue star correspond to dripping irrigation.

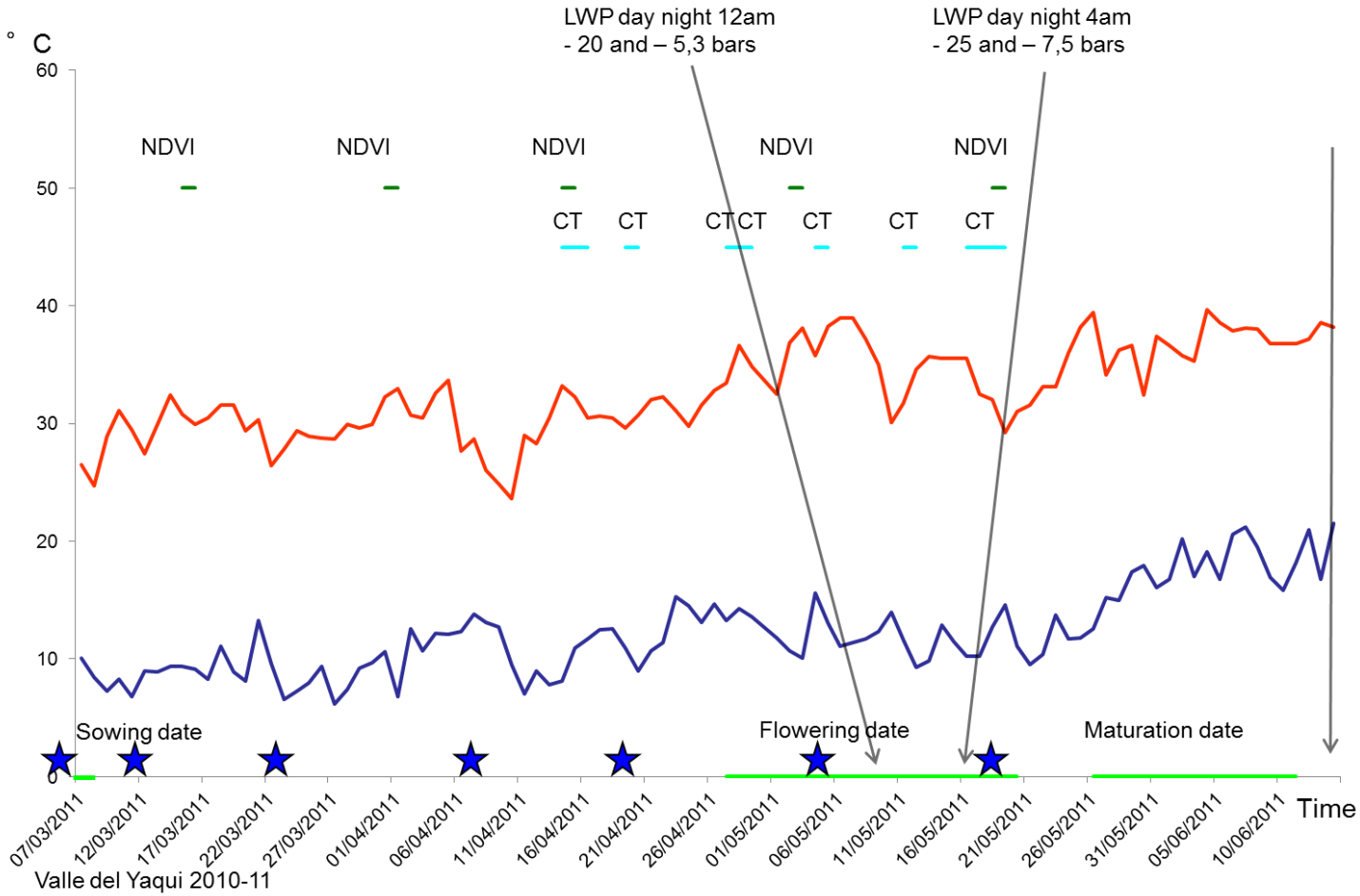


Figure 2 Representation of environmental conditions along the growth cycle in the heat irrigated experiment conducted in Mexico in 2011. CT (Canopy temperature), NDVI (normalized difference vegetative index), LWP (leaf water potential), blue stars correspond to flooding irrigation.

Appendix 3.1 List of molecular markers mapped in Gladius and Drysdale Pop1

Marker name	contig	Fwd primer sequence	Rvs primer sequence
cfb3200	ctg1310	GTATTCTGTTCCTGATCTTGCC	GACTGATGAGCCACGCTG
cfb43	ctg3169	AGCTTCCTCAAGAGCCATC	CCAAGTAAGCAAGAGGATGAG
cfb509	ctg1689	GCCTCACTCAACAACCATCA	ATCGAGAGAAGAGGCATTCG
cfb511	ctg1689	GGCAACAAATGGAAGGAAGA	GCGATGCACATAGTGGTGTC
cfb512	ctg1094	AGCGTCTTCCCTCAAACAGA	GCAGTGGAGAGGTCTTCTGG
cfb515	ctg730	GAGTTCTACCTGGCACTCGC	TCCAGATTTAAAGGCAGCTCA
cfb525	ctg486	CCAAGCCTCACATCTCTCTC	CCGCGAAGAAACAGAAGATT
gpw5007	N/A	AAAGTTGGTATGTGGCTCTGG	CAGTAAGCTGGGCTCGGTAG
gwm299	ctg660	ACTACTTAGGCCTCCCGCC	TGACCCACTTGCAATTCATC
wmc236	N/A	TGGTCACTATGGTAACCGAGGA	CCCTGGGTGATGAATAGACTTT
gpw7108	ctg1216		
wmm1966	ctg1216		
wmm1420	ctg716	N/A	
wmm1758	ctg0	N/A	
wmm448	ctg660	N/A	

In addition, the following markers obtained under material transfer agreements were also screened: gpw1027, gpw3233, gpw7108, gpw4143, and gpw7335 from INRA (Dr. Pierre Sourdille); the contig names correspond to the version 4 of the physical map of chromosome 3B.

Appendix 3.2 SNP markers used in the research with their corresponding names (Matthew Hayden, Pers. Comm.)

SNP name	SNP name
m3271	wsnp_Ex_c30368_39293103
m6587	wsnp_Ku_c1629_3206989
m7918	wsnp_Ra_c41135_48426638
m5202	wsnp_Ex_rep_c66331_64502558
m6651	wsnp_Ku_c18473_27773912
m3724	wsnp_Ex_c40595_47620787
m2691	wsnp_Ex_c22154_31342077
m92	wsnp_BE426287D_Ta_1_1
m5347	wsnp_Ex_rep_c67033_65490126
m7231	wsnp_Ku_c663_1368085
m7678	wsnp_Ra_c18153_27161629
m3260	wsnp_Ex_c3005_5548573
m7342	wsnp_Ku_c9009_15185650
m6861	wsnp_Ku_c29429_39332178
m7794	wsnp_Ra_c26083_35644783
m6919	wsnp_Ku_c33335_42844594
m3149	wsnp_Ex_c2820_5214711
m6920	wsnp_Ku_c33335_42844680
m3150	wsnp_Ex_c2820_5215394
m3726	wsnp_Ex_c4063_7344641
m3725	wsnp_Ex_c4063_7344449
m6238	wsnp_JD_c9805_10591233
m6482	wsnp_Ku_c13069_20938717
m2119	wsnp_Ex_c16304_24782232
m4412	wsnp_Ex_c6065_10623213
m7704	wsnp_Ra_c19522_28713505
m3716	wsnp_Ex_c40250_47352047
m316	wsnp_BE498786B_Ta_2_1
m5925	wsnp_JD_c2146_2955972
m6202	wsnp_JD_c8629_9594108
m6201	wsnp_JD_c8629_9593995
m5960	wsnp_JD_c2623_3541255
m6185	wsnp_JD_c8158_9193784
m1863	wsnp_Ex_c14147_22077948
m7285	wsnp_Ku_c7454_12836140
m1266	wsnp_CD897414B_Ta_2_1
m7860	wsnp_Ra_c32055_41111615
m1827	wsnp_Ex_c13906_21771680
m6200	wsnp_JD_c8629_9593896
m5995	wsnp_JD_c30422_23944042
m8326	wsnp_RFL_Contig2338_1839077
m5396	wsnp_Ex_rep_c67404_65986980
m4226	wsnp_Ex_c54357_57265797

m4039	wsnp_Ex_c4888_8713275
m4310	wsnp_Ex_c5657_9942445
m4040	wsnp_Ex_c4888_8714379
m6761	wsnp_Ku_c24414_34372822
m6165	wsnp_JD_c7325_8422075
m4755	wsnp_Ex_c7967_13522958
m7294	wsnp_Ku_c7784_13343904
m1886	wsnp_Ex_c14321_22290028
m5880	wsnp_JD_c17082_16025440
m5677	wsnp_Ex_rep_c70174_69125822
m6280	wsnp_JD_rep_c53979_36270300
m629	wsnp_BQ168706B_Ta_2_2
m628	wsnp_BQ168706B_Ta_2_1
m6380	wsnp_Ku_c10291_17065432
m6018	wsnp_JD_c35642_26554827
m5814	wsnp_JD_c1247_1802330
m6279	wsnp_JD_rep_c53979_36270209
m6381	wsnp_Ku_c10291_17065480
m5492	wsnp_Ex_rep_c68066_66815745
m377	wsnp_BE591466B_Ta_2_2
m2622	wsnp_Ex_c21499_30644485
m211	wsnp_BE489326B_Ta_2_2
m210	wsnp_BE489326B_Ta_2_1
m2728	wsnp_Ex_c22630_31827919
m6633	wsnp_Ku_c17718_26860963
m2494	wsnp_Ex_c19994_29025586
m1383	wsnp_Ex_c10717_17456391
m7905	wsnp_Ra_c38981_46788555
m6794	wsnp_Ku_c26257_36216976
m6677	wsnp_Ku_c19631_29148397
m3383	wsnp_Ex_c3227_5948436
m7595	wsnp_Ra_c12935_20587578
m6793	wsnp_Ku_c26257_36216869
m4452	wsnp_Ex_c6223_10857649
m2329	wsnp_Ex_c18624_27492167
m5886	wsnp_JD_c1816_2522844
m8273	wsnp_RFL_Contig1945_1118187
m537	wsnp_BG263758B_Ta_2_1
m6086	wsnp_JD_c5067_6187376
m610	wsnp_BQ159467B_Ta_2_1
m898	wsnp_CAP11_rep_c8708_3760250
m7534	wsnp_Ra_c10203_16850924
m2841	wsnp_Ex_c238_460841
m611	wsnp_BQ159467B_Ta_2_2
m3218	wsnp_Ex_c2920_5385184
m5826	wsnp_JD_c1316_1891903
m2351	wsnp_Ex_c1878_3541204
m5890	wsnp_JD_c1843_2562950
m6297	wsnp_JD_rep_c63654_40605158

m4613	wsnp_Ex_c7021_12096881
m3245	wsnp_Ex_c29631_38640100
m4863	wsnp_Ex_c9002_14999105
m6720	wsnp_Ku_c21818_31604716
m7035	wsnp_Ku_c4078_7436510
m7519	wsnp_Ku_rep_c73198_72796386
m7353	wsnp_Ku_c93664_84327484
m8196	wsnp_Ra_rep_c74606_72470419
m4085	wsnp_Ex_c5074_9009245
m3305	wsnp_Ex_c3096_5709257
m3306	wsnp_Ex_c3096_5709369
m6492	wsnp_Ku_c13311_21255428
m6493	wsnp_Ku_c13311_21255891
m5710	wsnp_Ex_rep_c70809_69689636
m8290	wsnp_RFL_Contig2073_1317762
m4218	wsnp_Ex_c5418_9575485
m5711	wsnp_Ex_rep_c70809_69690102
m4219	wsnp_Ex_c5418_9575513
m7692	wsnp_Ra_c18873_27993835
m8583	wsnp_RFL_Contig4320_5027794
m2573	wsnp_Ex_c21094_30222280
m5775	wsnp_JD_c10602_11238420
m4653	wsnp_Ex_c7291_12517871
m7131	wsnp_Ku_c50833_56310020
m3439	wsnp_Ex_c33463_41948471
m3833	wsnp_Ex_c4267_7700267
m2124	wsnp_Ex_c16378_24870688
m3835	wsnp_Ex_c4267_7700461
m3834	wsnp_Ex_c4267_7700325
m3244	wsnp_Ex_c29623_38630871
m8136	wsnp_Ra_rep_c108411_91697657
m6552	wsnp_Ku_c15149_23666345
m8137	wsnp_Ra_rep_c108411_91697852
m1332	wsnp_Ex_c10499_17162550
m4457	wsnp_Ex_c6245_10887043
m2613	wsnp_Ex_c21418_30554998
m1334	wsnp_Ex_c10499_17163260
m1333	wsnp_Ex_c10499_17162700
m6504	wsnp_Ku_c13721_21798677
m7870	wsnp_Ra_c3289_6166914
m1331	wsnp_Ex_c10499_17161734
m3256	wsnp_Ex_c29984_38961431
m2361	wsnp_Ex_c18926_27825028
m2204	wsnp_Ex_c17303_25979191
m3402	wsnp_Ex_c3257_6003626
m3601	wsnp_Ex_c3722_6786328
m3046	wsnp_Ex_c2639_4899517
m2661	wsnp_Ex_c21924_31095740
m2721	wsnp_Ex_c2250_4216508

m7221	wsnp_Ku_c6387_11197393
m7087	wsnp_Ku_c458_954940
m4942	wsnp_Ex_c9594_15882022
m8067	wsnp_Ra_c7615_13070849
m2720	wsnp_Ex_c2250_4216275
m6843	wsnp_Ku_c28381_38311164
m1769	wsnp_Ex_c13361_21059379
m6299	wsnp_JD_rep_c63810_40704815
m624	wsnp_BQ167580B_Ta_2_1
m81	wsnp_BE424246B_Ta_2_2
m3901	wsnp_Ex_c4457_8018164
m5941	wsnp_JD_c22783_19631797
m5209	wsnp_Ex_rep_c66349_64530060
m714	wsnp_CAP11_c2309_1201554
m8169	wsnp_Ra_rep_c70261_68008978
m3170	wsnp_Ex_c2850_5263978
m5432	wsnp_Ex_rep_c67645_66305241
m3669	wsnp_Ex_c39124_46489956
m1782	wsnp_Ex_c13505_21253168
m7679	wsnp_Ra_c18164_27178459
m5871	wsnp_JD_c16245_15468917
m6542	wsnp_Ku_c14875_23320708
m2063	wsnp_Ex_c15795_24169367
m1732	wsnp_Ex_c13154_20784674
m7973	wsnp_Ra_c48924_54032104
m3171	wsnp_Ex_c2850_5264093
m1974	wsnp_Ex_c15047_23217632
m6357	wsnp_JG_c522_325756
m1683	wsnp_Ex_c12781_20280572
m4497	wsnp_Ex_c64005_62986957
m1548	wsnp_Ex_c11893_19077166
m7561	wsnp_Ra_c11243_18269800
m1733	wsnp_Ex_c13154_20785032
m8594	wsnp_RFL_Contig4437_5225442
m1682	wsnp_Ex_c12781_20280445
m6900	wsnp_Ku_c31407_41142340
m2167	wsnp_Ex_c1676_3185400
m7680	wsnp_Ra_c18164_27178535
m4498	wsnp_Ex_c64005_62987015
m6513	wsnp_Ku_c14082_22272647
m4906	wsnp_Ex_c9390_15586085
m1541	wsnp_Ex_c11837_18996495
m1684	wsnp_Ex_c12781_20280815
m3273	wsnp_Ex_c3040_5615597
m125	wsnp_BE443288B_Ta_2_1
m8054	wsnp_Ra_c69_149518
m8053	wsnp_Ra_c69_149394
m3331	wsnp_Ex_c3130_5789888
m3330	wsnp_Ex_c3130_5789791

m3332	wsnp_Ex_c3130_5790163
m5016	wsnp_Ex_rep_c101457_86818610
m5013	wsnp_Ex_rep_c101457_86817938
m5015	wsnp_Ex_rep_c101457_86818160
m5014	wsnp_Ex_rep_c101457_86818055
m4778	wsnp_Ex_c8208_13870372
m6056	wsnp_JD_c4413_5541190
m4324	wsnp_Ex_c57007_58898157
m4600	wsnp_Ex_c700_1379957
m7542	wsnp_Ra_c10710_17570054
m6930	wsnp_Ku_c3371_6259457
m4311	wsnp_Ex_c56591_58653386
m4312	wsnp_Ex_c56591_58653455
m8185	wsnp_Ra_rep_c72670_70836439
m6273	wsnp_JD_rep_c50820_34666611
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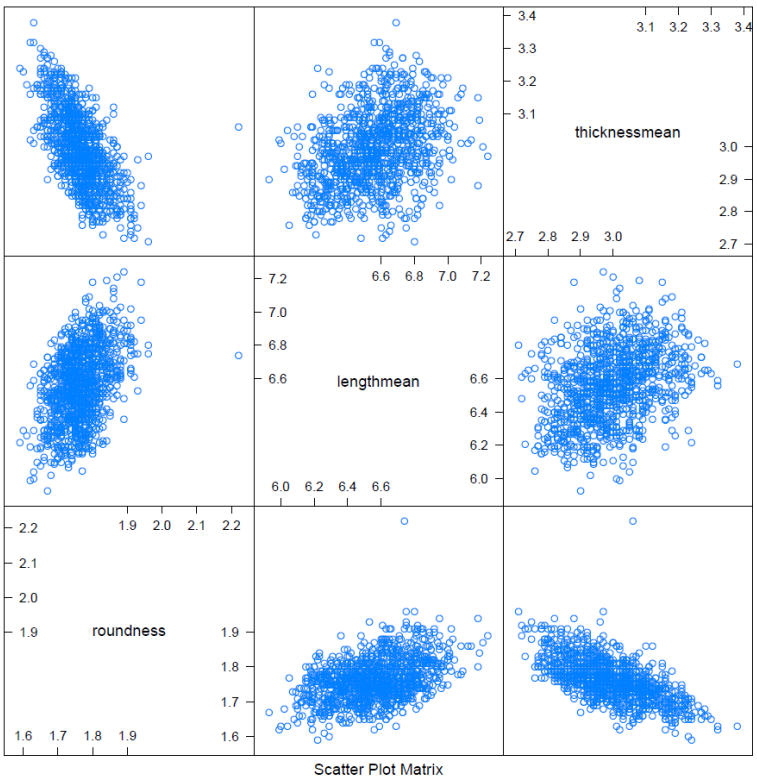
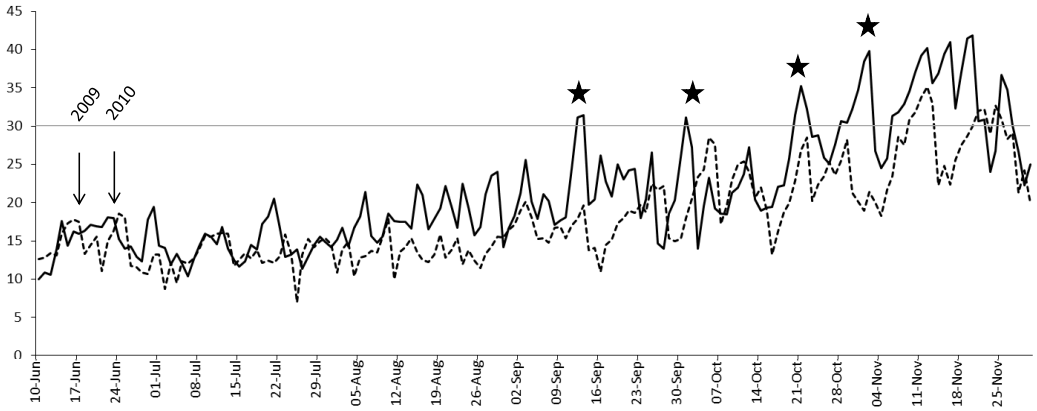
m1617	wsnp_Ex_c12369_19731179



Appendix 3.3 Scatter plot of grain characteristic traits including grain roundness, thickness and length.



Appendix 3.4 Daily average maximum temperature at Yanco (New South Wales, Australia) for 2009 (plain line) and 2010 (dash line). The two arrows correspond to the sowing data of the two well watered experiment conducted at Leeton. The black stars indicate where the temperature was higher than 30 degrees Celsius in 2009 compare to 2010.

Appendix 4.1: Descriptions of 29 environments were experiments were conducted on the wheat populations, showing locations, water supply from rainfall and/or irrigation, criteria used to select (RAC875/Kukri (Adapted from Chapter 2), Gladius/Drysdale (Pop1) and Excalibur/Kukri adapted from Edwards (2012)) lines for inclusion, numbers and types included, sowing densities, mean temperatures around flowering time.

Environment [a]	Location	Latitude	Longitude	Altitude (M)	Selection criterion [b]	Lines tested [c]	Sowing density seed m^{-2}	Rainfall and/or irrigation mm	Mean temperature around flowering time[d]	
									Sept	Oct
RAC875/Kukri population RK_DH and RK_RI										
AusRos07_NI_CS	Roseworthy (SA)	34°57' S	138°36' E	68	All	368 DH	200	153	21.8	25.4
AusRos08_NI_CS	Roseworthy (SA)	34°57' S	138°36' E	68	All	368 DH	200	223	20.8	25.2
AusPie07_NI_CS_1	Piednippie (SA)	32°68' S	134°31' E	35	Flowering time	260 DH	200	113	22.4	24.1
AusPie08_NI_CS	Piednippie (SA)	32°80' S	135°15' E	35	Flowering time	260 DH	200	212	22	25.6
AusHor08_NI_CS	Horsham (VIC)	35°21' S	138°74' E	132	Flowering time	260 DH	200	187	18.5	23.7
AusBoo07_NI_CS	Booleroo (SA)	32°88' S	138°35' E	342	Flowering time	260 DH	200	159	20.8	24.2
AusMin07_NI_CS	Minnipa (SA)	32°80' S	135°15' E	165	Flowering time	260 DH	200	86	23.9	26.1
AusStr08_NI_CS	Streaky Bay (SA)	32°80' S	134°22' E	27	Flowering time	260 DH	200	95	22	25.6
AusRob07_NI_CS	Robinvale (VIC)	34°61' S	142°81' E	89	Flowering time	260 DH	200	99	21.4	25.2
AusNun08_NI_CS	Nunjikompita (SA)	32°27' S	134°31' E	73	Flowering time	260 DH	200	96	22	25.6
									Feb	**March**
MexObr07_DI_CS	Ciudad de Obregon	27°28' N	109°56' W	38	All	368 DH	200	150	25.2	28.6
MexObr07_FI_CS	Ciudad de Obregon	27°28' N	109°56' W	38	All	368 DH	200	750	25.2	28.6
MexObr08_FI_CS	Ciudad de Obregon	27°28' N	109°56' W	38	Flowering time	255 DH	200	750	24.4	27
MexObr09_DI_CS	Ciudad de Obregon	27°28' N	109°56' W	38	Flowering time	255 DH	200	50	25.6	26.9
MexObr11_DI_CS	Ciudad de Obregon	27°28' N	109°56' W	38	Marker genotypes	34 DH/77 RI	200	150	24.5	29.3
MexObr12_DI_CS	Ciudad de Obregon	27°28' N	109°56' W	38	Marker genotypes	27 DH/40 RI	200	100	24.7	26.6
									April	**May**
MexObr08_FI_LS	Ciudad de Obregon	27°28' N	109°56' W	38	Flowering time	255 DH	200	900	30.7	35

MexObr09_FI_LS	Ciudad de Obregon	27°28' N	109°56' W	38	Flowering time	255 DH	200	1050	30.5	35.6
MexObr11_FI_LS	Ciudad de Obregon	27°28' N	109°56' W	38	Marker genotypes	34 DH/109 RI	200	1050	30.7	34.8
MexObr12_FI_LS	Ciudad de Obregon	27°28' N	109°56' W	38	Marker genotypes	27 DH/40 RI	200	900	30.5	37.8
									Oct	**Nov**
AusUrr09_SI-S_LS	Urrbrae (SA)	34°57' S	138°36' E	225	Recombinant	46 DH	133	N/A	21.8	27.5
AusUrr09_SI-W_LS	Urrbrae (SA)	34°57' S	138°36' E	225	Recombinant	46 DH	133	N/A	21.7	27.5
AusUrr09_SI-D_LS	Urrbrae (SA)	34°57' S	138°36' E	225	Recombinant	46 DH	133	N/A	21.7	27.5
Gladius/Drysdale population GD_RI										
MexObr10_DI_CS	Ciudad de Obregon	27°28’ N	109°56’ W	38	All (Pop1)	250 RI	200	150	-	-
AusLee09_FI_CS	Leeton (NSW)	34°57’ S	146°41’ E	140	All (Pop1)	250 RI	200	-	-	-
AusLee09_FI_LS	Leeton (NSW)	34°57’ S	146°41’ E	140	All (Pop1)	250 RI	200	-	-	-
AusLee10_FI_CS	Leeton (NSW)	34°57’ S	146°41’ E	140	All (Pop1)	250 RI	200	-	-	-
AusLee10_FI_LS	Leeton (NSW)	34°57’ S	146°41’ E	140	All (Pop1)	250 RI	200	-	-	-
Excalibur/Kukri population EK_RI										
AusPie07_NI_CS_2	Piednippie (SA)	32°68' S	134°31' E	35	All	233 DH	200	113	22.4	24.1

a Aus Australia, Mex Mexico, 07-11 2007 to 2011, DI drip irrigation , NI for not irrigated but rainfed, FI for flooding irrigation, SI for sprinkler irrigation, D drought, S Saturated, W Well watered, LS late sowing, CS conventional sowing.

b Marker genotypes: recombinant lines between the two loci *barc77* and *gwm114* and genotyped for *PPd-D1* and *barc13*

c DH doubled haploid lines, RI recombinant inbred lines.

d The temperature data for Mexico were collected at www.agroson.com.mx , whereas the temperature data for Australia were collected at http://www.bom.gov.au/.

Appendix 4.2 List of molecular markers mapped in the four wheat populations under *qYDH.3BL*

Marker name	*contig*	*Fwd primer sequence*	*Rvs primer sequence*
barc290	N/A	GCGACCAATTGATCCTAAAAGA	GCGCGATAGCTAGCAAAGAAAATG
barc77	ctg1310	GCGTATTCTCCCTCGTTTCCAAGTCTG	GTGGGAATTTCTTGGGAGTCTGTA
cfb3200	ctg1310	GTATTCTGTTCCTGATCTTGCC	GACTGATGAGCCACGCTG
cfb43	ctg3169	AGCTTCCTCAAGAGCCATC	CCAAGTAAGCAAGAGGATGAG
cfb503	ctg1094	CGATCCATCTGTAGGGCTGT	GGCTAGGCTTTGAACACTGC
cfb511	ctg1689	GGCAACAAATGGAAGGAAGA	GCGATGCACATAGTGGTGTC
cfb512	ctg1094	AGCGTCTTCCCTCAAACAGA	GCAGTGGAGAGGTCTTCTGG
cfb515	ctg730	GAGTTCTACCTGGCACTCGC	TCCAGATTTAAAGGCAGCTCA
cfb528	ctg1239	AATTGGACCCCCTTATCTGG	AGAGTGTGGCATTTTCTGGG
cfb560	ctg486	CAGATCCCGAGATCAACACC	TAGCCTGACCTGTGCCTTCT
cfb539	ctg647	GCAATGCCATCCTTGATTCT	ACGCGTCCGAGTTCAAAATA
gpw3233	ctg173	N/A	
gpw4143	ctg1484	N/A	
gpw7108	ctg1216	N/A	
gpw7335	ctg1094	N/A	
gwm1266		N/A	
gwm299	ctg660	ACTACTTAGGCCTCCCGCC	TGACCCACTTGCAATTCATC
wmc236	ctg647	TGGTCACTATGGTAACCGAGGA	CCCTGGGTGATGAATAGACTTT
wmm1420	ctg716	N/A	
wmm1758		N/A	
wmm1966	ctg1216	N/A	
wmm274	ctg1239	N/A	
wmm408	ctg1239	N/A	
wmm448	ctg660	N/A	
wmm454	ctg1239	N/A	
wmm480		N/A	
wmm517		N/A	
cfp49	ctg1691	ATGGCGTATCACAAACTGGTG	GGACCCCCTAAACATTGGTG
cfp50	ctg1691	CGCATCAAACAAGGGTTC	AGAATCACGGGGCAGGAG
cfp1237	ctg1691	GCCCTTTTCCTAGTAGTGGCATC	CTCTGTGTAACCCTAGCCCTCTC
cfp1556	ctg1691	TCAAGGTCGAGCGCGAGCAG	TCCATGTCAGAATCACGGGGCAG
cfp6008	ctg660	GTCTTCCACGCAAAGGAGAG	TCGAAACGAAAGCAGAGGAT
cfp6009	ctg660	CACGGGACGAAGTCTTCAAT	CATCATCCTCCCTTCCTTGA
cfp6016	ctg1216	TTTCCTCGCCCTCACTTCTA	TTTCCTCGCCCTCACTTCTA
cfp6018	ctg3169	CGAGGTTTGGAGTCCCTAGA	CAACAGTCCGACCAAAGGAT
cfp6029	ctg1691	TCCGGATACAGGACGAAGTC	GTACACGCAAGGGTTGGTTT
cfp6047	ctg61	CTCCTGGCTCCTCTCGTATG	GCGTTTTGACTCCGTCTGAT
cfs6009	ctg660	GCTCCTGTCATGAACCGAAT	TGCACTTAGGCCTTTTCCAC

In addition, the following markers obtained under material transfer agreements were gpw3233 and gpw4143, gpw7108 and the markers with the prefix wmm were from INRA (Dr. Pierre Sourdille). The contig names correspond to the version 4 of the physical map of chromosome 3B (Rustenholz et al. 2011)

Appendix 4.3 Summary of sequence comparison of the eighty-five predicted genes named after their contig sequences (e.g. CT_ correspond to contig ctg173) and CAT correspond to the category level described by Leroy et al. (2012), homology in rice and *Brachypodium* where orange and blue gene names correspond to synteny chromosomes in rice and *Brachypodium* respectively. The pink highlighted cell correspond to an e-value lower than $1.00e^{-20}$. The green highlighted cells correspond to genes with interest for their putative functions.

	BLASTx results to Rice protein sequences -MSU v7				BLASTx results to *Brachypodium* protein sequences			
Reference	Name	Description	e-value	Score	Name	Description	e-value	Score
CT_02_CAT04_6	LOC_Os01g47840.1	S-locus-like receptor protein kinase	0.00E+00	1239	Bradi2g46097.1		0	981
CT_02_CAT02_3	LOC_Os01g47900.1	S-locus-like receptor protein kinase	0.00E+00	1097	Bradi2g46180.1	protein kinase	0	1207
ER_01_CAT04_33	LOC_Os01g04070.1	verticillium wilt disease resistance protein	1.00E-108	386	Bradi2g61460.1		1E-172	514
ER_04_CAT04_9	LOC_Os01g04070.1	verticillium wilt disease resistance protein	0.00E+00	749	Bradi2g61460.1		0	1115
ER_04_CAT02_3	LOC_Os01g04070.1	verticillium wilt disease resistance protein	0	952	Bradi2g61460.1		0	1480
ER_04_CAT04_24	LOC_Os01g04070.1	verticillium wilt disease resistance protein	1.00E-109	394	Bradi2g61460.1		0	574
ER_04_CAT04_27	LOC_Os01g04070.1	verticillium wilt disease resistance protein	1E-141	501	Bradi2g61460.1		0	638
ER_05_CAT04_42	LOC_Os01g04070.1	verticillium wilt disease resistance protein	0	808	Bradi2g61460.1		0	1323
ER_01_CAT04_39	LOC_Os01g59180.1	OsFBX27 - F-box domain containing protein	1.00E-59	228	Bradi2g52810.1	F-Box	1E-72	230
ER_05_CAT02_3	LOC_Os01g71820.1	glycosyl hydrolases family 17	1.00E-127	451	Bradi2g60490.1	Glycosyl hydrolase (GH), subfamily GH17	3E-133	382
ER_05_CAT04_12	LOC_Os01g72160.1	glutathione S-transferase	1.00E-60	231	Bradi1g60587.1		4E-67	209
ER_04_CAT04_15	LOC_Os03g29350.1	von Willebrand factor type A domain containing protein	7.00E-11	63.5	Bradi1g60207.1		9E-15	69.3
ER_07_CAT04_15	LOC_Os03g62250.1	zinc finger, C3HC4 type domain containing protein	0.001	40.8	Bradi2g61450.1	RING, subfamily zinc finger (C3HC4-type RING finger) family protein	4E-44	145

ER_04_CAT04_18	LOC_Os04g34010.1	aluminum-activated malate transporter	9E-20	92.8	Bradi5g09690.1		2E-25	98.6
ER_07_CAT04_18	LOC_Os05g25310.1	acyl-CoA synthetase protein	8.00E-73	270	Bradi3g03730.1	fatty-acyl-CoA synthase activity	2E-87	271
ER_01_CAT04_27	LOC_Os05g25430.1	receptor-like protein kinase	6E-21	99.4	Bradi2g31490.1	CrRLK1L	2E-13	70.5
ER_01_CAT04_24	LOC_Os05g25450.1	TKL_IRAK_CrRLK1L-1.3 CrRLK1L homolog	0.00E+00	668	Bradi2g31490.1	CrRLK1L	0	652
ER_05_CAT04_30	LOC_Os05g25450.1	TKL_IRAK_CrRLK1L-1.3 CrRLK1L homolog	0	912	Bradi2g31490.1	CrRLK1L	0	905
ER_01_CAT04_9	LOC_Os07g02500.1	expressed protein	6E-22	102	Bradi4g33990.1		2E-104	309
ER_05_CAT04_27	LOC_Os07g09110.1	OsFBX219 - F-box domain containing protein	6.00E-36	149	Bradi1g28737.1		3E-24	103
ER_06_CAT04_15	LOC_Os09g31019.1	ubiquitin fusion protein	2.00E-24	108	Bradi4g38540.1	ubiquitin-protein ligase activity	5E-32	107
ER_05_CAT04_33	LOC_Os12g10930.1	NL0E	2.00E-53	207	Bradi2g61467.1		3E-79	247
BCN_02_CAT04_30	LOC_Os01g71820.1	glycosyl hydrolases family 17	1E-108	390	Bradi2g60557.1		5E-116	342
BCN_02_CAT04_18	LOC_Os01g72130.1	glutathione S-transferase	2E-16	81.6	Bradi2g60650.1		1E-18	75.5
BCN_02_CAT04_27	LOC_Os01g72130.1	glutathione S-transferase	1.00E-16	82.4	Bradi1g60587.1		1E-18	76.6
BCN_02_CAT02_9	LOC_Os01g72130.1	glutathione S-transferase	9E-42	166	Bradi1g60587.1		5E-52	165
BCN_02_CAT04_39	LOC_Os01g72160.1	glutathione S-transferase	7.00E-76	281	Bradi2g60760.1		1E-86	256
BCN_02_CAT01_3	LOC_Os02g17280.1	gamma-secretase subunit APH-1B	3.00E-93	339	Bradi3g10110.1	endopeptidase activity	7E-122	347
BCN_02_CAT04_15	LOC_Os03g57050.1	expressed protein	1.00E-06	51.2	Bradi1g35380.1		1E-6	48.5
BCN_02_CAT04_60	LOC_Os09g19790.1	puromycin-sensitive aminopeptidase	1E-150	530	Bradi4g28767.2		0	538
BCN_02_CAT02_6	LOC_Os10g33910.1	mitochondrial import inner membrane translocase subunit Tim16	8.00E-32	132	Bradi2g61480.1		8E-54	164

BCN_02_CAT04_78	LOC_Os11g14410.1	polygalacturonase	2.00E-23	106	Bradi2g04550.1	Glycosyl hydrolase (GH), subfamily GH28	7E-10	57.4
LG_03_CAT04_6	LOC_Os01g72810.1	secreted glycoprotein	1.00E-116	416	Bradi4g41920.1	protein kinase family protein, subfamily SD-2b	8E-76	252
LG_03_CAT03_3	LOC_Os01g72820.2	CRS1/YhbY domain containing protein	1.00E-137	484	Bradi2g61340.1		0	556
LG_03_CAT04_18	LOC_Os01g72834.1	RNA recognition motif containing protein	6E-53	205	Bradi2g61350.1	RRM domain containing protein	7E-79	241
LG_01_CAT04_36	LOC_Os02g52650.1	chlorophyll A-B binding protein	7.00E-51	197	Bradi3g58020.1		4E-67	204
LG_01_CAT04_42	LOC_Os03g06840.1	GRF zinc finger family protein	4.00E-6	49.7	Bradi5g10840.1	zinc ion binding	1E-9	54.7
LG_01_CAT01_3	LOC_Os03g37640.1	MATE efflux family protein	0.00E+00	666	Bradi1g15670.1	antiporter activity	0	658
LG_01_CAT04_45	LOC_Os03g37640.1	MATE efflux family protein	1E-179	626	Bradi1g15670.1	antiporter activity	0	616
LG_05_CAT04_3	LOC_Os11g37120.1	nucleolar protein 5	1.00E-52	205	Bradi1g17550.1		4E-33	123
KA_01_CAT01_3	LOC_Os06g22440.1	SAM dependent carboxyl methyltransferase	1.00E-114	410	Bradi2g60170.1		3E-166	468
KA_01_CAT04_57	LOC_Os07g13634.1	cytokinin-N-glucosyltransferase 1	9.00E-42	167	Bradi1g53560.1	UDP-galactose	6E-66	208
KA_01_CAT04_63	LOC_Os07g13634.1	cytokinin-N-glucosyltransferase 1	1.00E-110	397	Bradi1g53560.1	UDP-galactose	1E-139	401
KA_01_CAT04_66	LOC_Os07g13634.1	cytokinin-N-glucosyltransferase 1	6.00E-42	167	Bradi1g53560.1	UDP-galactose	2E-67	212
KA_01_CAT04_69	LOC_Os07g13634.1	cytokinin-N-glucosyltransferase 1	1.00E-113	406	Bradi1g53560.1	UDP-galactose	4E-147	423
KA_01_CAT04_48	LOC_Os08g27540.2	expressed protein	4.00E-12	70.1	Bradi5g25380.1		4E-14	72.4
KA_01_CAT04_42	LOC_Os08g43020.1	transferase family protein	2.00E-30	129	Bradi2g60898.1		2E-53	174
KA_01_CAT04_45	LOC_Os08g43040.2	transferase family protein	1.00E-97	355	Bradi2g60898.1		4E-154	447
IV_01_CAT04_132	LOC_Os01g73110.1	expressed protein	2.00E-34	144	Bradi2g61490.1		2E-49	166
IV_01_CAT04_75	LOC_Os02g42940.	MSP domain containing	9.00E-15	81.	Bradi5g16840.	beta-tubulin binding	5E-17	81.3

	1	protein		3	3			
IV_01_CAT04_153	LOC_Os02g56130.1	PCNA - Putative DNA replicative polymerase clamp	2.00E-12	69.7	Bradi3g54630.1	MutLalpha complex binding	2E-14	69.3
IV_01_CAT01_3	LOC_Os03g32170.1	NAD dependent epimerase/dehydratase family protein	0.00E+00	671	Bradi1g16000.1		0	687
IV_01_CAT04_108	LOC_Os03g42410.1	B3 DNA binding domain containing protein	0.000006	47	Bradi4g03000.1	ABI3VP1 transcription factor	0.012	33.5
IV_01_CAT04_66	LOC_Os04g30030.1	cysteine-rich receptor-like protein kinase 12 precursor	7.00E-61	232	Bradi4g09457.1		3E-75	238
IV_01_CAT04_156	LOC_Os04g39070.1	OsFBX139 - F-box domain containing protein	9.00E-33	139	Bradi3g60270.1	F-Box	3E-40	150
IV_03_CAT04_3	LOC_Os04g39080.1	OsFBX140 - F-box domain containing protein	2.00E-26	118	Bradi3g60270.1	F-Box	3E-27	111
IV_01_CAT04_12	LOC_Os07g13634.1	cytokinin-N-glucosyltransferase 1	1E-113	406	Bradi1g53560.1	UDP-galactose	4E-147	423
IV_01_CAT04_15	LOC_Os07g13634.1	cytokinin-N-glucosyltransferase 1	6E-42	167	Bradi1g53560.1	UDP-galactose	2E-67	212
IV_01_CAT04_18	LOC_Os07g13634.1	cytokinin-N-glucosyltransferase 1	1.00E-110	397	Bradi1g53560.1	UDP-galactose	1E-139	401
IV_01_CAT04_24	LOC_Os07g13634.1	cytokinin-N-glucosyltransferase 1	9.00E-42	167	Bradi1g53560.1	UDP-galactose	6E-66	208
IV_01_CAT04_45	LOC_Os07g13634.1	cytokinin-N-glucosyltransferase 1	1E-167	586	Bradi1g53560.1	UDP-galactose	0	650
IV_01_CAT04_33	LOC_Os08g27540.2	expressed protein	4.00E-12	70.1	Bradi5g25380.1		4E-14	72.4
IV_01_CAT04_39	LOC_Os08g43020.1	transferase family protein	2.00E-30	129	Bradi2g60898.1		3E-53	174
IV_01_CAT04_36	LOC_Os08g43040.2	transferase family protein	4.00E-97	353	Bradi2g60898.1		7E-153	444
IV_03_CAT04_6	LOC_Os10g08500.1	retrotransposon protein,	9.70E-01	29.6	Bradi1g58746.1		0.019	30.4
IV_01_CAT01_6	LOC_Os10g41780.3	chlorophyllide a oxygenase, chloroplast precursor	0	815	Bradi2g61500.1	iron ion binding	0	884
AIK_01_CAT02_12	LOC_Os01g39960.1	lycopene epsilon cyclase, chloroplast precursor	0.00E+00	846	Bradi2g41890.1		0	903
AIK_01_CAT01_6	LOC_Os01g39970.1	protein kinase domain containing protein	0.00E+00	1013	Bradi2g41900.1	protein kinase family	0	1107
AIK_01_CAT01_9	LOC_Os01g40050.	peptidyl-prolyl cis-trans	1.00E-135	476	Bradi2g41910.	ubiquitin-protein	0	518

	1	isomerase			1	ligase activity		
AII_01_CAT01_3	LOC_Os01g40094.1	protein phosphatase 2C	1E-177	619	Bradi2g41950.1	protein serine/threonine phosphatase activity	0	685
AII_01_CAT01_6	LOC_Os01g40110.1	ZOS1-11 - C2H2 zinc finger protein	1E-112	402	Bradi2g41957.1		2E-154	434
AII_01_CAT04_45	LOC_Os01g40190.1	retrotransposon protein, putative, unclassified	3.00E-40	165	Bradi2g41970.1		8E-64	224
AIK_01_CAT04_48	LOC_Os01g40240.1	expressed protein	1.00E-74	278	Bradi2g42010.1	F-Box	3E-129	378
AIK_01_CAT04_42	LOC_Os01g40250.1	down-regulated in metastasis family protein	0.00E+00	2024	Bradi2g42017.1		0	2214
AII_01_CAT04_117	LOC_Os01g53580.1	3-5 exonuclease domain-containing protein	4.00E-10	61.6	Bradi1g47470.1	DNA helicase activity	5E-9	53.5
AII_01_CAT04_90	LOC_Os01g67480.1	helix-loop-helix DNA-binding domain containing protein	5.00E-10	60.5	Bradi2g57800.1	bHLH transcription factor	6E-21	85.9
AII_01_CAT04_15	LOC_Os03g48140.1	myosin	1.00E-178	623	Bradi2g41977.2		0	639
AII_01_CAT04_18	LOC_Os03g48140.1	myosin	0.00E+00	1720	Bradi2g41977.2		0	1785
AIK_02_CAT04_36	LOC_Os04g05104.1	retrotransposon protein, putative, unclassified	3E-15	79.3	Bradi5g01030.1		1E-54	185
AIK_02_CAT04_33	LOC_Os04g12110.1	expressed protein	7.00E-09	58.2	Bradi5g01030.1		1E-13	68.2
AII_01_CAT04_123	LOC_Os06g13750.1	expressed protein	3.00E-09	62	Bradi4g16250.1		4E-18	82
AIK_02_CAT04_21	LOC_Os07g07194.2	MAC/Perforin domain containing protein	1.00E-82	303	Bradi2g61640.1		3E-104	312
AIK_02_CAT04_27	LOC_Os10g02360.1	OsWAK98 - OsWAK-RLCK receptor-like cytoplasmic kinase	0.00E+00	642	Bradi2g38370.1	WAK receptor-like protein kinase, subfamily WAKL-OS	8E-88	283
AIK_02_CAT04_6	LOC_Os11g17014.1	NB-ARC domain containing protein	1.00E-117	421	Bradi4g25780.1	NBS LRR	6E-134	412
AIK_02_CAT04_18	LOC_Os11g17014.1	NB-ARC domain containing protein	2E-22	102	Bradi4g25770.1		5E-27	94.7

Appendix 4.4 Summary of sequence comparison of the eighty-five predicted genes named after their contig sequences (e.g. CT_ correspond to contig ctg173) and CAT correspond to the category level described by Leroy et al. (2012), differentially expressed (DE) genes between RAC985 and Kukri, SNP discovery R for RAC875, K for Kukri, G for Gladius, D for Drysdale, and E for Excalibur, physical contigs (PC) (figure 4).

	Transcript evidence		SNP polymorphisms			
Reference	***Triticum***	**DE**	R vs. K	G vs. D	E vs. K	PC
CT_02_CAT04_6	√	-	√	-	-	ctg173
CT_02_CAT02_3	√	-	√	-	√	ctg173
ER_01_CAT04_33	-	-	-	-	-	ctg660
ER_04_CAT04_9	√	-	-	-	-	ctg660
ER_04_CAT02_3	√	-	-	-	-	ctg660
ER_04_CAT04_24	√	-	-	-	-	ctg660
ER_04_CAT04_27	-	-	-	-	-	ctg660
ER_05_CAT04_42	√	-	-	-	-	ctg660
ER_01_CAT04_39	√	-	-	√	-	ctg660
ER_05_CAT02_3	√	√	-	√	-	ctg660
ER_05_CAT04_12	√	-	-	-	-	ctg660
ER_04_CAT04_15	√	-	-	-	-	ctg660
ER_07_CAT04_15	√	-	√	√	√	ctg660
ER_04_CAT04_18	-	-	-	-	-	ctg660
ER_07_CAT04_18	√	-	√	√	√	ctg660
ER_01_CAT04_27	√	-	√	√	√	ctg660
ER_01_CAT04_24	√	-	√	√	-	ctg660
ER_05_CAT04_30	√	-	-	-	-	ctg660
ER_01_CAT04_9	√	-	-	√	-	ctg660
ER_05_CAT04_27	√	-	-	-	-	ctg660
ER_06_CAT04_15	-	-	-	-	-	ctg660
ER_05_CAT04_33	-	-	-	-	-	ctg660
BCN_02_CAT04_30	√	-	-	-	-	ctg61
BCN_02_CAT04_18	-	-	-	-	-	ctg61
BCN_02_CAT04_27	-	-	-	-	-	ctg61
BCN_02_CAT02_9	√	-	-	-	-	ctg61
BCN_02_CAT04_39	√	-	-	-	-	ctg61
BCN_02_CAT01_3	√	√	√	√	√	ctg61
BCN_02_CAT04_15	-	-	-	-	-	ctg61
BCN_02_CAT04_60	-	-	-	-	-	ctg61
BCN_02_CAT02_6	√	-	√	√	√	ctg61
BCN_02_CAT04_78	-	-	√	-	√	ctg61
LG_03_CAT04_6	√	-	-	-	-	ctg2680
LG_03_CAT03_3	√	-	-	√	-	ctg2680
LG_03_CAT04_18	√	-	√	√	-	ctg2680
LG_01_CAT04_36	√	-	√	-	-	ctg2680
LG_01_CAT04_42	√	-	-	-	-	ctg2680
LG_01_CAT01_3	√	-	√	√	-	ctg2680
LG_01_CAT04_45	√	√	√	√	-	ctg2680
LG_05_CAT04_3	√	-	√	√	-	ctg2680

KA_01_CAT01_3	√	-	-	-	-	ctg1689
KA_01_CAT04_57	√	-	-	-	-	ctg1689
KA_01_CAT04_63	√	-	-	-	-	ctg1689
KA_01_CAT04_66	√	-	√	-	-	ctg1689
KA_01_CAT04_69	√	-	-	√	-	ctg1689
KA_01_CAT04_48	-	-	-	-	-	ctg1689
KA_01_CAT04_42	-	-	-	-	-	ctg1689
KA_01_CAT04_45	-	-	-	-	-	ctg1689
IV_01_CAT04_132	√	√	-	-	-	ctg1691
IV_01_CAT04_75	√	-	-	-	√	ctg1691
IV_01_CAT04_153	-	-	√	-	-	ctg1691
IV_01_CAT01_3	√	-	-	-	-	ctg1691
IV_01_CAT04_108	√	-	-	-	√	ctg1691
IV_01_CAT04_66	√	-	-	-	-	ctg1691
IV_01_CAT04_156	√	-	√	√	-	ctg1691
IV_03_CAT04_3	√	-	√	√	-	ctg1691
IV_01_CAT04_12	√	-	√	√	-	ctg1691
IV_01_CAT04_15	√	-	-	-	-	ctg1691
IV_01_CAT04_18	√	-	-	-	-	ctg1691
IV_01_CAT04_24	√	-	-	-	-	ctg1691
IV_01_CAT04_45	√	-	-	-	-	ctg1691
IV_01_CAT04_33	-	-	-	-	-	ctg1691
IV_01_CAT04_39	-	-	-	-	-	ctg1691
IV_01_CAT04_36	-	-	-	-	-	ctg1691
IV_03_CAT04_6	√	-	√	√	-	ctg1691
IV_01_CAT01_6	√	√	-	-	-	ctg1691
AIK_01_CAT02_12	√	√	-	-	-	ctg647
AIK_01_CAT01_6	√	-	-	-	-	ctg647
AIK_01_CAT01_9	√	√	√	-	√	ctg647
AII_01_CAT01_3	√	√	√	-	-	ctg647
AII_01_CAT01_6	√	√	-	-	-	ctg647
AII_01_CAT04_45	√	-	-	-	-	ctg647
AIK_01_CAT04_48	√	-	-	-	-	ctg647
AIK_01_CAT04_42	√	-	-	√	-	ctg647
AII_01_CAT04_117	-	-	-	-	-	ctg647
AII_01_CAT04_90	√	√	-	-	-	ctg647
AII_01_CAT04_15	√	-	-	-	-	ctg647
AII_01_CAT04_18	√	√	-	-	-	ctg647
AIK_02_CAT04_36	-	-	-	-	-	ctg647
AIK_02_CAT04_33	-	-	-	-	-	ctg647
AII_01_CAT04_123	√	-	-	√	-	ctg647
AIK_02_CAT04_21	√	-	-	-	-	ctg647
AIK_02_CAT04_27	√	-	-	-	-	ctg647
AIK_02_CAT04_6	√	-	-	-	√	ctg647
AIK_02_CAT04_18	√	-	-	-	-	ctg647

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