The genetic basis of barley (*Hordeum vulgare* L.) adaptation to Australian environment

By

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To my beloved wife 'Hamere',

my daughters 'Meti' & 'Kume',

and my sons 'Firaol' & 'Oliyad'

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Abstract

The main aim of this study was to identify genomic regions controlling adaptive traits and yield under Australian environment. Three doubled haploid mapping populations developed from inter-crossing three locally adapted elite genotypes (Commander, Fleet and WI4304) were used for the study. The parents were selected based on their long-term performances in southern Australia and are similar in maturity. Field trials were conducted at Minnipa (South Australia), Roseworthy (South Australia) and Swan Hill (Victoria) in 2012 and 2013 cropping seasons. Phenotypic evaluation comprised maturity (Zadoks), early vigour, leaf rolling, leaf waxiness, normalized difference vegetation index (NDVI), chlorophyll content (SPAD), grain plumpness, and grain yield. Three high-density genetic linkage maps were constructed using Genotyping by Sequencing (GBS) and single nucleotide polymorphisms (SNPs) for major phenology genes controlling photoperiod response and vernalization sensitivity. QTL mapping identified 13 maturity QTL, 18 QTL for other adaptive traits including three QTL for leaf rolling, six for leaf waxiness, three for early vigor, four for NDVI, and two QTL for SPAD. Seventeen QTL for grain plumpness and 18 yield QTL explaining from 1.2% to 25.0% of phenotypic variation were found across populations and environments. Significant QTL x environment interaction was observed for all maturity, grain plumpness and yield QTL except *QMat.CF-5H.1*, *QPlum.FW-4H.1* and *QYld.FW-2H.1*. Seven of the 13 maturity QTL are coincident with known phenology genes. The major phenology genes *Ppd* and *Vrn* were not associated with variation in grain plumpness and yield in this study, and adjustment for maturity effect through co-variance analysis had no major effect on yield QTL. Adjustment for phenology genes confirmed six yield per se QTL that are independent of phenology genes. Six new yield QTL were identified in close proximity to phenology genes after phenology adjustment, with stable expression and major effects across environments, explaining up to 57.4% of phenotypic variance. Yield QTL common between two or all three populations were identified on chromosomes 2H and 6H. A yield QTL on chromosome 2H coincident with the *HvCEN/EPS2* locus was identified in CW and FW populations. Controlled environment experiments was conducted under long day and short day light conditions using two contrasting recombinant lines selected from the yield QTL region on Chromosome 2H from each population. The result suggested that the yield QTL identified in CW and FW populations on 2H is independent of phenological variation. Further study is required to verify whether this yield QTL is related to *HvCEN/EPS2* itself or whether a gene closely linked to the *HvCEN* locus is responsible for the observed yield

variation. The three interlinked populations with high-density linkage maps described in this study are a significant resource for examining the genetic basis for barley adaptation in low to medium rainfall Mediterranean type environments. The identification of a QTL for increased yield that is not associated with maturity differences provides an opportunity to apply marker-based selection for grain yield.

Chapter 1: General introduction

Barley (*Hordeum vulgare* L.) is an important grain crop with a potential to substantially contribute to the increasing global food and feed demand. It is a versatile crop in terms of its diverse uses as healthy food, feed, malting and distilling purposes. Barley has a high level of adaptability to stressful conditions, including cold, drought, alkaline, and saline soils (Schulte et al. 2009), which enables its cultivation over many regions of the world. It is the fourth most widely grown cereal in the world after wheat, maize and rice with over 49.1 million hectares planted to this crop in 2013 (FAOSTAT 2014).

In Australia, barley is the second important crop after wheat (ABARES 2014), mainly grown in the southern Australia region. The current productivity of the crop in Australia is 2.05 t/ha (ABARES 2014), which is less than the world average of 2.91 t/ha (FAOSTAT 2014). The barley growing areas of Australia have a Mediterranean-type climate pattern characterized by cool winters and hot summers, and low and erratic rainfall especially during anthesis and the grain filling stages of the crop (Turner 2004). Under such environments, phenology, modulated by photoperiod response genes (Ppd-H1 & Ppd-H2) and vernalization requirement genes (Vrn-H1, Vrn-H2 & Vrn-H3), play crucial roles in determining crop development and adaptability.

There is no doubt that the observed increase in average world productivity of barley from less than 1.5 t/ha in 1961 to 2.91 t/ha in 2013 (Fig.1.1) is attributable to the combinations of genetic improvement, improved agronomic management, and increasing precision in the use of inputs. However, the crop area sown to barley production has been decreasing since 1979 (Fig. 1.1), which might be associated with low level of investment in barley improvement relative to wheat, maize and rice. Further increases in productivity from the shrinking crop land is required to meet the ever increasing food and feed demand associated with the soaring human population (United Nations 2015).

The use of locally adapted, high yielding varieties represents the most feasible approach to increase sustainably productivity. This requires the complementation of crop breeding programs with modern genomic tools. Conventional breeding through direct phenotypic selection has greatly contributed to the improvement of economically important crops for traits controlled by a few major genes or for large effect QTL. However, this fails to address

complex quantitative traits controlled by a large number of minor effect genes or QTL subject to the confounding effects of the environment and the genotype x environment interaction (Moose & Mumm 2008). Genetic dissection through quantitative trait loci (QTL) mapping will help identify the genomic regions underlying quantitative traits such as yield and adaptation. The identified QTL could then be deployed in marker-assisted selection (MAS) to select genotypes carrying the traits of interest without the confounding effects due to environmental influences.

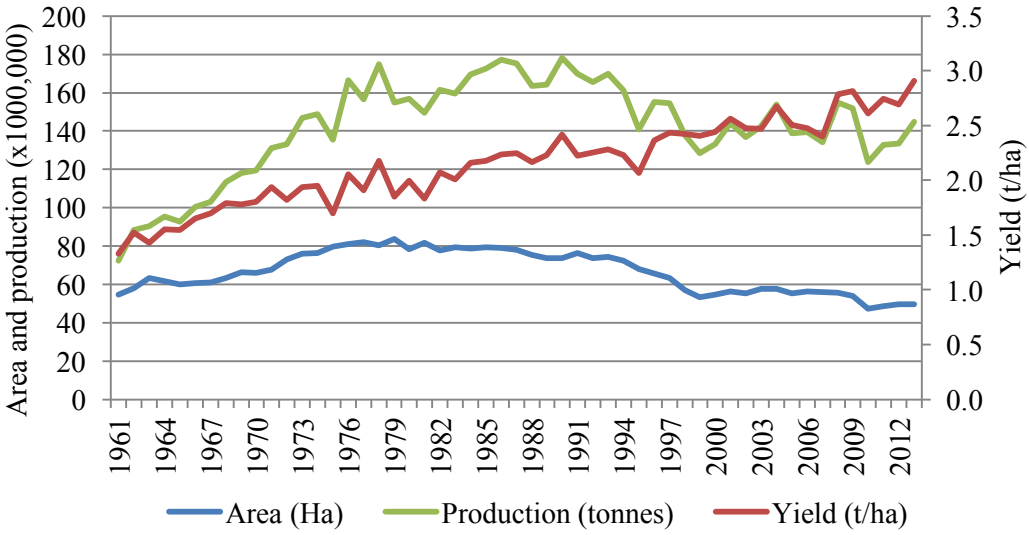

Fig. 1.1 Total area (ha), total production (t) and productivity of barley from 1961 to 2013

The current and future potential of new genomic tools for modern crop breeding have been broadly discussed by (Langridge & Fleury 2011). Genetic linkage maps are important tools to understand and dissect the genetic control of complex quantitative traits by providing the platform for QTL analysis (Fleury et al. 2010). Mapping populations developed from crossing locally adapted, elite germplasm are useful to identify genes or QTL controlling adaptation with minimal confounding effects of the major genes that differentiate different types of germplasm such as winter *versus* spring type and adapted *versus* unadapted germplasm. However, to date the barley mapping populations used in Australia have been developed from crosses between lines showing wide differences and most were targeted for improvement of quality and disease resistance, rather than adaptation and yield (Langridge & Barr 2003). The literature review section of this thesis (Chapter 2) focusses on existing barley mapping populations. Three doubled haploid (DH) mapping populations that were developed

from inter-crossing of three Australian elite genotypes (Commander, Fleet and WI4304) have been used for the current study.

The objectives of this research project were: (1) to construct the genetic linkage maps of the three doubled haploid populations (Chapter 3), (2) to identify QTL controlling maturity and other developmental and adaptive traits of barley (Chapter 4), and (3) to identify QTL controlling yield and grain plumpness of barley (Chapter 5).

Chapter 2: Literature review

2.1 Introduction

Barley (*Hordeum vulgare* L.) belongs to the Poaceae family, the tribe Triticeae, and the genus Hordeum (Bennett & Smith 1976). It is self-pollinated with less than 1% outbreeding (von Bothmer & Komatsuda 2011), and is a diploid species (2n= 2X =14) with a haploid genome of 5.1 Gb (Dolezel et al. 1998; Mayer et al. 2012). Barley has been widely used in genomics and genetics studies because of its diploid nature, high phenotypic diversity, ease of hybridization, and inducing mutations that facilitate chromosome analysis and mapping (Graner, Kilian & Kleinhofs 2011).

Barley is adapted to a wide range of production environments in different geographical regions world. Its adaptation is influenced by environmental factors of which day length (photoperiod response) and cold temperature (vernalization requirement) play major roles. Advances made in barley genetics and genomics have resulted in the identification and characterization of major genes controlling phenology and other important traits that drive barley adaptation to different production environments.

Moreover, developments in statistical techniques and software concomitant with new genomics tools, including dense molecular linkage maps, have enabled genetic dissection of complex traits such as yield through quantitative trait loci (QTL) analysis. Construction of molecular genetic linkage maps require mapping populations developed from parental genotypes differing in the target traits, genotyping platforms and statistical technics and software. Tremendous developments and changes have been seen in all of these aspects over the past decades. Molecular markers have advanced from the earliest Restriction Fragment Length Polymorphism (RFLP) markers to the current Single Nucleotide Polymorphism (SNP) markers (Schlötterer 2004); genotyping methods have moved from gel-based PCR methods to the current Genotyping By Sequencing (GBS) and the KASP assay platforms. In line with these technological developments, there has also been a change of emphasis in the nature of mapping populations used for genetic analysis, from the traditional bi-parental populations to association mapping and multi-parental populations.

2.2 Phenological control of adaptation and yield in cereals

Phenology, as defined in Merriam-Webster Dictionary, is "a branch of science dealing with the relations between climate and periodic biological phenomena (as bird migration or plant flowering)". Crop phenology allows matching crop development with availability of environmental resources such as water and radiation, and influences yield and adaptation to a particular environment (Richards 1991). The life cycle of crop plants involves a series of phenological events, which are divided in to distinct stages of vegetative and reproductive development. Proper timing of the critical developmental stages such as flowering (heading) time in relation to the environment is crucial for adaptation and yield of cereals. This is especially important in Mediterranean environments where terminal moisture stress is a common yield limiting factor in crops such as barley (Cuesta-Marcos et al. 2009).

2.2.1 Flowering time: an important adaptive trait of cereals

Flowering time is an important phenological trait determining adaptation to a particular environment and yield of crop plants (Borràs-Gelonch et al. 2010; Cuesta-Marcos et al. 2009; Richards 1991). It determines the duration of developmental phases, and indirectly affects dry matter production, the number of structures (*e.g.*, tillers, spikes and grains) that contribute to final yield and dry matter partitioning (Boyd 1996).

In cereals, grain is the most economically important part, and improving grain yield is the primary objective of crop breeders and agronomists. In grain crops such as wheat (*Triticum spp*.) and barley (*Hordeum vulgare* L.), suitable heading time under a particular environment is decisive for grain production, both in quantity and quality. This is critically important in Mediterranean type environments like southern Australia where rainfall is unpredictable and erratic in distribution, exposing crops to events of cyclic and terminal moisture stresses in most years. On the other hand, too early flowering in good rainfall seasons often results in yield penalty since the plants enter reproductive phase before adequate structures that contribute to the final yield are formed, and before sufficient dry matter for grain filling is accumulated (weak source strength), despite the season's potential. The optimal heading date in a particular environment is one that strikes a balance between sufficient vegetative development duration and sufficient grain filling duration after heading without facing terminal stress from the environment. The duration of pre-heading vegetative phase

determines yield potential through its effect on the number and size of plant structures that directly or indirectly contribute to yield, including tillers, branches, leaves, plant height, etc., depending on the species.

2.2.2 The genetic and physiological basis of flowering in barley

Flowering time is a complex quantitative trait controlled by a complex interplay of genetic networks that are triggered by specific environmental cues. Day length (photoperiod) and temperature (vernalization) are the major environmental factors controlling flowering time by triggering genetic responses. The underlying genetic mechanisms have been well studied in the model species *Arabidopsis thaliana*, followed by the monocots like rice), barley, and wheat. In cereals such as wheat and barley, the control of flowering is also controlled by narrow sense earliness (*earliness per se*) (Kikuchi and Handa 2009), in addition to photoperiod sensitivity and vernalization requirement. Earliness per se (eps) is the control of flowering time that is independent of both photoperiod sensitivity and vernalization requirements (Takahashi and Yasuda 1971). Genetic variation for response to photoperiod response and vernalization requirement has been the driving force for barley adaptation to a wide range of environments (Distelfeld et al. 2009). The underlying genes range from major genes controlling gross variation such as growth habit (winter *vs* spring types) to novel allelic variations existing within locally adapted elite germplasm.

Genetic studies conducted for more than two decades identified major genes controlling variation in photoperiod sensitivity and vernalization response in rice, wheat and barley, following the discovery of such genes in Arabidopsis. The *PHOTOPERIOD RESPONSE 1* (*Ppd-H1*) (Laurie et al. 1994) and *PHOTOPERIOD RESPONSE 2* (*Ppd-H2*) (Faure et al. 2007; Kikuchi et al. 2009) are two major genes controlling photoperiod sensitivity in barley, while *VERNALIZATION RESPONSE* (*VRN)* is controlled by three major genes (*Vrn-H1*, *Vrn-H2*, and *Vrn-H3*) (Trevaskis et al. 2003; Trevaskis et al. 2007; Trevaskis et al. 2006; Yan et al. 2006; Yan et al. 2004; Yan et al. 2003). *Vrn1* encodes MADS box genes that control vernalization-induced flowering in cereals. The barley *Vrn-H1* accelerates the transition from vegetative to reproductive development by enhancing the expression of *FLOWERING LOCUS T1 (HvFT1/Vrn3*) in long days, and down-regulating *Vrn-H2* that represses *HvFT1/Vrn-H3* (Trevaskis et al. 2007; Trevaskis et al. 2006). *VRN2* encodes a protein containing a zinc finger motif and a CCT domain (Yan et al. 2004).

Other genes involved in flowering pathways include the phytochrome pathway genes (*HvPhyA*, *HvPhyB* and *HvPhyC*) (Szucs et al. 2006), the five *FLOWERING LOCUS T (FT*-like) genes (*HvFT1-HvFT5*) (Faure et al. 2007), the barley *CONSTANS* genes (*HvCO1-HvCO8*) (Griffiths et al. 2003), and *GIGANTEA* (*HvGI*) (Dunford et al. 2005). *APETALA2* (*HvAP2*) also plays a role in barley phenology, mainly in determining the size and shape of barley inflorescence by regulating the duration of inflorescence internode elongation (Houston et al. 2013). *EARLINESS PER SE 2* (*EPS2)* is a gene that pleiotropically controls flowering time and other important traits independently of photoperiod and vernalization in wheat and barley (Laurie et al. 1994). The candidate gene for *EPS2* is the *CENTRORADIALIS* (*HvCEN*), which is an FT family member that regulates the winter versus spring growth habit in barley (Comadran et al. 2012). The barley FT-like genes *HvFT1, HvFT2, HvFT3,* and *HvFT4* are homologous to the rice *OsFTL2*, *OsFTL1*, *OsFTL10*, and *OsFTL12*, respectively, while no rice equivalent is found for *HvFT5* (Faure et al. 2007). *HvFT1* is a candidate for *VRN-H3* and is expressed under long-day conditions. It is the main barley FT-like gene involved in switching the shoot apex from vegetative to flowering, while *HvFT3* is a candidate gene for *Ppd-H2*, which affects flowering time under short day conditions (Faure et al. 2007). GIGANTEA (*GI*) is a plant specific nuclear protein with diverse physiological functions including flowering time regulation, light signalling, hypocotyl elongation, control of circadian rhythm, sucrose signalling, starch accumulation, chlorophyll accumulation, transpiration, cold tolerance, drought tolerance, and miRNA processing (Mishra and Panigrahi 2015). The chromosomal locations of most of the known genes controlling barley phenology are depicted in Fig. 2.1.

2.2.3 Integration of photoperiod and vernalization pathways to induce flowering in barley

In temperate cereals such as wheat and barley, the interaction between the photoperiod and the vernalization pathways, coupled with the phytochrome and circadian clock genes dictate the shift from vegetative development to flowering. In barley, the CO-like proteins, mainly HvCO1, are involved in the activation of FT-like genes, mainly *HvFT1*, in response to long days (Campoli et al. 2012) to accelerate flowering, in which *Ppd-H1* is also required for transcriptional activation of the FT-like genes (Turner et al. 2005) (Fig. 2.2).

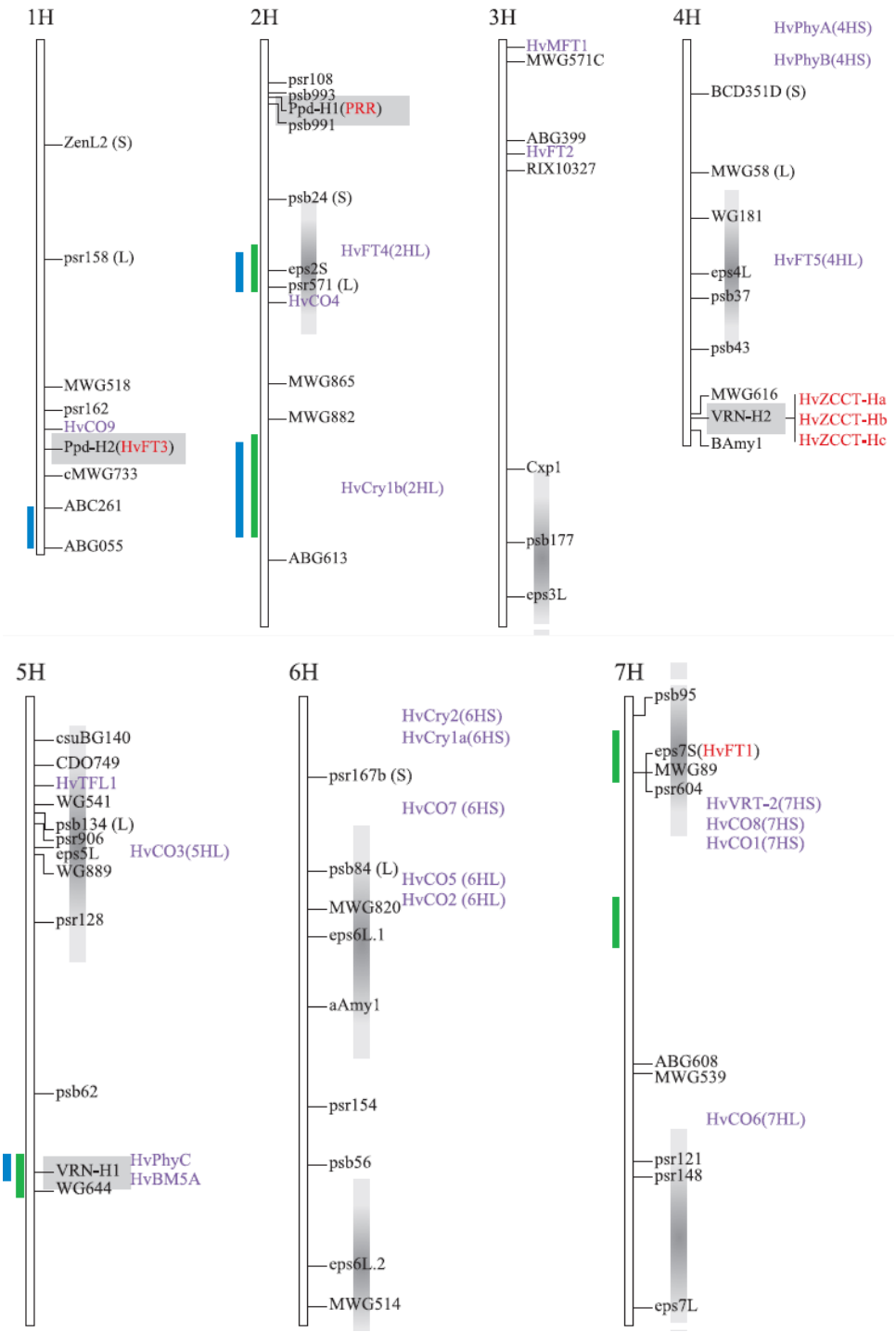

Fig. 2.1 The genetic map and chromosomal locations of photoperiod response genes in barley. The QTLs shown are from the data reported by Laurie et al. (1995) and Sameri and Komatsuda (2004). The positions of the QTLs related to flowering time are shown by bold black, blue and green lines. The QTLs indicated in black in the shadowed boxes were detected under several conditions. Blue and green lines indicate the results of autumn- or spring-sowing experiments (Sameri and Komatsuda 2004), respectively. The photoperiod response genes shown in red were reported to be consistent with QTLs. The photoperiod response genes shown in violet are located at their approximate positions on barley chromosomes.

Source: Kikuchi and Handa (2009)

Over-expression of *HvCO1* under long day accelerates flowering, while natural genetic variation in *Ppd-H1* controls flowering independently of *HvCO1*, which suggests that *Ppd-H1* may bypass the established CO-FT interaction in Arabidopsis to induce flowering under long day conditions (Andres and Coupland 2012). Vernalization increases the transcription of *VERNALIZATION 1* (*VRN1*) (Trevaskis et al. 2003; Trevaskis et al. 2006; Yan et al. 2003), which promotes inflorescence development and represses transcription of *VRN2* (Yan et al. 2004). Exposure to short days also represses VRN2 and allows *FT1* expression, thus promoting flowering during the summer. VRN2 blocks *FT1* expression under long days, while its expression is repressed during the winter by vernalization via VRN1 (Andres and Coupland 2012).

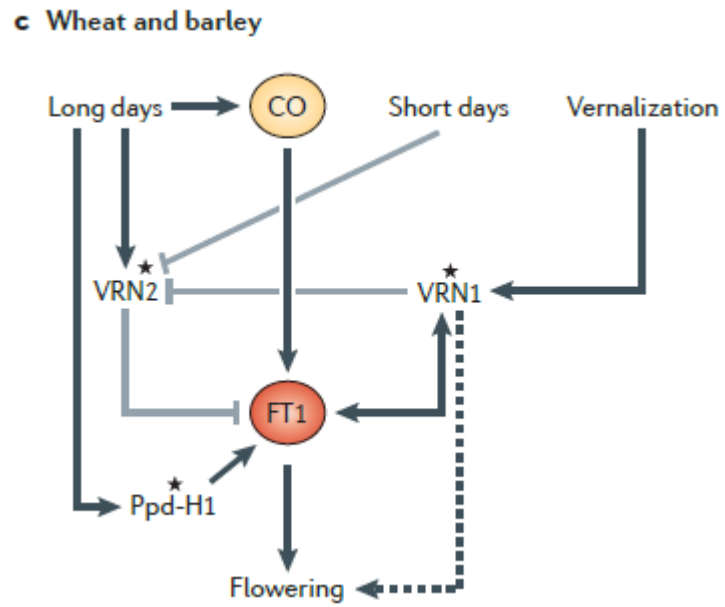

Fig. 2.2 Flowering time regulation by day length and vernalization in wheat and barley
Source**:** Andres and Coupland (2012)

PHYTOCHROME (HvPhy) and *CIRCADIAN CLOCK ASSOCIATED (HvCCA)* genes clearly affect barley heading time pathway through interaction with other genes, such as *HvPhyC*, which induces early heading by up-regulating *HvFT1* and bypassing *HvCO1* under long day (Nishida et al. 2013). *EAM8* is a barley ortholog of the Arabidopsis circadian clock regulator *EARLY FLOWERING3 (ELF3),* which accelerates the transition from vegetative to reproductive growth and inflorescence development (Faure et al. 2012). *ELF3/EAM8* contributes to photoperiod-dependent flowering in barley, suppressing flowering under non-inductive photoperiods by blocking gibberellic acid production, which is an important floral

promoting signal in barley, and *FT1* expression (Boden et al. 2014). *FT1* promotes expression of floral identity genes in the developing apex, while *VRN1* promotes the transition of the vegetative apex to inflorescence development, where gibberellic acid and *FT1* are also required for the completion of inflorescence development and flowering (Boden et al. 2014) (Fig. 2.3).

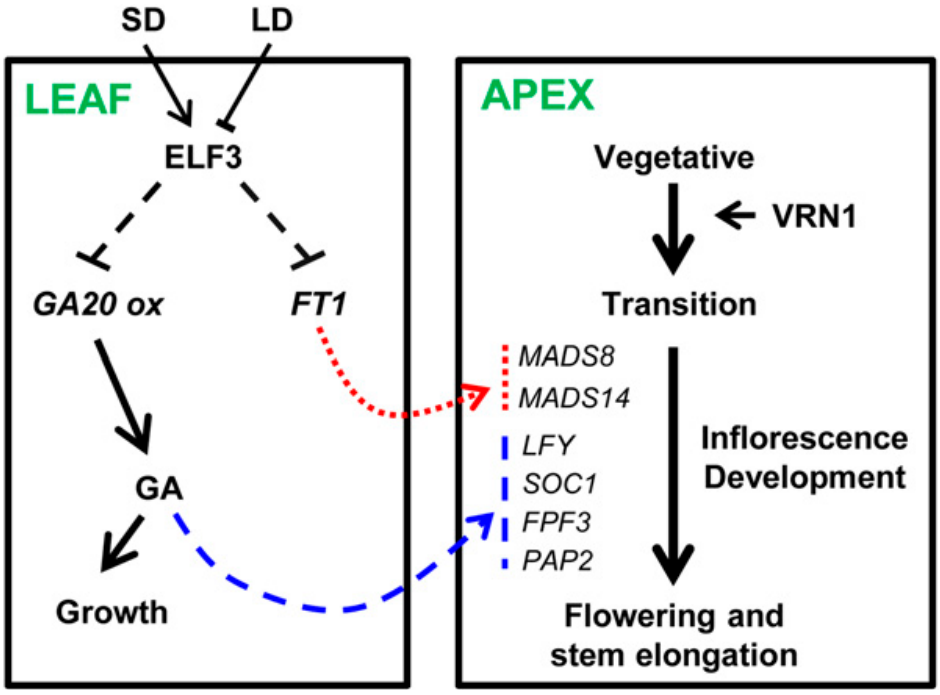

Fig. 2.3 Model of ELF3 regulation of flowering in spring barley. SD = short day, LD = long day, GA 20-ox = gibberellin 20-oxidase- one of the enzymes that catalyse the late steps in the formation of active gibberellic acid Ait-Ali et al. (1999), MADS = a conserved sequence motif which comprise the MADS-box gene family (Schwarz-Sommer et al. 1990), LFY = LEAFY protein- a transcription factor that promotes early floral meristem identity in synergy with APETALA1, FPF3 = FLORAL PROMOTING FACTOR3, SOC1 = SUPPRESSOR OF CONSTANS1, PAP2 = PHOSPHATIDIC ACID PHOSPHATASE2.

Source: Boden et al. (2014) (descriptions of acronyms and references added)

The GI-CO-FT interaction plays a crucial role in regulating the photoperiod pathway in Arabidopsis under long day condition (Higgins et al. 2010), but the role of GI in barley (*HvGI*) is not clear (Alqudah et al. 2014). A more complete model of barley flowering time involving the photoperiod, vernalization and circadian clock pathway genes is depicted in Fig. 2.4 below.

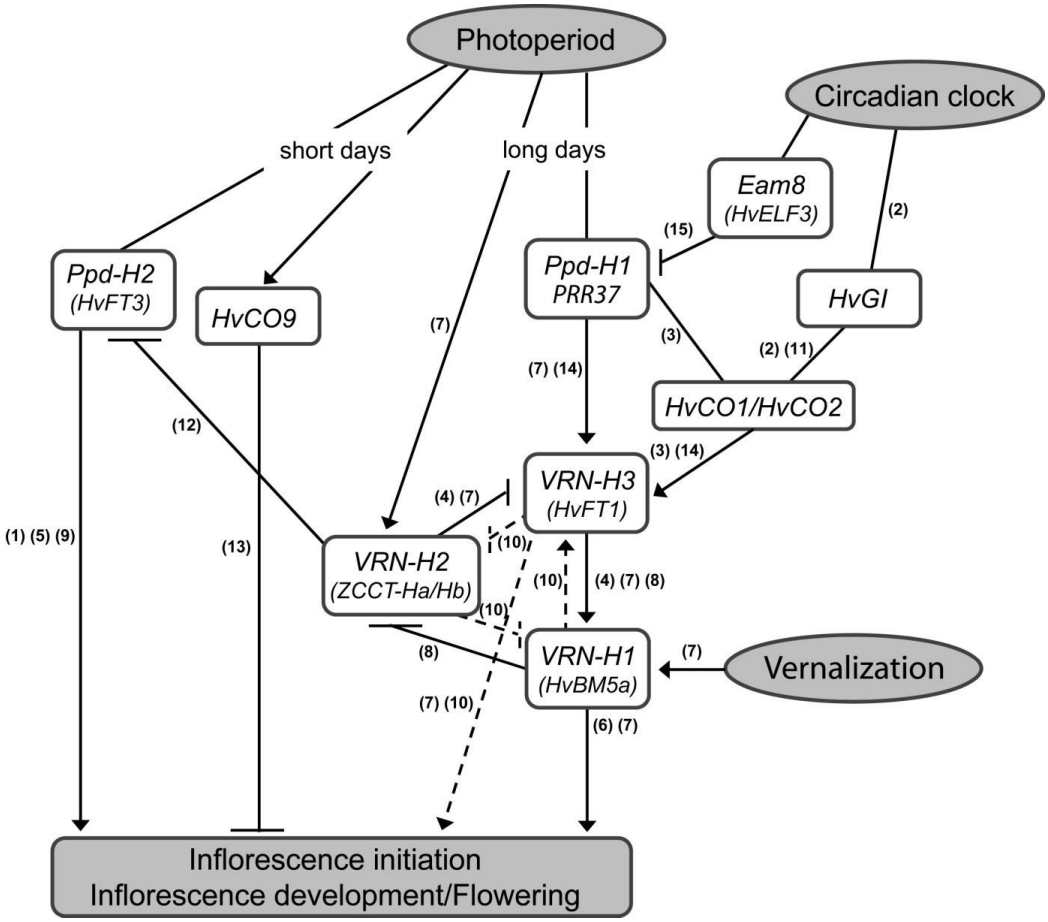

Fig.2.4 Flowering time model in barley showing the interactions between photoperiod and vernalization pathways. Numbers in brackets indicate literature in which experimental evidences support this model; (1) Laurie et al. (1995); (2) Dunford et al. (2005); (3) Turner et al. (2005); (4) Yan et al. (2006); (5) Faure et al. (2007); (6) Shitsukawa et al. (2007); (7) Hemming et al. (2008); (8) Li and Dubcovsky (2008); (9) Kikuchi et al. (2009); (10) Shimada et al. (2009); (11) Shin-Young et al. (2010); (12) Casao et al. (2011); (13) Kikuchi et al. (2012); (14) Campoli et al. (2012); (15) Faure et al. (2012b). Dashed lines indicate alternative models of gene interactions.

Source: Drosse et al. (2014)

2.2.4 Fine tuning the genetic control of heading time in barley

The genetic mechanisms underlying the control of heading time in barley described above are based on studies conducted on total number of days to heading. Alqudah et al. (2014) fine-tuned the study by dissecting pre-anthesis development in to four major stages: awn primordium, tipping, heading and anther extrusion, and four sub-phases as shown in Fig. 2.5.

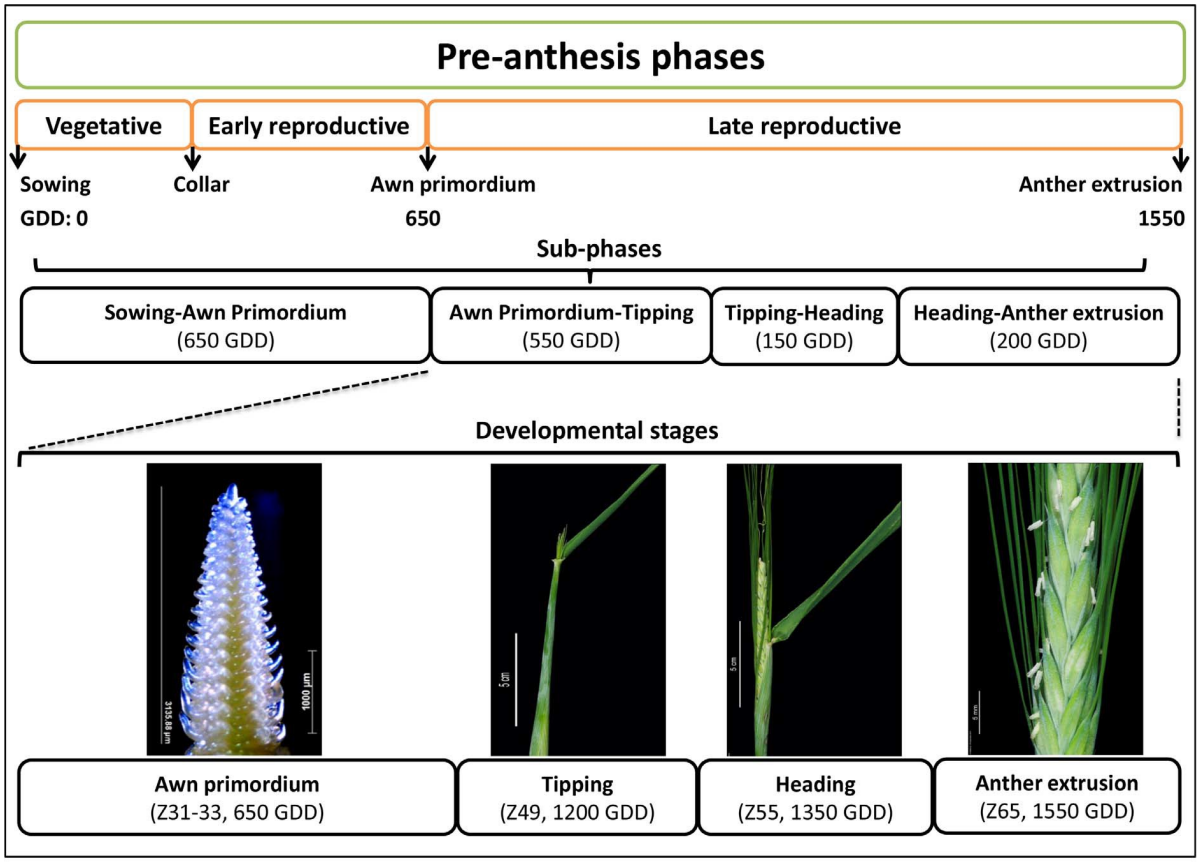

Fig. 2.5 Barley pre-anthesis phases and developmental stages

Source**:** Alqudah et al. (2014)

By using genome wide association mapping of a worldwide spring barley collection comprising photoperiod-sensitive (*Ppd-H1*) and reduced photoperiod-sensitivity (*ppd-H1*) accessions under long day condition in green house, they identified novel QTL in addition to the known major genes regulating heading time under field conditions. Based on these findings, a new genetic network model including newly identified genes (*e.g.*, different *CO*-like genes) that belong to different heading time pathways in barley has been proposed for both photoperiod groups (Fig. 2.6) (Alqudah et al. 2014).

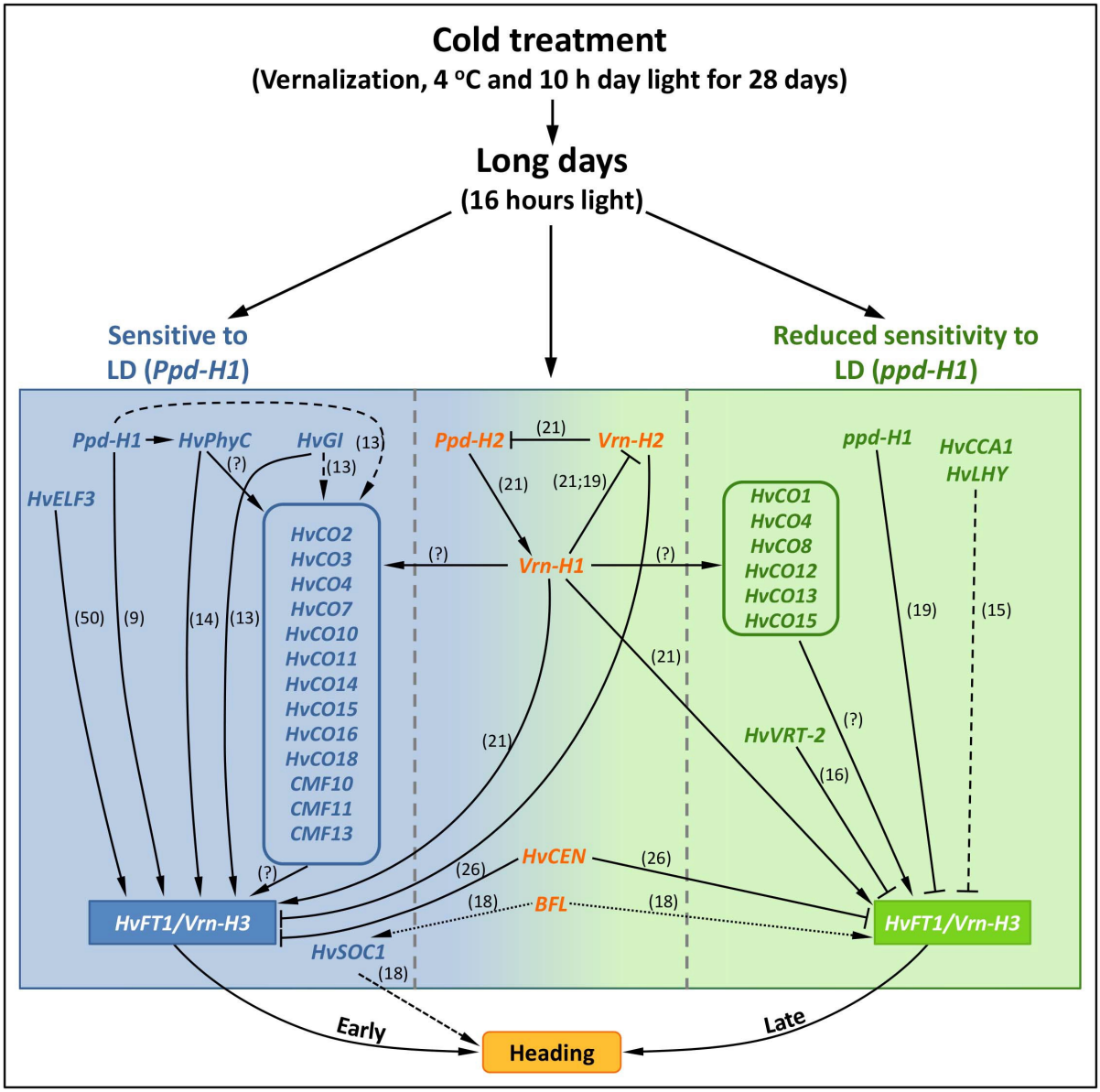

Fig.2.6 A new model of heading-time regulation in photoperiod sensitive (Ppd-H1) and reduced photoperiod sensitivity (ppd-H1) groups under long day (LD) condition. Arrow heads indicate promotion of heading, whereas flat arrow heads indicate delay of heading. Genes with known roles in the regulation of heading time in barley are shown by continuous lines. Known interaction from Arabidopsis is shown in dashed lines. Known interaction from rice is shown in round dotted lines. Ambiguous interaction is indicated by a question mark. Numbers in parenthesis show the reference to published interaction. *BFL*=BARLEY FLORICAULI LEAFY, *HvCCA1*= Barley CIRCADIAN CLOCK ASSOCIATED 1, CMF = CCT Motif Family genes, *HvLHY*= Barley Late Elongated Hypocotyl gene, *HvVRT2* = Barley Vegetative to Reproductive Transition gene 2.

Source: Alqudah et al. (2014)

2.3 Barley mapping populations for genetic studies

2.3.1 Bi-parental mapping populations

Bi-parental mapping populations are generated from crossing two genetically contrasting genotypes for the target traits of interest. Different types of bi-parental populations exist, depending on the genetic structure of the final population and on how the subsequent generations descend from the F_1 to produce the mapping population. These include F_2 populations, backcross (BC) populations, recombinant inbred lines (RILs), near isogenic lines (NILs), doubled haploid (DH) lines, and advanced intermating lines (AILs). Bi-parental mapping populations are classified as ephemeral or immortal depending on their genetic constitution and stability. The ephemeral populations include F_2 and BC populations that harbour large proportions of heterozygosity and are genetically unstable. The immortal populations comprise the DH, the RILs, the NILs, and the AILs in which individual plants in the population are nearly homozygous and are genetically stable.

Bi-parental mapping populations are easy to develop and they have been the basis for much of our current understanding of the genetic control of important traits in various crops including barley. In barley, the identification of major phenology genes, including the photoperiod response genes (*Ppd-H1* and *Ppd-H2*) and the vernalization response genes (*Vrn-H1*, *Vrn-H2*, and *Vrn-H3*) that govern adaptation to different regions and environments, has been achieved through genetic analysis of bi-parental populations. A summary of barley mapping populations that have been specifically used in low rainfall environments is given in Table 2.1. These are discussed in the following sections with emphasis on the nature of the germplasm used for crossing, the population size and genotyping method used.

2.3.1.1 Germplasm

The barley populations summarized in Table 2.1 represent different type of populations including those generated from wild x unadapted, wild x adapted, spring x winter and adapted x adapted.

All mapping populations used in Australia, with the exception of Mundah x Keel and Tallon x Kaputar, were developed from crosses between locally adapted and unadapted germplasm, including the extremes of Australian Spring x UK Winter as in Sloop x Halcyon (Table 2.1).

These populations have been used to identify and study the effects of genes that exert large effects for global adaptation or gross differences between the different types of germplasm, such as the major phenology genes known in barley (*Ppd-H1, VrnH1* and *Vrn-H2*). However, such populations have large confounding effects of the genetic background and the major genes that delineate macro-scale germplasm pools do not control regional adaptation of elite local germplasm.

2.3.1.2 Population size

The size of the mapping population affects the accuracy and resolution of genetic linkage maps. Using large mapping populations enables to achieve high resolution to very small genetic distances between polymorphic markers, and to identify weak genetic linkages. Population size is also dictated by the objectives of the study and the type of population. For example, fine mapping to clone a gene requires much larger population than construction of linkage maps. Larger F_2 populations are required compared to the DH or BC populations to achieve the required mapping resolution (Xu 2010). Most of the barley mapping populations listed in Table 2.1 had a population size of less than 200 individuals, except the ISR42-8 x Scarlett, HS584 x Brenda AB-QTL, W89001002003 x I60049, Orria x Plaisant and the HEB-25 NAM populations, the extreme being the Tallon x Kaputar population which had only 65 individuals.

2.3.1.3 Genotyping

High-density genetic linkage map is important for efficient dissection of QTL underling complex traits. With the exceptions of the wild barley introgression lines (S42IL), the HEB-25 NAM, and the Orria x Plaisant RIL populations, all the barley mapping populations used in the 2-4 t/ha environments between 1996 and 2014 have been genotyped largely with RFLP, AFLP, RAPD, SSR, STS, DArT markers (Table 2.1). The number of markers used to genotype these populations range from as small as 54 markers in Mundah x Keel to 665 markers in Arta x Keel (Table 2.1). Only the three recent populations Orria x Plaisant (382 SNPs), S42IL (4201 SNPs), and HEB-25 NAM (5,709 SNPs) have benefited from the latest high throughput SNP genotyping technologies with high marker coverage in the latter two populations (Table 2.1).

Table 2.1 Barley bi-parental mapping populations used in 2-4 t/ha environments

Population	Lines	Genotyping	Germplasm	Reference
Steptoe x Morex	150 DH	292 RFLP, RAPD, Isozymes & SAP	American 6-row Spring x American 6-row Spring	Kleinhofs et al. (1993)
Mundah x Keel	110 RIL	54 AFLP, RFLP & SSR	Australian Spring x Australian Spring	Long et al. (2003)
Tallon x Kaputar	65 DH	258 AFLP & SSR	Australian Spring x Australian Spring	Cakir et al. (2003)
Sloop x Halcyon	166 DH	257 AFLP, RFLP, SSR & SNP*	Australian Spring x UK Winter	Read et al. (2003)
Amagi Nijo x WI2585	139 DH	100 RFLP & SSR	Japanese Spring x Australian Spring	Pallotta et al. (2003)
Clipper x Sahara	150 DH	215 RFLP, SSR & others	Australian Spring x North African Landrace	Karakousis et al. (2003)
Galleon x Haruna Nijo	112 DH	435 AFLP, RFLP, SSR & others	Australian Spring x Japanese Spring	Karakousis et al. (2003)
Chebec x Harrington	120 DH	348 AFLP, RFLP, SSR & others	Australian Spring x Canadian Spring	Barr, Karakousis et al. (2003)
Alexis x Sloop	111 DH	291 AFLP, RFLP, SSR & other	European Spring x Australian Spring	Barr, Jefferies et al. (2003)
	153 RIL	214 AFLP, RFLP, SSR & other		
Arta x Hsp41-1	194 RIL	189 AFLP & SSR	Syrian Winter Landrace x Wild	Baum et al. (2003)
VB9524 x ND11231-12	180 DH	211 AFLP & SSR	Australian Spring x North American Spring	Emebiri and Moody (2006)
ISR42-8 x Scarlett	301 BC2DH	98 SSR	Wild x European Spring	von Korff et al. (2006)
HS584 x Brenda AB-QTL	207 BC3 DH	108 SSR	Wild x European Spring	Li et al. (2006)
Beka x Mogador	120 DH	215 RFLP, STS, RAPD & SSR	European Spring x European Winter	Cuesta-Marcos et al. (2009)
Tadmor x ER/Apm	158 RIL	165 RFLP, STS, RAPD & SSR	Syrian Landrace x North African Spring	von Korff et al. (2008)
Henni x Meltan	118 DH	269 AFLP	Northern European Spring x Northern European Spring	Borràs-Gelonch et al. (2010)
Merit x H93174006	127 RIL	209 DArT & SSR	Canadian Spring x Canadian Spring	Chen, Chang and Anyia (2012)
W89001002003 x I60049	200 RIL	104 SSR	6 row Canadian Spring x 6 row Canadian Spring	Chen, Chang and Anyia (2012)
Arta x Keel	188 RIL	665 DArT, SSR & STS	Syrian Winter Landrace x Australian Spring	Rollins et al. (2013)
Orria x Plaisant	217 RIL	382 SNP	6 row Spanish Facultative x 6 row European Winter	Mansour et al. (2014)
Nure x Tremois	118 DH	543 DArT & other	Southern European Winter x European Spring	Tondelli et al. (2014)
S42IL		4,201 SNP	Wild x Unadapted	Honsdorf, Nora et al. (2014)
HEB-25 NAM	1,420 BC1 RIL	5,709 SNP	25 Wild x European Spring (Barke)	Maurer et al. (2015)

*only one SNP

Although bi-parental mapping populations have been successfully used to dissect the genetic control of global adaptation in barley, they allow genetic analysis of only two alleles and their interactions with two genetic backgrounds. The use of interconnected populations developed from inter-crossing of elite germplasm allows increasing allelic diversity while still representing the germplasm of the local breeding programs. Such interconnected populations comprising 17 small DH populations derived from crosses among 14 heterogeneous cultivars have been use to validate heading date QTL in barley in Spain (Cuesta-Marcos, Alfonso et al. 2008).

There has been a growing interest in alternative population structures, including advanced backcross quantitative trait loci (AB-QTL) analysis, nested association mapping (NAM), and genome wide association mapping panels (GWAS). Recently, more complex population structures, such as multi-parent advanced generation inter-cross (MAGIC), are being implemented in barley, wheat, rice and other crops. Detailed comparison of the different types of mapping populations based on various features is available in Bohra (2013).

2.3.2 Advanced backcross quantitative trait loci (AB-QTL) analysis

Elite germplasm of crop plants has desirable characters such as high yield, quality threshability, and non-shattering. They are however, constrained by narrow genetic variation due to a long history of selective breeding. Conversely, unadapted wild germplasm represents a huge reservoir of un-utilized genetic variation but also carries many undesirable traits. In traditional plant breeding approaches, the use of wild germplasm has been limited to the transfer of major genes, mainly for disease and insect resistance through repeated backcrossing to a recurrent (adapted) parent to recover most of the adapted genetic background. A single gene transferred from wild germplasm can still be associated with undesirable genes due to linkage drag. Transferring quantitative characters from wild germplasm had not been attempted in the conventional backcross breeding schemes due to problems of epistasis and compounded linkage drag. However, molecular linkage maps help reduce this problem by enabling selection of individuals carrying recombinant chromosomes with minimal linkage drag (Tanksley & Nelson 1996). Populations used for QTL studies in adapted germplasm such as the F_2, BC1, and RIL populations cannot be directly used for identification and transfer of useful QTL from unadapted germplasm due to limitations described in (Tanksley & Nelson 1996). AB-QTL analysis has been developed as a solution

to this problem in which QTL analysis is delayed until advanced generations of backcrossing (BC2, BC3 and more generations), and aim to support the discovery and transfer of useful QTL alleles from unadapted donor lines into elite germplasm (Tanksley & Nelson 1996; Wang, B & Chee 2010).

Earlier applications of AB-QTL analysis in genetics and breeding of various crops including tomato, rice, barley, wheat, maize and cotton have been thoroughly reviewed in Wang & Chee (2010). The review included the works of Pillen, Zacharias and Leon (2003); von Korff et al. (2004); von Korff et al. (2005); Li et al. (2006); von Korff et al. (2006); Yun et al. (2006); Gyenis et al. (2007); von Korff, Maria et al. (2007), Schmalenbach, Korber and Pillen (2008), and Schmalenbach, Leon and Pillen (2009), on different traits including yield and other agronomic traits, quality and disease resistance.

Wang, et al. (2010) used 301 BC2DH lines and a set of 39 introgression lines (S42ILs) to study the genetic mechanisms underlying flowering time in barley. BC2DH lines were developed from a cross between the German spring barley cultivar Scarlett (*H. vulgare* L.) as a recurrent parent and the wild accession ISR42-8 (*H. vulgare ssp*. *spontaneum*) as described in von Korff et al. (2004). They found seven candidate genes associated with flowering time QTL in population S42 and four exotic alleles that exhibited significant effects on flowering time in S42ILs.

Saal et al. (2011) used the same 301 BC2DH lines of the spring barley BC2DH population S42 described in Wang et al. (2010) to study localization of QTL x nitrogen interaction effects for yield-related traits. The traits studied were the number of ears per m^2, days to heading, plant height, thousand grain weight and grain yield. They reported 82 QTL for these traits. Sayed et al. (2012) have also used AB-QTL analysis using the same population and found eight QTL for proline content and leaf wilting under drought stress conditions. Both the Scarlet (adapted) and ISR42-8 (wild) contributed favourable alleles for proline content and leaf wilting whereby the exotic allele increased proline content by 54%.

Recently, Honsdorf et al. (2014) studied the juvenile drought stress tolerance of wild barley using 55 wild barley introgression lines (ILs) of the S42IL library and the elite barley cultivar Scarlett. They used an improved genetic map of the population S42 introgression lines (S42IL) that they generated with 4,201 SNPs from a Genotyping By Sequencing (GBS)

platform and found 21 QTL where the exotic allele increased biomass and relative water content under drought.

2.3.3 Association mapping

QTL analysis approaches have been extended from the traditional bi-parental based linkage analysis to genome wide association studies (GWAS) to harness allelic variation present in natural populations or in broad panels of unrelated lines. GWAS exploits linkage disequilibrium to localise QTL in diverse natural populations (Cavanagh et al. 2008; Mackay, I & Powell 2007). In bi-parental linkage analysis, allelic variation is limited to the maximum possible segregation between the two parents of the particular population and the amount of genetic recombination is limited by the generations of inter-crossing used to develop the population. GWAS utilizes diverse genetic variation in natural populations and takes advantage of historic recombination events to identify QTL. While linkage mapping provides population specific QTL, GWAS tests multiple alleles for their association with the trait and can be directly deployed for QTL discovery (Bohra 2013), and requires very large samples to have sufficient power to detect the genomic regions of interest (Huang et al. 2015).

GWAS has been successfully applied in human genetics to detect the genomic regions associated with various human diseases (Visscher et al. 2012), and in plants including Arabidopsis, maize and rice (Brachi, Morris & Orevitz 2011). A number of studies using GWAS for different physiological traits, morphological traits, agronomic traits, disease resistance and quality traits of barley have been reported over the last few years and these are summarized in Table 2.2.

The accuracy of GWAS is affected by such factors including sample size, composition of the mapping panel, statistical approaches to overcome genetic confounding and methods to identify and account for complex genetic architectures (Korte & Farlow 2013). The population structure of the studied panel must be taken into account to avoid false associations (Visscher et al. 2012).

2.3.4 Nested association mapping (NAM)

Nested association mapping (NAM) involves crossing several founder genotypes with a common parent. The resulting F_1s are either self-pollinated to develop RILs, or used to generate doubled haploid populations. NAM integrates the advantages of linkage analysis and association mapping in a single, unified mapping population to dissect complex traits (Yu et al. 2008). It has advantage over both linkage mapping and association mapping in that it benefits from both historic and recent recombination events to require only low marker density, provide high allele richness, high mapping resolution, and high statistical power, but without the disadvantages of either linkage analysis or association mapping (Yu et al. 2008). The first example of QTL identification through NAM was used to study the genetic architecture of maize flowering time (Buckler et al. 2009).

Schnaithmann, Kopahnke and Pillen (2014) used an explorative barley NAM population (HEB-5) to map QTL for leaf rust resistance. The population consisted of 295 BC1S1 lines developed from crossing and backcrossing five exotic barley donors with the elite barley cultivar 'Barke'. Maurer et al. (2015) have recently reported another barley NAM population (HEB-25) that was developed by crossing 25 wild barley genotypes with one elite barley cultivar (Barke). They have used this population to dissect the genetic architecture of flowering time in barley and have identified eight QTL controlling this trait, the strongest effects being associated with the *Ppd-H1* locus.

2.3.5 Multi-parent advanced generation inter-cross (MAGIC) populations

Multi-parent advanced generation inter-cross (MAGIC) involves inter-mating of multiple inbred founders for several generations prior to creating inbred lines, resulting in a diverse population whose genomes are fine-scale mosaics contributed from all founders (Huang et al. 2015). Multi-parental populations provide more equilibrated allelic frequencies than GWAS panels and higher recombination rates than bi-parental populations (Pascual et al. 2015).

An early example reported by McMichael et al. (2005) used 837 DH lines from Chieftan/Barque//Manley/VB9104. The variety 'Flagship' was a commercial line developed from this population, which combined the Canadian malting quality conferred by the complex locus on the long arm of chromosome 5H, with the thermostable β-amylase from VB9104. It also combined *Rph20* leaf rust resistance from Chieftan, *Rp14* spot form net

blotch resistance and *Rha2* cereal cyst nematode resistance from Barque with the phenotype and adaptation of the two Australian parents, VB9104 and Barque. Genotyping of the DH population was conducted using 290 simple sequence repeats (SSR) markers. While the four parents were not closely related, only 20 of the SSR markers exhibited four unique alleles. The inability to assign markers unequivocally to a parent was a major limitation to conducting genetic analysis in this population. The evolution of genotyping techniques has since resolved this limitation and multi-parent populations that combine high genetic recombination and diversity are now available.

Sannemann et al. (2015) reported the first eight parent advanced generation inter-cross (MAGIC) doubled haploid (DH) population to proof the concept of MAGIC population structure for QTL mapping in barley. They used 533 DH lines derived from inter-mating of eight German spring barley genotypes (Ackermanns Bavaria, Ackermanns Danubia, Barke, Criewener 403, Heils Franken, Heines Hanna, Pflugs Intensiv and Ragusa). The DH lines were genotyped with 4,550 SNPs and used to identify QTL for the major flowering-time genes *Vrn-H1*, *Vrn-H3*, *HvGI*, *Ppd-H1*, *HvFT2*, *HvFT4*, *Co1* and the plant height genes linked to *sdw1* with high precision.

Huang *et al*. (2012) used a four-parent MAGIC population of 1,579 RILS derived from inter-mating between four Australian wheat cultivars (Yitpi, Baxter, Chara and Westonia), and constructed the genetic linkage map of MAGIC population using a total of 1162 markers comprising of 826 DArT, 283 SNPs and 53 SSR markers. The usefulness of the constructed multi-parent linkage map for QTL mapping was demonstrated using phenotypic data for plant height and hectolitre weight. Similarly, Milner *et al*. (2015) used 338 durum wheat recombinant inbred lines (RILs) derived from four durum wheat (*Triticum turgidum ssp. durum*) breeding lineages differing in their origin and phenotypes (Neodur, Claudio, Colosseo and Rascon/2*Tarro) to construct a linkage map spanning 2664 cM with 7594 SNPs. This map was successfully used to dissect QTL underlying plant height, heading date, maturity and yield. A more complex eight-parent MAGIC population for wheat was developed by Mackay, IJ et al. (2014), which comprised 1091 F7 winter wheat lines derived from systematic inter-mating of eight UK wheat cultivars (Alchemy, Brompton, Claire, Hereward, Railto, Robigus, Sossons, and Xi19). They used the same crossing scheme outlined by Cavanagh et al. (2008) for eight-parent MAGIC population development (Fig.2.7).

The population was genotyped using the Illumina Infinium iSelect 90,000 SNP wheat array, and was found to be highly recombined. It was recommended that the population could be used as a platform for QTL fine mapping and gene isolation. Other MAGIC populations used in wheat, rice, chickpea, pigeon pea, peanut and maize have been reviewed recently by (Huang et al. 2015).

QTL analysis in MAGIC populations requires advanced computational tools that takes in to account all possible patterns of allele segregation unlike the analysis software developed for bi-parental crosses which only needs to consider the polymorphic marker data of the two parents (Huang & George 2011). The haplotype approach enables full exploitation of the potential of the multi-parent population and directly assigns parental alleles at significant genetic positions (Sannemann et al. 2015).

Table 2.2 Summary of genome wide association studies in barley

Association panel	Genotyping	Traits studied	Reference
500 UK barley cultivars	1536 SNPs	15 morphological traits	Cockram et al. (2010)
318 wild barley accessions	558 Dart & 2878 SNPs	Spot blotch resistance	Roy et al. (2010)
224 diverse collection of spring barley	1536 SNPs	Heading date, plant height, thousand grain weight, starch content, crude protein content	Pasam et al. (2012)
615 barley cultivars	1536 SNPs	10 agronomic and 32 morphological traits	Wang, M et al. (2012)
76 barley genotypes	1033 SNPs	13 agronomic traits	Locatelli et al. (2012)
185 cultivated and 38 wild genotypes from different countries and continents	710 DArT, 61 SNPs & 45 SSR	yield, yield components, developmental, physiological and anatomical traits	Varshney et al. (2012)
184 genotypes representing the Mediterranean region gene pool	1536 SNPs	Frost tolerance	Visioni et al. (2013)
192 spring barley genotypes of different geographic origins	954 SNPs	Salt tolerance	Long, NV et al. (2013)
298 Ethiopian and Eritrean barley landraces	7842 SNPs	Kernel weight and grain zinc and iron concentration	Mamo, Barber and Steffenson (2014)
174 European spring and winter barley cultivars	839 DArT markers	Grain yield and 18 quality traits	Matthies et al. (2014)
>3000 lines/cultivars	3072 SNPs	Stem rust race TTKSK resistance	Zhou et al. (2014)
360 elite genotypes from the Northern Region Barley Breeding Program in Australia	3244 DArT	Leaf rust (Puccinia hordei)	Ziems et al. (2014)
≈770 lines replicated over four years	3072 SNPs	5 agronomic traits	Pauli et al. (2014)
156 winter barley genotypes	3212 SNPs	Drought tolerance and stress induced leaf senescence	Wehner et al. (2015)
109 German winter barley genotypes	3886 SNPs	Yield and quality	Gawenda et al. (2015)
100 accessions from international barley core selected collection	1336 SNPs	Cadmium concentration in different organs of barley	Wu, Sato and Ma (2015)
288 tow-rowed and 288 six-rowed spring barley accessions	3072 SNPs	Culm cellulose content	Houston et al. (2015)

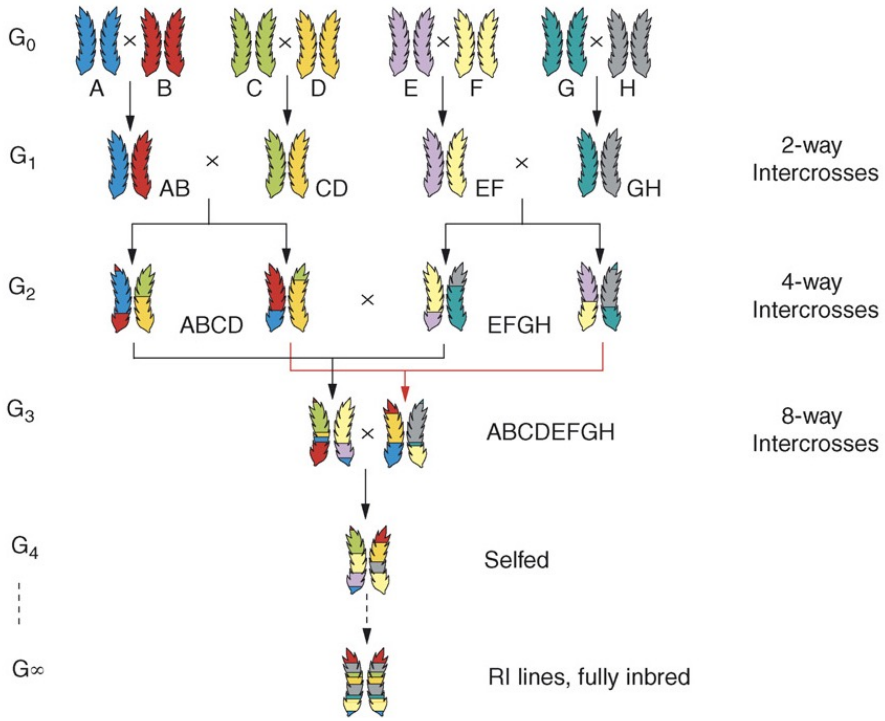

Fig. 2.7 Crossing scheme for eight-parent MAGIC population

Source: Cavanagh et. al. 2008

2.4 Conclusions

Phenology is the major determinant of barley adaptation and yield in temperate cereals like barley and wheat. Synchronization of flowering time with suitable time of the season in terms of photoperiod, temperature and moisture is an important goal of barley breeders, especially in Mediterranean type environments such as southern Australia. Photoperiod response and vernalization requirement are two important mechanisms through which the environment influences barley flowering time, and the major genes underlying these mechanisms have been identified.

Establishing mapping populations is an important prerequisite for genetic linkage map construction, thereby to dissect the genetic basis of complex traits such as phenology, adaptation and yield. Bi-parental mapping populations are easier to establish but are limited to analysis of only two alleles and interaction between two genetic backgrounds. Bi-parental linkage mapping gives low map resolution, unless very large populations are used and is less accurate for detecting QTL positions than more complex structures. AB-QTL analysis provides the opportunity for allele mining from the reservoir of variation existing in wild germplasm, but does not show the differences between adapted and benchmark germplasm.

Association mapping has the advantage of using existing natural genetic variation and historic recombination events and provides dense genetic recombination maps for more precise QTL detection. However, population structure and other confounding effects need to be taken into account to ensure the power of association mapping. Nested association mapping combines the advantages of linkage mapping and association mapping while reducing their limitations. MAGIC populations provide higher equilibrated allele frequencies than the GWAS populations and are suitable for fine mapping and detection of small effect QTL but require greater time and effort to produce.

The use of carefully selected, tailor made populations is imperative for the study of the genetics of adaptation to a particular environment. The three interconnected DH populations used in the current study have been developed from adapted elite Australian germplasm (Commander, Fleet and WI4304) that are similar in maturity but have different merits in terms of important traits for yield and adaptation. The detailed description of each parental line is given in the materials and methods section of chapter 3. The populations have been developed with the aim of creating allelic recombinations that enable identification of novel QTL for adaptation to the Australian environment.

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Chapter 3: Construction of genetic linkage maps for Commander x Fleet, Commander x WI4304 and Fleet x WI4304 doubled haploid populations

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Construction of genetic linkage maps for Commander x Fleet, Commander x WI4304 and Fleet x WI4304 doubled haploid populations

3.1 Abstract

Three genetic linkage maps of doubled haploid populations were constructed using Genotyping by Sequencing (GBS) and Single Nucleotide Polymorphisms (SNPs) for known phenology genes. The mapping populations involved reciprocal crosses between three Australian elite genotypes Commander (C), Fleet (F) and WI4304 (W). The constructed linkage maps comprise 2179, 2892, and 2252 markers in CF, CW and FW populations, respectively. The markers were grouped to seven, eight, and nine linkage groups, spanning a total length of 1304.3 cM, 1404.4 cM, and 1274.9 cM, respectively in the CF, CW and FW maps. Segregation distortion percentages were similar in the three populations and varied between chromosomes and between reciprocal crosses. These genetic linkage maps are the first high density genetic maps developed from adapted x adapted crosses in Australia and will serve as a platform for genetic dissection of complex traits such as yield and adaptation in Australian barley breeding programs.

3.2 Introduction

Genetic linkage maps lay the basis for genetic analysis of biological traits. Molecular linkage maps provide information on the genomic locations of molecular markers and the relative distances between them within linkage groups. This information is essential to study the genetic control of traits through QTL analysis. The important steps required for genetic linkage map construction includes the selection of suitable parental lines, developing the mapping populations, genotyping of individuals in the populations and construction of the linkage maps using statistical tools and software.

The choice of parental lines is dictated by the traits targeted for genetic analysis, so that the parental pairs have the required genetic polymorphism for the molecular markers or genes linked to the traits of interest. Different types of populations could be used for genetic linkage map construction including the F2 populations, backcross (BC1), and permanent populations such as the doubled haploids (DHs), recombinant inbred lines (RILs), and backcross inbred lines (BILs) (Xu 2010). Mapping populations developed from elite breeding materials are

important to dissect the genetic architecture of quantitative traits and to identify QTL, which can be deployed directly in breeding programs (Wurschum 2012). Population size also plays an important role as it affects the resolution of the genetic linkage maps and the precision of genetic dissection of quantitative traits.

Genetic map distances are estimated from the recombination fraction between two loci and are measured in Morgans or centi Morgans (cM) units. Recombination frequencies are converted to genetic map distances using mapping functions that estimate the influence of an even number of recombination events between two adjacent loci. The two commonly used mapping functions (Haldane and Kosambi) differ on the issue of crossover interference in the estimation of map distances. Interference refers to the condition in which the occurrence of a crossover event in one region of a chromosome affects the probability of occurrence of another crossover event in the adjacent region (Hillers 2004). The Haldane's mapping function (Haldane 1919) assumes no interference between crossovers, thus considers that recombination events in adjacent regions of the chromosome are independent of each other. The Kosambi's mapping function (Kosambi 1944*)* on the other hand considers the interference in the estimation of map distances.

Barley *(Hordeum vulgare* L.), a diploid species with a genome size of 5.1Gb (Dolezel *et al.* 1998) has been used for genetic studies for several decades. Different mapping populations of barley have been developed which vary with regard to the type of the populations, population size, and the target traits for genetic analysis and the target environments for which they were developed. The genetic linkage maps developed using these populations also vary depending on the number and type of markers used. Most of the previous genetic studies in Australia have used mapping populations with small population size and low marker saturation (Barr, Jefferies, et al. 2003; Barr, Karakousis, et al. 2003; Cakir et al. 2003; Karakousis, Barr, Kretschmer, Manning, Jefferies, et al. 2003; Karakousis, Barr, et al. 2003b; Long et al. 2003; Pallotta et al. 2003; Read et al. 2003). Moreover, these previous populations have been developed from crosses involving Australian varieties with exotic materials or landraces (Langridge & Barr 2003). In the current study, three new doubled haploid (DH) populations developed from inter-crossing elite Australian germplasm have been used for genetic linkage map construction using a high throughput Genotyping by Sequencing (GBS) platform.

3.3 Materials and methods

3.3.1 Plant materials

Three doubled haploid (DH) populations of barley developed from reciprocal crosses among three Australian genotypes were used. The genotypes include two elite varieties (Commander and Fleet) and one advanced breeding line (WI4304). Commander (Keel/Sloop//Galaxy) is a malting variety with large grain size and is high yielding in southern Australia. WI4304 (Riviera/ (Puffin/Chebec)-50//Flagship) is a malting quality breeding line with high osmotic adjustment and high net photosynthesis under drought conditions (Le 2011). Fleet (Mundah/Keel//Barque) is a feed variety characterized by high water use efficiency, long coleoptile, and adaptation to deep sandy soils. Initially, 257, 269, and 422 lines were genotyped from the Commander x Fleet (CF), Commander x WI4304 (CW) and Fleet x WI4304 (FW) populations, respectively. These initial sets include some abnormal genotypes with deformed morphologies that do not seem to have arisen from normal recombinations between the parental alleles. Moreover, some DH lines were found to be clonal individuals with exactly the same genotypes. The abnormal lines were removed and the clonal individuals in each clonal group were considered as one genotype in the construction of the genetic linkage map.

3.3.2 DNA extraction and genotyping

Genomic DNA was extracted from young leaves using the Phenol/Chloroform method (Rogowsky *et al.* 1991). DNA concentration and quality was checked using a Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies, Inc. Wilmington, USA) and standardized using PicoGreen (Ahn, Costa & Emanuel 1996). The three populations were genotyped using Genotyping by Sequencing (GBS) to identify markers for genetic map construction. The GBS library was prepared using available protocols (Elshire *et al.* 2011; Poland *et al*. 2012). The DNA samples where digested using two restriction enzymes (PstI and MspI) for complexity reduction, barcoded and multiplexed. Each GBS library containing 96 DNA samples (96-plex) was run on a single lane of Illumina HiSeq2000 for sequencing. The populations were also genotyped for the phenology genes using Polymerase Chain Reaction (PCR) and High Resolution Melting (HRM (Table 3.1) and the KBioscience Competitive Allele-Specific Polymerase chain reaction (KASPar) assay (Table 3.2). The KASP protocol used is available

online from LGC genomics (http://www.lgcgroup.com/). The PCR protocols and programs used for genotyping the PCR and HRM based genotyping of phenology genes are given in Table S3.1. The PCR products were separated by gel electrophoresis using 2% agarose and the resulting bands were scored. The phenology genes used include the photoperiod response gene (*Ppd-H1)*, the vernalization sensitivity gene (*Vrn-H2*), HvZCCTHc, *HvAP2*, *HvFT5*, *HvFT5_1_724, HvTFL1*, *HvCO2*, *HvCO1, HvGI*, *HvPhyB* and *HvPhyC* (Tables 3.1 & 3.2). *Ppd-H1*, *Vrn-H2*, *HvCO2* and *HvZCCTHc* were selected based on previously reported polymorphism between the parents (Le 2011), while all the other genes were selected after initial screening for polymorphism using a subset of the DH lines and the parents.

3.3.3 Data analysis and linkage map construction

The GBS raw data were analysed using the Universal Network Enabled Analysis Kit (UNEAK) pipeline in TASSEL (Lu *et al.* 2013). Heterozygous markers were converted to missing values and markers with more than 20% missing data were removed. Genetic linkage maps were constructed using 2179, 2892, and 2252 GBS markers, respectively, in CF, CW and FW populations and the phenology genes listed above. The linkage maps include 229, 228 and 299 DH lines, respectively, in the CF, CW and FW populations. The marker genotype data were inspected for missing data, segregation distortion, duplicate markers and clonal individuals using the appropriate functions and settings in R/qtl (Broman 2010).

The linkage maps were constructed using MSTmap for R (Taylor 2015). Map distances were calculated using the Kosambi mapping function (Kosambi 1944). The created maps were manually curated to remove unexpected double crossovers before being used for QTL analysis. The marker sequences were aligned to the barley physical map databases (POPSEQ and IBSC 2012) (**http://floresta.eead.csic.es/barleymap/**) to assign the markers to the correct chromosomes and to align the chromosomes in the correct orientations. Chromosome charts were generated using windows QTL cartographer 2.5 (Wang, Basten & Zeng 2012).

3.3.4 Construction of consensus genetic maps

The presence of common markers among different genetic linkage maps provides the opportunity for map integration. Consensus linkage maps constructed through integration of

different high-density maps provide increased marker density and genome coverage compared to the individual maps, and facilitate the use of markers across different germplasm (Muñoz-Amatriaín et al. 2011). The three individual genetic linkage maps constructed in this study (*i.e.*, CF, CW and FW maps) share significant number of markers since they are constructed from interlinked populations. These three individual linkage maps were integrated to a consensus genetic map using MergeMap v1.2 (Wu et al., 2011; Wu, 2008b), a software that implements an efficient algorithm for resolving conflicts in the marker order among individual maps by deleting the smallest set of marker occurrences (Wu et al., 2011). Equal weights (weight = 1.00) were given to all of the three individual maps as they were constructed with approximately similar precision.

Genetic distances between markers in the consensus genetic map are usually inflated relative to the individual maps due to an algorithmic anomaly of the coordinate system used in MergeMap (Close et al. 2009). Due to this, previous studies used scaling factors to normalize the chromosomal lengths after consensus map construction. Close et al. (2009) used the arithmetic mean of individual linkage groups to determine an appropriate scaling factor for each linkage group in the consensus map. Muñoz-Amatriaín et al. (2011) determined the appropriate scaling factor by dividing the arithmetic mean of the genetic distances in individual genetic maps by that of the consensus genetic map.

In this study, we followed similar procedures and determined the scaling factors for each linkage group, by dividing the genetic distances of the consensus genetic map by the arithmetic mean of the genetic distances of individual linkage maps. We found scaling factors of 0.74, 0.78, 0.80, 0.74, 0.72, 0.86, 0.63, and 0.75, respectively for chromosomes 1H, 2H, 3H, 4H, 5H, 6H, and 7H, with an average of 0.75±0.07. The lengths of each chromosome in the consensus map were normalized by multiplying the estimated marker positions in each linkage group of the consensus map by the respective scaling factors given above.

3.4 Results

3.4.1 Individual genetic linkage maps constructed

Across the three populations, the total number of unique GBS markers was initially 5287. Of these, 2827 markers (53.5%), 3699 markers (70%), and 3321 markers (62.8%) in CF, CW

and FW populations were polymorphic between the parental pairs. From the 2827 (CF), 3699 (CW) and 3321 (FW) polymorphic markers, those with more than 20% missing data were removed and the remaining markers, comprising of 2340 in CF, 3084 in CW, and 2738 in FW populations were used for initial map construction. Markers that were unsuitable for map construction due to segregation distortion were further removed in the diagnostic step of R/qtl using the threshold of P< 1e-10 (Broman 2010).

The final linkage maps comprise 2179 markers for the CF, 2892 markers for the CW, and 2252 markers for the FW populations, respectively (Table 3.3 and Figs. 3.2, 3.3 & 3.4). The 2179 markers (2172 GBS markers and 7 phenology gene markers) in CF were distributed in seven linkage groups representing the seven barley chromosomes and covered a total length of 1304.3 cM. The 2892 markers (2884 GBS markers and 8 phenology genes) in CW were distributed in eight linkage groups covering a total length of 1404.4 cM. The 2252 markers (2247 GBS markers and 5 phenology genes) in the FW were distributed in nine linkage groups covering a total length of 1274.9 cM. Two of the nine linkage groups comprise only one and three markers (Table 3.4).

Large linkage distances with no marker were observed in all of the three populations. In CF, a maximum linkage distance of 58 cM (94-152 cM) was observed on chromosome 4H. In CW, a maximum linkage distance of 34 cM (129-163 cM) was observed on chromosome 5H, while in FW, a maximum of 55 cM (18-73 cM) was observed on chromosome 2H. All chromosomes with linkage distances greater than 10 cM are shown in Fig 3.1.

3.4.2 Segregation distortion

Six hundred seventy (30.8%) markers from 2179 markers in CF have shown significant segregation distortion (P<0.05), of which 376 markers (17.3%) favoured the Commander allele while the remaining 294 markers (13.5%) favoured the Fleet allele. In CW population, 963 (33.3%) markers from 2892 showed significant segregation distortion, of which 542 markers (18.7%) favoured the Commander allele while the remaining 421 markers (14.6%) favoured the WI4304 allele. In FW population, 772 (34.3%) markers from 2252 showed significant segregation distortion (P<0.05), of which 366 markers (16.3%) favoured the Fleet allele and the remaining 406 markers (18.0%) favoured the WI4304 allele.

Comparison of reciprocal crosses showed that in the CF map, higher segregation distortion was associated with the DH lines in which Fleet was the female parent (FC-DH…) than for the lines in which Commander was the female parent (CF-DH…) (Fig. S3.1). In the CW map, markers on chromosomes 3H and 5H showed higher segregation distortion for the DH lines in which Commander was the female parent (CW-DH…) while in chromosomes 1H, 2H, 6H and 7H, the segregation distortion was higher for the lines in which WI4304 was the female parent. Nearly similar segregation distortion between the reciprocal crosses was observed for markers on chromosome 4H (Fig. S3.2). In the FW map, higher segregation distortion was observed in DH lines with Fleet as the female parent (FW-DH…) on chromosomes 1H and 3H, while the distortion was higher for the DH lines with WI4304 as the female parent (WF-DH…) on chromosomes 2H, 4H and 5H. Nearly similar segregation distortion was observed between the reciprocal crosses for the markers on chromosomes 6H and 7H (Fig. S3.3). In some chromosomes (Fig. 3.6), the markers with significant segregation distortion are clustered in certain regions and are not uniformly distributed along the chromosome.

High segregation distortion percentages were observed in chromosomes 1H, 2H, 5H, and 6H in CF; 1H, 2H, 5H and 7H in CW, and in 2H, 5H and 6H in FW population. The lowest segregation distortion percentages were observed in chromosomes 3H and 4H in all of the three populations and in chromosome 7H in CF and FW populations (Fig. 3.5). A summary of marker genotype frequencies across all individuals in each of the three populations is given in Table 3.4.

3.4.3 Consensus linkage map

The number of common markers between pairs of populations includes 1218 markers between CF and CW, 1446 markers between CW and FW, and 743 markers between CF and FW. The constructed consensus genetic map of the three populations comprises a total of 3901 markers and 1618 non-redundant markers spanning a total length of 1668.4 cM (before normalization) and 1254 cM (after normalization) (Table 3.3). The marker order in the consensus map is consistent with the order in the individual maps.

The genome coverage increased by 60% and the distance between markers reduced by 42% in the consensus map relative to the average of the three individual maps. Chromosome charts based on the consensus genetic maps are given in the Appendix section (Fig.S3.4).

Table 3.1 Primer details of flowering genes genotyped using PCR and HRM

Target gene	Primers	Primer sequence	product size (bp)	Marker type	Genotyping method used	Reference
HvCO2	HvCO2-164-F	TTTTGGAGAAGGAAGCTGGA	651	SNP	HRM	Wang, G *et al*. (2010)
	HvCO2-814-R	TTCCATAATTGCTCCCTTGC				
HvZCCTHc	HvZCCTHcF	CACCATCGCATGATGCAC	194	fragment presence (+) or absence (-)	PCR	von Zitzewitz *et al*. (2005)
	HvZCCTHcR	TCATATGGCGAAGCTGGAG				
Vrn-H2	ZCCT.06	CCTAGTTAAAACATATATCCATAGAGC	306	fragment presence (+) or absence (-)	PCR	Wang, G *et al*. (2010)
	ZCCT.07	GATCGTTGCGTTGCTAATAGTG				
Ppd-H1	PPDH1-3	GGTTTCTTTTGGTTTCTGGC	274	SNP	HRM	Le (2011)
	PPDH1-4	GGATAAACTTGAATCAACTGTTG				

Table 3.2 Phenology genes genotyped using KASP

Type	SNP ID	Gene	Mutation	Source
SNP	HvCO1_39	HvCO1	[A/G]	Barley Phenology SNP Database v1.0
SNP	HvGI_3818	HvGI	[C/T]	Barley Phenology SNP Database v1.0
SNP	HvFT5.1_167	HvFT5	[A/G]	Barley Phenology SNP Database v1.0
SNP	HvFT5.1_724	HvFT5	[G/T]	Barley Phenology SNP Database v1.0
SNP	HvTFL1_239	HvFTL1	[C/T]	Barley Phenology SNP Database v1.0
SNP	HvPhyC_3415	HvPhyC	[C/T]	Barley Phenology SNP Database v1.0
SNP	HvAP2_672	HvAP2	[G/T]	Barley Phenology SNP Database v1.0
SNP	HvPhyB_1235	HvPhyB	[A/G]	Barley Phenology SNP Database v1.0

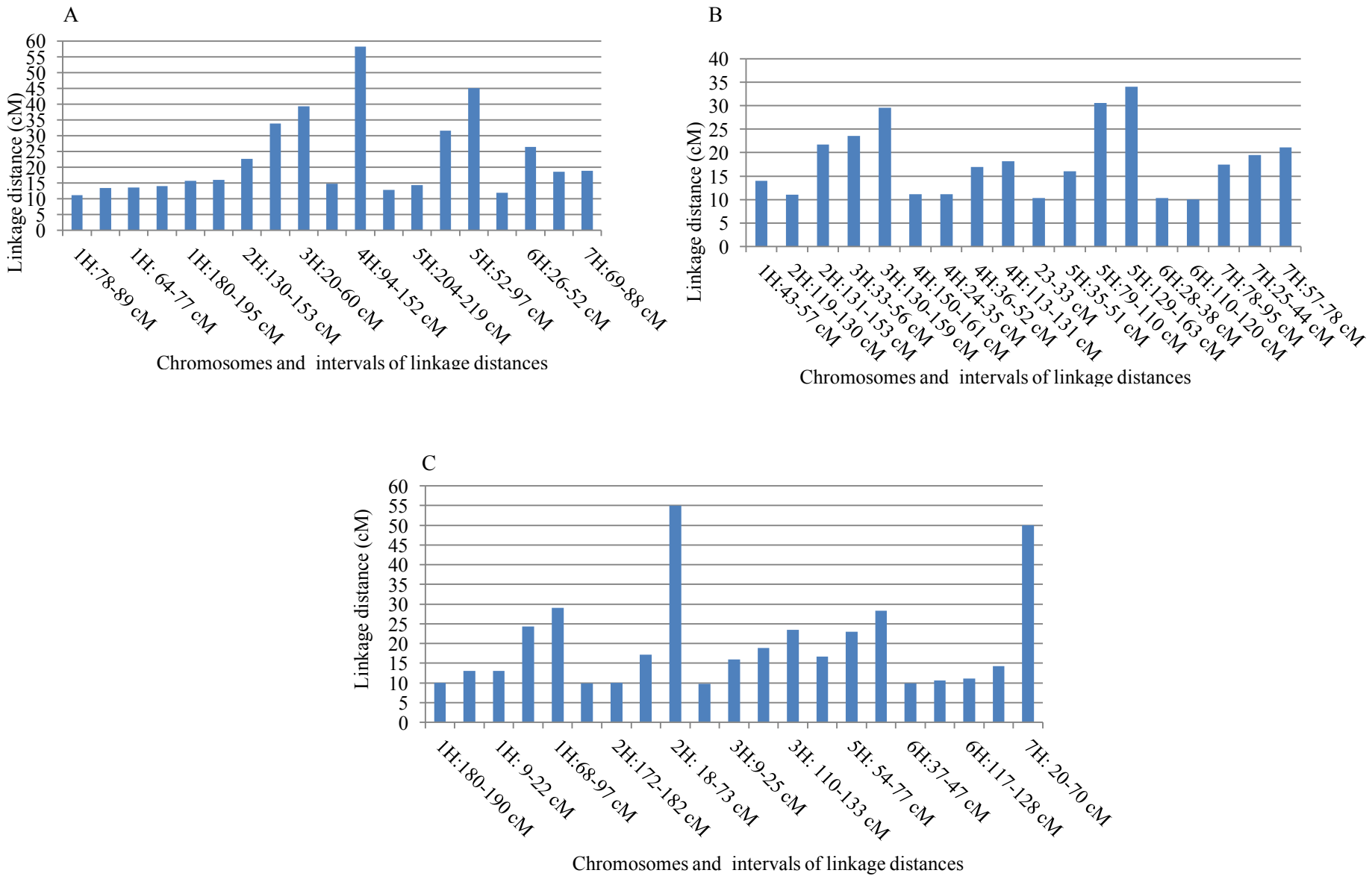


Fig. 3.1. Genomic regions with large gaps of greater than 10 cM shown against the chromosomes and the intervals of the gaps exist CF, CW and FW populations.

Table 3.3 Summary statistics of genetic linkage maps of CF (A), CW (B), FW (C) populations and the consensus genetic map (D)

A.

Chrom.	Length (cM)	Number of markers	Number of non-redundant markers*	Distance between markers	
				Mean	95% percentile of distances
1H	207.0	218	84	0.95	5.3
2H	188.2	426	137	0.44	2.3
3H	198.6	369	113	0.54	1.9
4H	192.5	268	76	0.72	3.8
5H	219.3	242	89	0.91	2.8
6H	137.9	298	95	0.46	1.8
7H	163.8	358	105	0.46	1.8
Genome	1307.3	2179	615	0.60	2.6

B.

Chrom.	Length (cM)	Number of markers	Number of non-redundant markers*	Distance between markers	
				Mean	95% percentile of distances
1H	180.8	260	99	0.70	4.2
2H	226.9	656	177	0.35	1.5
3H	217.1	547	132	0.40	1.5
4H	185.3	313	94	0.59	3.0
5H	240.3	500	143	0.48	1.5
6H	151.9	335	116	0.45	2.3
7H	200.6	274	88	0.73	3.4
7Ha	1.5	7	3	0.21	0.7
Genome	1404.4	2892	852	0.49	2.3

C.

Chrom.	Length (cM)	No. of markers	No. of non-redundant markers*	Distance between markers	
				Mean	95% percentile of distances
1H	201.6	218	105	0.92	3.6
2H	256.5	489	213	0.52	1.8
3H	188.6	368	103	0.51	2.5
4H	164.2	336	144	0.49	2.6
5H	201.8	387	177	0.52	1.9
6H	160.6	351	151	0.46	2.2
7H	100.6	99	41	1.02	1.9
X1	0.0	1	1	0.00	*
X2	0.9	3	2	0.30	1.2
Genome	1274.9	2252	937	0.57	2.3

*only one marker at a locus is considered from a number of co-located markers
Chrom = Chromosome, X1 and X2 are groups of unlinked markers

D.

Chrom.	Length (cM)		No. of markers	No. of non-redundant markers*	Distance between markers	
	Original	Normalized			Mean	95% percentile of distances
1H	232.6	171.4	368	179	0.47	2.39
2H	285.5	222	839	352	0.26	1.27
3H	251.4	200.1	654	224	0.31	1.30
4H	208.2	154.9	466	195	0.33	1.38
5H	285.3	204.4	625	273	0.33	1.26
6H	199.3	171.1	507	228	0.34	1.46
7H	206.1	130.1	442	167	0.29	1.19
Genome	1668.4	1254	3901	1618	0.32	1.46

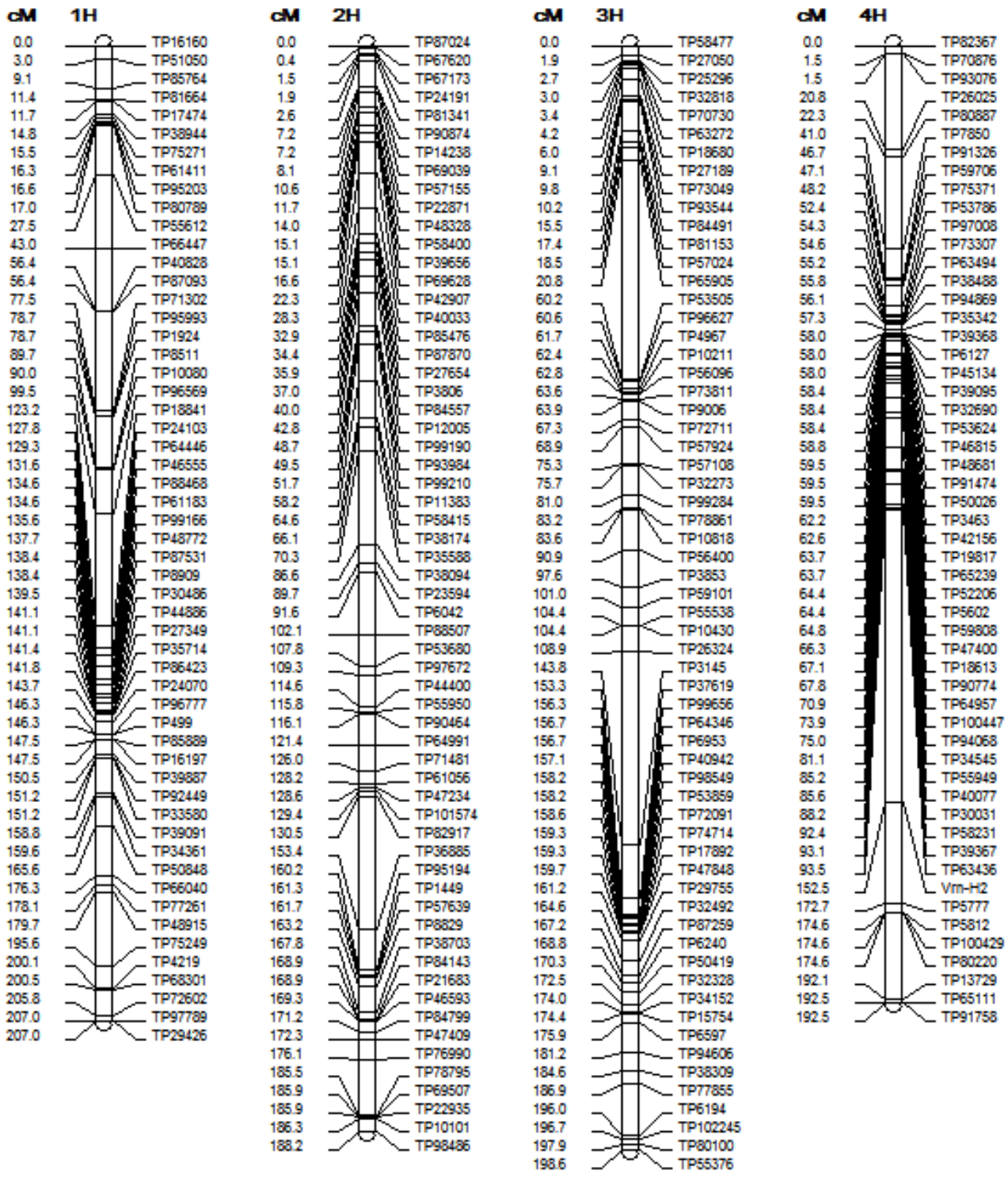
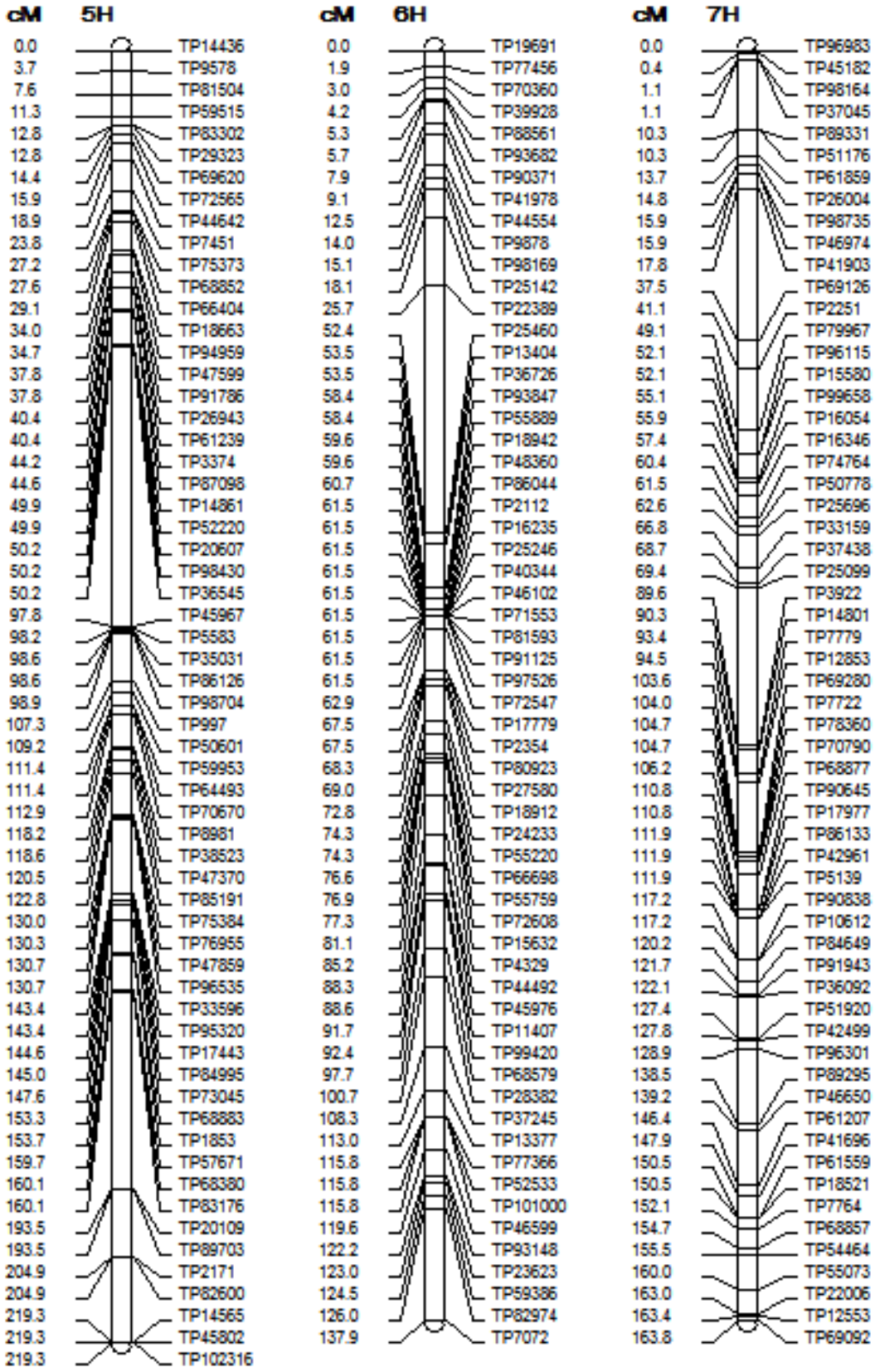


Fig.3.2 Genetic linkage map of Commander x Fleet DH population

Fig. 3.2 (*continued*)



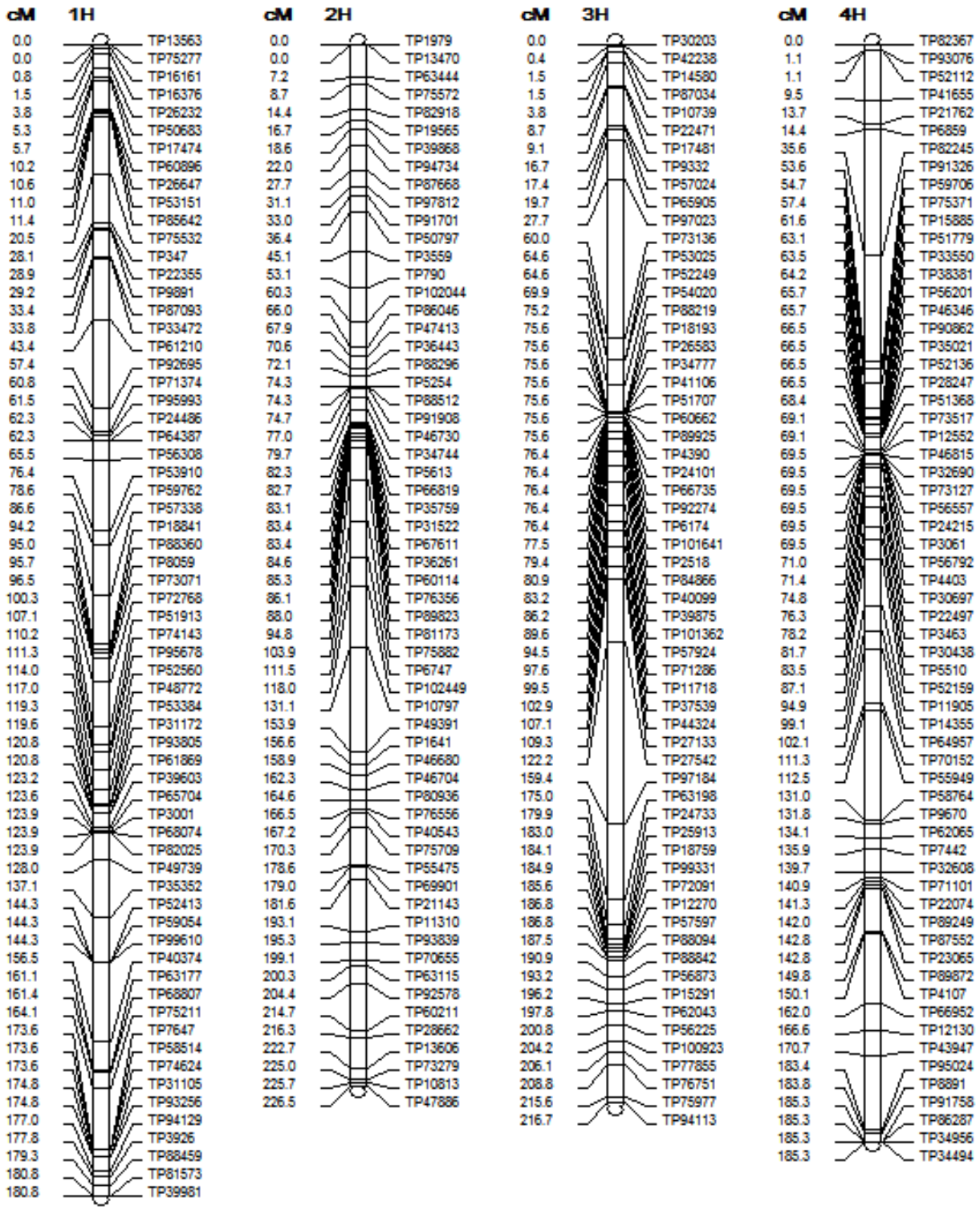
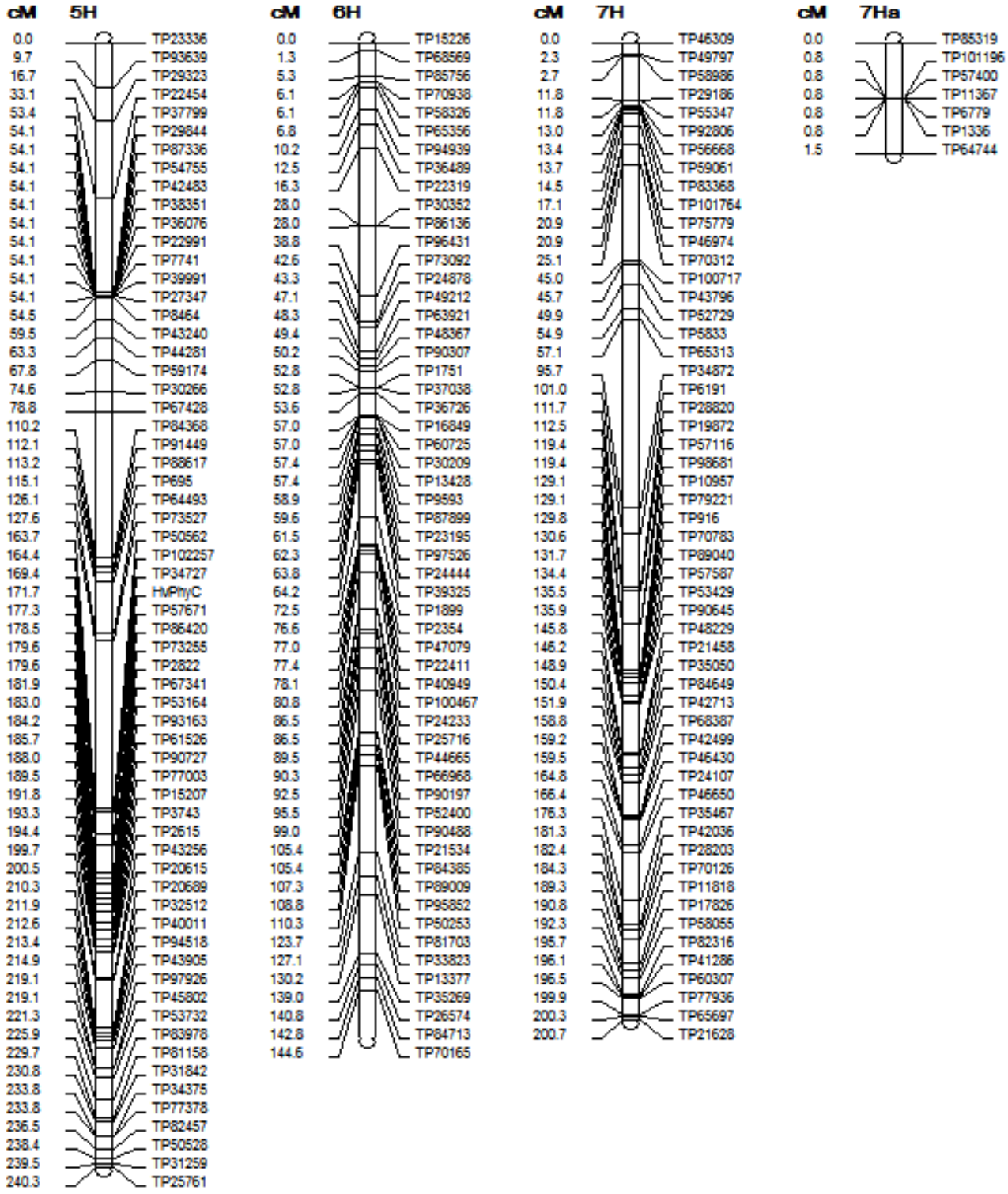


Fig.3.3 Genetic linkage map of Commander x WI4304 DH population

Fig. 3.3 (*continued*)



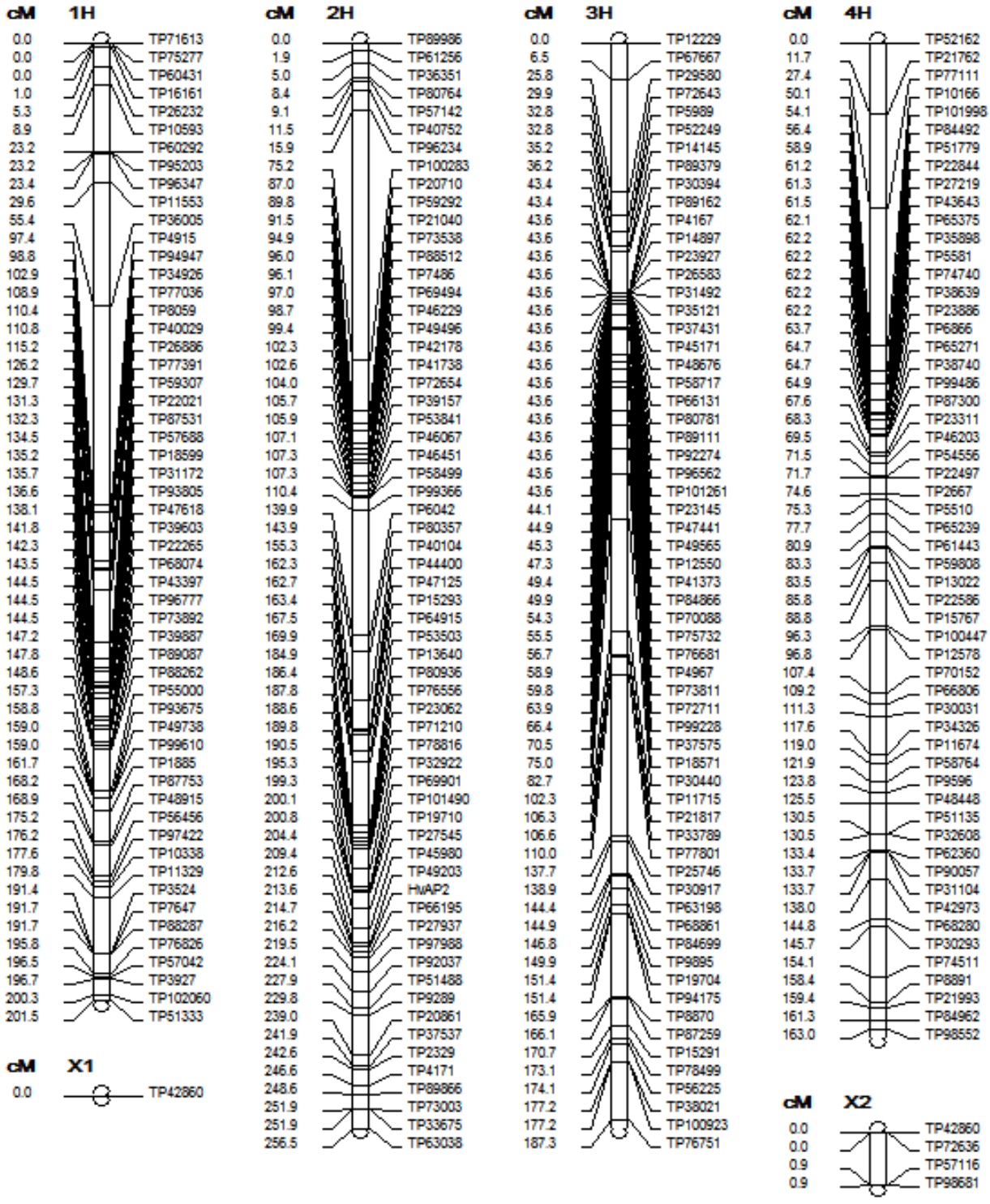


Fig.3.4 Genetic linkage map of Fleet x WI4304 DH population

Fig. 3.4 (*continued*)

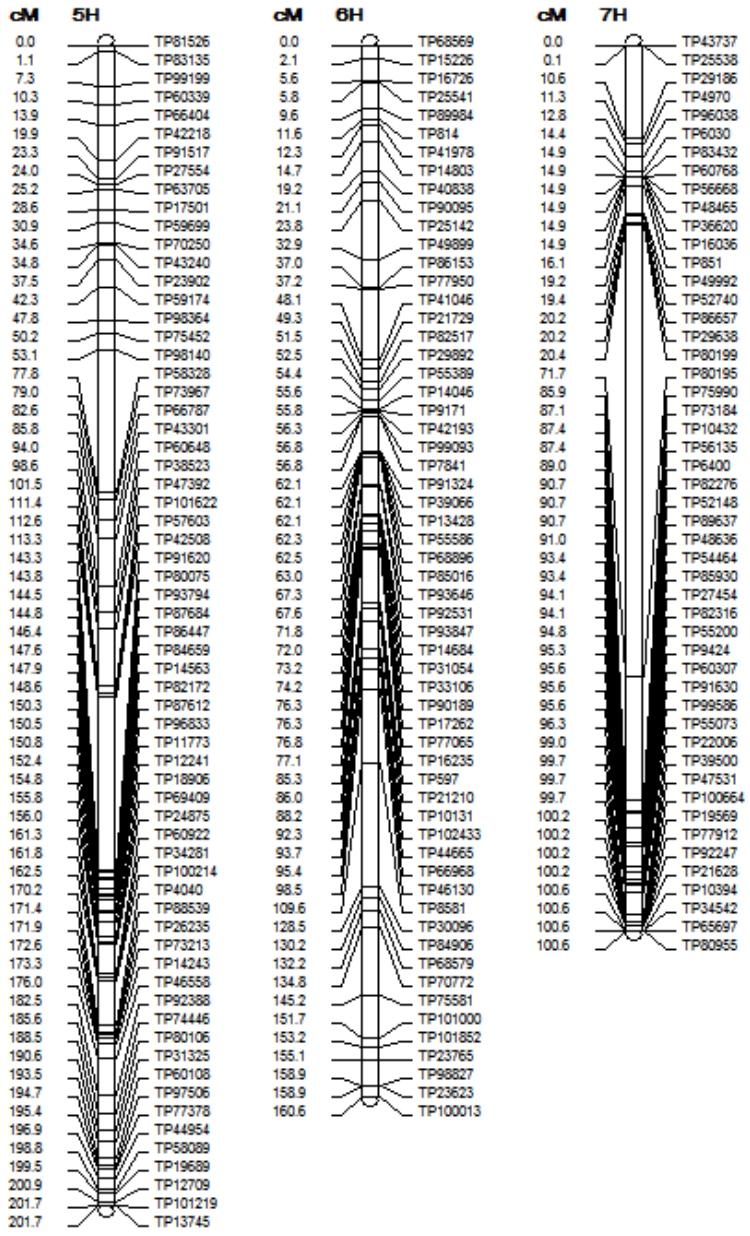


Table 3.4 Summary of marker genotype frequencies across all individuals in CF, CW and FW population

CF			CW			FW		
Marker genotype	Number*	%	Marker genotype	Number*	%	Marker genotype	Number*	%
AA	246238	0.50	AA	326725	0.49	AA	329534	0.49
BB	241283	0.48	BB	316210	0.48	BB	330723	0.49
Missing	11470	0.02	Missing	16441	0.03	Missing	13390	0.02
Total	**498991**	**1.00**	**Total**	**332651**	**1.00**	**Total**	**673647**	**1.00**

*=over all individuals and markers

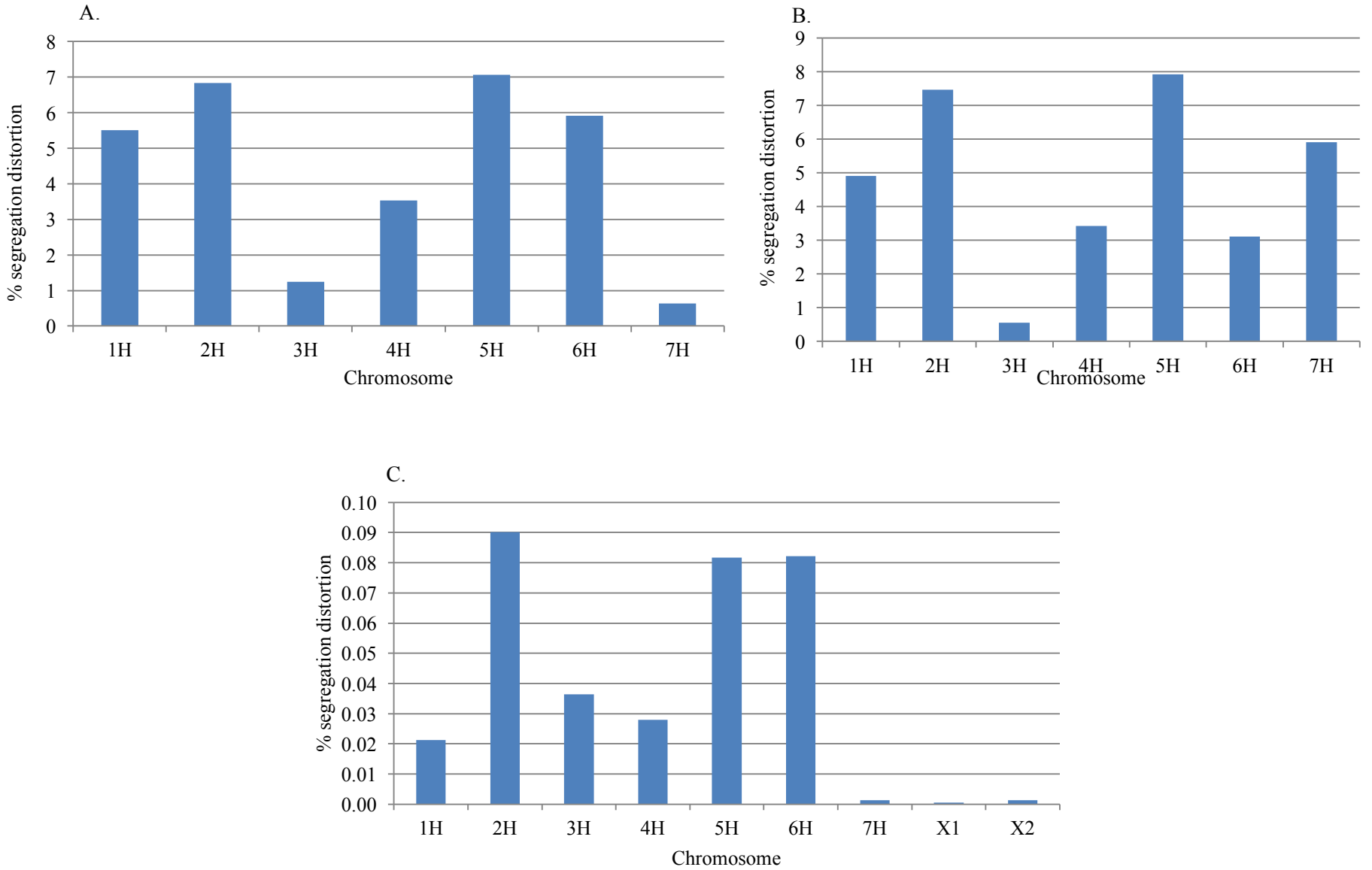


Fig.3.5 Percentage of markers with significant segregation distortion in each chromosome of CF (A), CW (B) and FW (C) populations

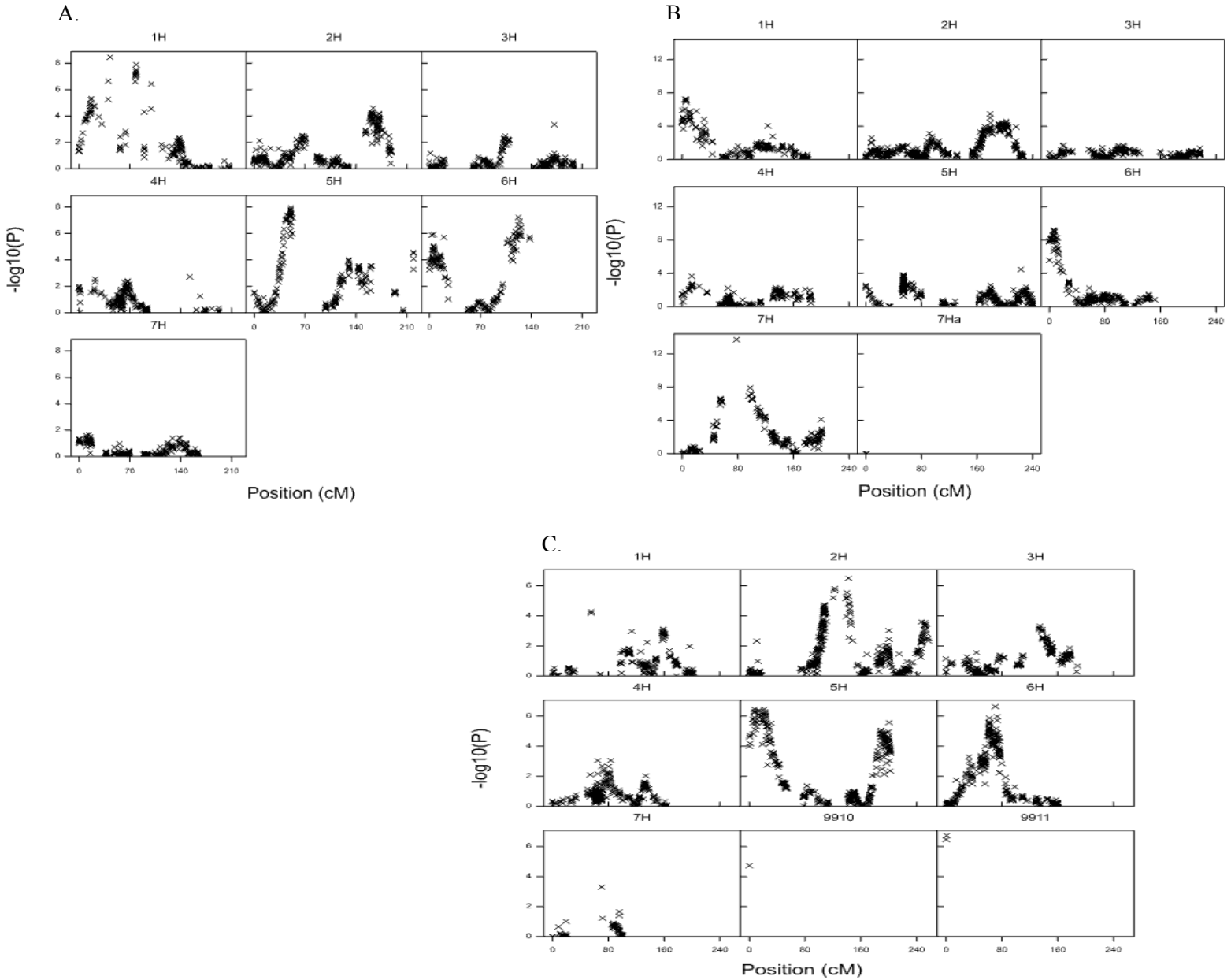


Fig.3.6 Regions of the genome showing significant segregation distortion in CF (A), CW (B) and FW (C) populations

3.5 Discussion

Previous genetic linkage maps of barley used in Australia were constructed using populations developed from adapted x non-adapted crosses with a large confounding effect of the genetic background. Such populations are not directly relevant to breeding programs aiming at the genetic dissection of complex traits such as yield and adaption. The three populations used for construction of the three genetic linkage maps reported here were developed from elite x elite crosses that were specifically targeted for dissecting the genetic control of yield and adaptation in the Mediterranean type environment of southern Australia. The marker order on the three genetic linkage maps is consistent with the order reported for the barley physical map (POPSEQ) (Mascher et al. 2013).

The relatively lower level of polymorphism obtained in CF (53.5%) population compared to the other two populations (CW and FW) was expected as the parents (Commander and Fleet) are related by descent both having Keel in their pedigrees. Given that these three populations were developed from crosses between lines from one breeding program, the level of polymorphism obtained (53.5% in CF, 70% in CW and 62.8% in FW) is considered high enough compared with previous reports. Graner, et al. (1991) reported a polymorphism of only 26% for the cross between the distantly related barley genotypes Igri and Franka. In wheat, Edwards (2012) reported polymorphisms of 13% and 25% respectively, for DArT and SSR markers in the Excalibur/Kukri mapping population.

The percentages of segregation distortion observed in the three populations were similar (30.8% in CF, 33.3% in CW and 34.3% in FW) and were below the percentage reported by Graner, et al. (1991). High segregation distortion percentages were associated to chromosomes 2H and 5H consistently in all of the three populations. Other chromosomes with high segregation distortion percentages were 1H, 6H and 7H, though these were not consistent across all populations. Conversely, chromosomes 3H and 4H have shown the lowest segregation distortion percentages consistently for all of the three populations. Segregation distortion from *in vitro* culture derived DH populations has been described as a common phenomenon (Graner, et al. 1991; Manninen 2000; Thompson et al. 1991) and is associated with differential response of the parental gametes to the *in vitro* culture environment. Markers were differently distorted between the reciprocal DH populations except the markers on chromosome 4H in CW, and on chromosomes 6H and 7H in the FW

populations. Segregation distortion is controlled by genetic factors and cytoplasmic effects are inferred when there are differences in segregation distortion between the reciprocal populations, while similar distortion in the reciprocal populations indicates the effect of nuclear genetic factors (Reflinur et al. 2014).

The large linkage distances observed in the genomes of all the three populations, especially on chromosomes 2H, 4H and 5H in CF; on 3H and 5H in CW, and on 2H and 7H in FW are mainly due to lack of polymorphism between the parents in these genomic regions. However, some of the markers that were discarded during the map construction steps due to high proportion of missing data (more than 20%) may belong to the regions where these large linkage distances were observed. The consensus genetic map constructed from the three individual maps increased the marker coverage and improved map resolution by reducing the distances between markers.

In conclusion, the CF, CW and FW genetic linkage maps are the first high density genetic maps developed from adapted x adapted crosses in Australia and will serve as a platform for genetic dissection of complex traits such as yield and adaptation in Australian barley breeding programs.

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Chapter 4: Genetic analysis of developmental and adaptive traits in three doubled haploid populations of barley (*Hordeum vulgare* L.) [Published Article]

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Genetic analysis of developmental and adaptive traits in three doubled haploid populations of barley (*Hordeum vulgare* L.)

Understanding the genetic mechanisms underlying crop adaptation to a particular environment would create a platform for designing sound breeding programs that enable development of adapted varieties. Precise phenotyping and high throughput genotyping are crucial for dissection of complex traits such as adaptation. Mapping populations developed from locally adapted elite germplasm enable identification of novel alleles controlling adaptation and other important traits through QTL analysis.

Maturity, early vigor, normalized difference vegetation index (NDVI) and leaf chlorophyll content (SPAD), leaf waxiness, and leaf rolling are important traits that affect barley adaptation in drought prone environments such as southern Australia. This paper presents genetic analysis of these traits based on field experiments conducted under six environments in Southern Australia, using three interconnected doubled haploid populations developed from Australian elite germplasm.

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Name of Principal Author (Candidate)	Bulti Tesso Obsa		
Contribution to the Paper	Collected and analysed phenotypic data, KASP genotyped the SNP, analysed the QTL and wrote the manuscript		
Overall percentage (%)			
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	May 25. 2016

Co-Author Contributions

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

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

Name of Co-Author	Jason Eglinton		
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ORIGINAL ARTICLE

Genetic analysis of developmental and adaptive traits in three doubled haploid populations of barley (*Hordeum vulgare* L.)

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Abstract

Key message **Study of three interconnected populations identified 13 maturity QTL of which eight collocate with phenology genes, and 18 QTL for traits associated with adaptation to drought-prone environments.**

Abstract QTL for maturity and other adaptive traits affecting barley adaptation were mapped in a drought-prone environment. Three interconnected doubled haploid (DH) populations were developed from inter-crossing three Australian elite genotypes (Commander, Fleet and WI4304). High-density genetic maps were constructed using genotyping by sequencing and single nucleotide polymorphisms (SNP) for major phenology genes controlling photoperiod response and vernalization requirement. Field trials were conducted on the three DH populations in six environments at three sites in southern Australia and over two cropping seasons. Phenotypic evaluations were done for maturity, early vigour, normalized difference vegetation index (NDVI) and leaf chlorophyll content (SPAD), leaf waxiness and leaf rolling. Thirteen maturity QTL were identified, all with significant QTL × environment interaction with one exception. Eighteen QTL were detected for other adaptive traits across the three populations, including three QTL for leaf rolling, six for leaf waxiness, three for early vigour, four for NDVI, and two QTL for SPAD. The three interlinked populations with high-density linkage maps described in this study are a significant resource for examining the genetic basis for barley adaptation in low-to-medium rainfall Mediterranean type environments.

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Introduction

Plant development is influenced by the combined effects of genotype, environment and their interaction. Phenological adjustment, mainly driven by temperature and photoperiod, plays crucial roles in synchronizing growth and reproductive cycles of crops with environmental variation over the growing season (Fowler et al. 2001).

Many barley phenology QTL have been mapped, some coincident with known photoperiod response, vernalization, and earliness per se loci (Laurie et al. 1995). The photoperiod response genes (*Ppd-H1* and *ppd-H2*) and the vernalization requirement genes (*Vrn-H1*, *Vrn-H2* and *Vrn-H3*) are important determinants of flowering time (Cockram et al. 2007). Flowering time models have been improved with the identification of candidate genes at these loci and the study of gene interactions in response to vernalization and photoperiod (Alqudah et al. 2014). Less well understood are the earliness per se loci, which influence barley phenology independently of vernalization and photoperiod.

Ppd-H1 confers early flowering under long days while its recessive allele *ppd-H1* conditions late flowering in barley (Laurie et al. 1994). *Ppd-H2* promotes flowering in winter

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barley cultivars that have not satisfied their vernalization requirement under both short and long days (Casao et al. 2011). The gene for *Ppd-H1* is a photoperiod response regulator (*PPR7*) and photoperiod responsiveness is associated with increased expression of the *FLOWERING LOCUS T* (*FT*) *HvFT1/Vrn-H3* (Turner et al. 2005). *FT* represents a family of "florigen" factors that induce or repress flowering in plants. This includes *TERMINAL FLOWER 1* identified in barley (Kikuchi et al. 2009). Also associated with circadian expression are the barley *CONSTANS* gene *HvCO1* which up regulates *HvFT1* (Griffiths et al. 2003; Deng et al. 2015), the *GIGANTEA* gene (Dunford et al. 2005) and two *ZCCT* genes (Trevaskis et al. 2006).

Vrn1 regulates vernalization-induced flowering in cereals, with *Vrn2* and *Vrn3* its downstream targets (Trevaskis et al. 2007; Deng et al. 2015). *Vrn1* is induced by vernalization and accelerates the transition from vegetative to reproductive development by enhancing the expression of *HvFT1/Vrn3* in long days, and down-regulating *Vrn2* that represses *HvFT1/Vrn3* (Trevaskis et al. 2006, 2007).

Earliness per se 2 (*EPS2*) is an important gene on chromosome 2H affecting flowering time independently of photoperiod and vernalization. This gene affects other agronomic traits including tiller biomass, tiller grain weight, ear grain number, and plant height (Laurie et al. 1994). The *CENTRORADIALIS* (*HvCEN*) gene which is the candidate gene for *EPS2* is an FT family member and regulates the winter versus spring growth habit of barley (Comadran et al. 2012).

APETALA2 (*HvAP2*) plays a role in determining the size and shape of barley inflorescence by regulating the duration of inflorescence internode elongation (Houston et al. 2013) and is responsible for cleistogamous flowering (Nair et al. 2010). Another family of flowering regulators is the red/far-red light phytochromes with the barley genes, *HvPhyA*, *HvPhyB* and *HvPhyC* described by Szucs et al. (2006).

In addition to phenology, other morpho-physiological traits including early vigour, leaf rolling, leaf waxiness and chlorophyll content are putative traits for adaptation to drought-prone environments. Early vigour ensures rapid early development of leaf area and aboveground biomass, thus reducing evaporation of water from the soil and contributes to improved yield through maximizing use of available environmental resources (ter Steege et al. 2005; Tiyagi et al. 2011). Leaf rolling is an adaptive response to drought through increased stomatal resistance in response to decreasing leaf water potential (O'Toole and Cruth 1980). Leaf rolling reduces leaf surface area, thus reducing exposure to solar radiation and minimizing water loss through transpiration (Clarke 1986). Moderate leaf rolling has been proposed to increase grain yield by maintaining photosynthetic activity (Zhang et al. 2009).

Epicuticular wax acts as a barrier for self-defence against external stresses and is a putative adaptive trait to drought tolerance (Zhou et al. 2013). Waxiness is genetically controlled and *eceriferum* (*cer*) mutants which influence β-diketone and hydroxy-β-diketone synthesis in spike and internode epicuticular waxes of barley have been characterized (von Wettstein 1972). *Waxy spike 1* (*wxs1*) located on chromosomes 7H and 2H are genes controlling spike waxiness in barley. High epicuticular wax accumulation, that is positively correlated with yield, has been reported under terminal water stress in barley (Febrero et al. 1998; González and Ayerbe 2009).

The senescence process of crop plants is essential for efficient nutrient remobilization during grain filling (Christiansen and Gregersen 2014). Maintenance of green colour until grain filling is called the stay green trait, and coupled with stress avoidance mechanisms, has been proposed as an important trait for improved grain plumpness and overall yield of cereals by prolonging photosynthesis (Thomas and Howarth 2000). Senescence is an adaptive strategy used by plants to respond to seasonal environmental cues such as changes in photoperiod (Thomas and Ougham 2015), and may be induced prematurely under drought, leading to reduced crop yield (Gregersen et al. 2013). Although stay green is linked to phenology genes, delaying flowering time, was reported to explain from 5.4 to 15.4 % of variation for senescence in barley but was independent of flowering time (Emebiri 2013).

Most previous genetic studies of barley in Australia have used mapping populations developed from Australian germplasm crossed with exotic varieties or land races with the main focus of improving malt quality, while also targeting disease resistance and tolerance to abiotic stresses as secondary traits (Langridge and Barr 2003). In the current study, three new doubled haploid (DH) populations developed from adapted × adapted Australian germplasm have been used. The main aim of the study was to understand the genetic basis for adaptation of elite barley germplasm under the Mediterranean type environment of Australia. The specific objectives were to map quantitative trait loci (QTL) associated with maturity and adaptive traits including early vigour, leaf rolling, leaf waxiness, and leaf chlorophyll content using multi-environment field trials and high-throughput genotyping platforms.

Materials and methods

Plant materials

Three F_1-derived DH populations of barley were developed from pair-wise reciprocal crosses among three genotypes, including two elite Australian varieties (Commander and Fleet) and an advanced breeding line (WI4304). Commander (Keel/Sloop//Galaxy) is a malting variety

Table 1 Description of trial environments

Trial	Location	Year	Latitude	Longitude	Seeding date	LTARF (mm)[a]	Annual RF (mm)	CSRF (mm)	CSRF as % of LTA
MRC12	Minnipa	2012	32.84°S	135.15°E	10.06.2012	280 [b](184)	237	151	82
MRC13	Minnipa	2013			20.05.2013		316	197	107
RAC12	Roseworthy	2012	34.54°S	138.74°E	27.06.2012	446 [c](298)	337	230	77
RAC13	Roseworthy	2013			14.06.2013		417	302	102
SWH12	Swan Hill	2012	35.18°S	143.37°E	13.06.2012	329 [d](188)	234	111	59
SWH13	Swan Hill	2013			28.05.2013		299	198	105

MRC Minnipa Research Centre (South Australia), *RAC* Roseworthy Agricultural Research Centre (South Australia), *SWH* Swan Hill testing site (Victoria), *RF* rainfall, *CSRF* cropping season rainfall (May–October), *LTARF* long-term average rainfall

[a] Values in parenthesis show the long-term average rainfall during the cropping season (May–October)

[b] Total of mean monthly rainfall at Minnipa from 1996 to 2014

[c] Total of mean monthly rainfall at Roseworthy from 1885 to 2014

[d] Total of mean monthly rainfall at Swan Hill from 1898 to 2014

representing an established benchmark for grain yield and grain size in medium rainfall environments of Australia (www.nvtonline.com.au). Fleet (Mundah/Keel//Barque) is a feed variety characterized by high water use efficiency, a long coleoptile, and adaptation to deep sandy soils. WI4304 (Riviera/(Puffin/Chebec)-50//Flagship) is a malting quality breeding line with high osmotic adjustment and high net photosynthesis under drought conditions (Le 2011). The parents were selected to have similar maturity to dissect the genetic basis of adaptation to the Australian environment with minimal confounding effect of maturity. The doubled haploid populations comprise 229 lines from Commander/Fleet (CF), 228 lines from Commander/WI4304 (CW), and 299 lines from Fleet/WI4304 (FW).

Field trials

Six trials were conducted at three field sites in southern Australia (Roseworthy, Minnipa and Swan Hill) for two seasons (2012 and 2013). The detailed description of the test environments is given in Table 1 and Fig. S1. Each field trial was an un-replicated design with gridded checks of the parents and other reference varieties every eight plots. The trials were managed according to the recommendations for barley production in the region except for slight variations in sowing dates. The weather data for the closest weather stations were obtained online from the Australian Bureau of Meteorology (http://www.bom.gov.au/climate/data/).

Soil samples were taken at three developmental stages (emergence, anthesis and physiological maturity) to determine moisture content using a soil coring auger. The samples were taken from three random locations in each trial field at 20–60 cm soil depth at Roseworthy, 80 cm at Swan Hill, and 120 cm at Minnipa. The samples from the same depth for each field were combined and the percent moisture content was determined using the gravimetric method (Black et al. 1965) and is given in Fig. S2. Soil physical and chemical analyses were conducted at the Soil and Plant Analysis Laboratory (CSBP, Western Australia), and the results are shown as Table S1.

Phenotyping

Maturity was assessed as decimal growth stage based on Zadoks scale (Zadoks et al. 1974) at all sites in both years. The Zadoks scale is a 0–99 standardized scale of cereal development divided into ten principal growth stages from germination to ripening. In 2013, early vigour and plant greenness were scored at Roseworthy, leaf waxiness at Minnipa, and leaf rolling at Swan Hill. Early vigour was scored using a 1–5 scale, where 5 is the most vigorous. Average chlorophyll content was measured on the flag leaves of three randomly selected plants per plot at the early milk stage of grain development (Zadoks' score 73) using a handheld SPAD-502 chlorophyll meter (Konica-Minolta, Tokyo, Japan). Normalized difference vegetation index (NDVI) was measured on plot basis at the early milk stage of grain development to assess the variation in stay green character using a digital GreenSeeker® Handheld Crop Sensor (Trimble Navigation Limited, USA). Leaf waxiness was scored visually at full heading stage (Zadoks' score 59) using a scale of 1–3, where a score of 3 represents the highest wax deposition on the leaf surface. Leaf rolling was scored visually at booting stage (Zadoks' score 49) using a scale of 1–5, 5 represents full leaf rolling based on the procedure of O'Toole and Cruth (1980).

Genotyping and linkage map construction

Genomic DNA was extracted from young leaves using the phenol/chloroform method (Rogowsky et al. 1991). DNA concentration and quality was checked using a Nanodrop

ND-1000 spectrophotometer (NanoDrop Technologies, Inc. Wilmington, USA) and standardized using PicoGreen (Ahn et al. 1996). The three populations were genotyped using genotyping by sequencing (GBS) to identify markers for genetic map construction. The GBS library was prepared using the protocols described in Elshire et al. (2011) and Poland et al. (2012). The DNA samples were digested using two restriction enzymes (PstI and MspI) for complexity reduction, barcoded and multiplexed. Each GBS library containing 96 DNA samples (96-plex) was run on a single lane of Illumina HiSeq2000 for sequencing. The GBS raw data were analysed using the Universal Network Enabled Analysis Kit (UNEAK) pipeline in TASSEL (Lu et al. 2013). Heterozygous markers and those with more than 20 % missing data were removed.

The populations were also genotyped for several phenology genes using polymerase chain reaction (PCR) and high resolution melting (HRM) (Table S2) and the KBioscience Competitive Allele-Specific Polymerase chain reaction (KASP) assay (Table S3). The KASP protocol used is available online from LGC genomics (http://www.lgcgroup.com/). The PCR protocols and programs used for genotyping the PCR and HRM-based genotyping of phenology genes are given in Table S2. The PCR products were analysed by gel electrophoresis using 2 % agarose and the resulting bands were scored. The phenology genes used include the photoperiod response gene (*Ppd-H1*), the vernalization sensitivity gene (*Vrn-H2*) and its related genes *HvZCCTHc*, *HvAP2*, *HvFT5*, *HvFT5_1_724*, *HvTFL1*, *HvCO2*, *HvCO1*, *HvGI*, *HvPhyB* and *HvPhyC* (Tables S2 and S3). *Ppd-H1*, *Vrn-H2*, *HvCO2* and *HvZCCTHc* were selected based on polymorphisms between the parents (Le 2011) while all the other genes were selected after initial screening for polymorphism using a subset of the DH lines and the parents. The markers for *Vrn-H1*, *HvFT2*, *HvFT3*, *HvFT4* and *HvCEN* were monomorphic in the populations (data not shown) and therefore were not mapped.

Genetic linkage maps were constructed using 2178, 2892, and 2252 GBS markers, respectively, in CF, CW and FW populations and the phenology genes listed above. The linkage maps include 229, 228 and 299 DH lines, respectively, in the CF, CW and FW populations. The marker genotype data were inspected for missing data, segregation distortion, duplicate markers and clonal individuals using the appropriate functions and settings in R/qtl (Broman 2010). The linkage maps were constructed using MSTmap for R (Taylor 2015). Map distances were calculated using the Kosambi mapping function (Kosambi 1944). The maps were manually curated to remove unexpected double crossovers before being used for QTL analysis. The marker sequences were aligned to the barley physical map databases (POPSEQ and IBSC 2012) (http://floresta.eead.csic.es/barleymap/) to assign the markers to the correct chromosomes and to align the chromosomes in the correct orientations.

Statistical analysis

Descriptive statistics, ANOVA and correlation analyses were performed on the Zadoks' score, early vigour, leaf rolling, leaf waxiness, SPAD and NDVI values using GenStat version 17 (VSN International Ltd, 2014). Generalized heritability was estimated based on the definition of (Cullis et al. 2006) as follows:

$$h^2g = 1 - \frac{\text{PEV}}{2\sigma^2 g},$$

where PEV is the predicted error variance and $\sigma^2 g$ is the genotypic variance.

The yield and maturity scores of the reciprocal crosses were compared using Z-test. As the test did not show significant differences between the groups (data not shown), the two groups were treated as one population for further phenotypic and genetic analyses.

QTL analysis

GenStat version 17 was used for QTL analysis of Zadoks' score, early vigour, leaf rolling, leaf waxiness, SPAD, and NDVI data. A genome-wide scan was performed using simple interval mapping (SIM) to detect candidate QTL positions, followed by composite interval mapping (CIM) with cofactors. Genetic predictors were estimated with a step size of 2 cM, and the minimum cofactor proximity and minimum distance to declare independent QTL were set to 30 and 20 cM, respectively. Repeated iterations of CIM were performed until no further change in the selected QTL was observed. The method of Li and Ji (2005) was used with genome-wide significance level of $\alpha = 0.05$ as a threshold to reject the null hypothesis of no QTL effect. This method is based on the effective number of independent tests proposed by Cheverud (2001), and was designed to control the experiment-wise significant level and the false discovery rate in multi-locus analyses as an alternative to the computationally intensive methods such as permutation test (Li and Ji 2005).

Results

Variation in plant development

Variation for maturity in the three DH populations and their parents (Commander, Fleet and WI4304) is given in Table 2. WI4304 was the earliest in maturity among the parents, though it was similar to Fleet at Roseworthy in

Table 2 Variability and heritability of Zadoks' score, early vigour, and chlorophyll content (SPAD), NDVI, leaf waxiness and leaf rolling in three populations

	Zadoks' score						Early vigour (RAC13)	SPAD (RAC13)	NDVI (RAC13)	Leaf waxiness (MRC13)	Leaf rolling (SWH13)
	MRC12	RAC12	SWH12	MRC13	RAC13	SWH13					
Commander (C)	42[b]	56[b]	47[b]	56[b]	51[c]	48[b]	3.33	23.65[a]	0.15[a]	2.5[b]	1.5[b]
Fleet (F)	42[b]	57[a]	49[a]	57[b]	54[b]	50[a]	3.26	24.18[a]	0.12[c]	3.0[a]	3.0[a]
WI4304 (W)	46[a]	57[a]	49[a]	59[a]	57[a]	51[a]	3.43	15.81[b]	0.12[b]	2.0[c]	2.5[a]
F probability	<0.001	0.013	<0.001	<0.001	<0.001	<0.001	0.47 ns	0.02	<0.001	<0.001	<0.001
CF mean	42	57	48	56	52	49	3.10	22.78	0.14	2.5	2.5
CF minimum	39	51	41	49	47	44	1.00	6.83	0.09	1.0	1.0
CF maximum	49	59	51	59	57	55	4.50	35.27	0.21	3.0	5.0
SD	2.27	1.38	2.19	2.20	2.07	2.16	0.84	5.18	0.03	0.58	0.98
Heritability	0.54	0.52	0.73	0.82	0.62	0.77	0.32	0.57	0.46	0.89	0.43
CW mean	42	57	46	56	52	48	3.30	23.59	0.14	2.2	3.2
CW minimum	37	49	39	49	47	43	2.00	11.30	0.09	1.5	1.0
CW maximum	49	59	51	59	57	53	5.00	35.90	0.20	3.0	5.0
SD	2.30	1.57	2.56	2.22	2.02	1.70	0.12	4.49	0.02	0.37	1.1
Heritability	0.68	0.93	0.77	0.79	0.73	0.67	0.64	0.48	0.57	0.90	0.45
FW mean	43	56	47	57	53	49	3.12	30.29	0.13	2.3	2.8
FW minimum	37	53	39	51	49	43	1.50	14.53	0.09	1.5	1.0
FW maximum	49	59	51	65	61	57	5.00	45.03	0.21	3.0	5.0
SD	3.46	1.50	2.71	3.22	2.14	2.77	0.63	5.13	0.02	0.4	0.84
Heritability	0.66	0.85	0.92	0.78	0.65	0.99	0.60	0.85	0.61	0.94	0.80

The superscript letters correspond to significant differences at $P < 0.05$ using LSD multiple comparison test

MRC12 = Minnipa 2012, RAC12 = Roseworthy 2012, SWH12 = Swan Hill 2012, MRC13 = Minnipa 2013, RAC = Roseworthy 2013, SWH13 = Swan Hill 2013, SD = standard deviation

2012 and at Swan Hill in both years (Table 2; Fig. 1). Commander and Fleet were not significantly different from each other at Minnipa in either year. All the three genotypes differed significantly from each other ($P < 0.001$) in 2013 at Roseworthy.

The three DH populations showed significant transgressive segregation for maturity. The narrowest range of six and the widest range of 14 decimal scales in Zadoks' score were observed in FW population in RAC12 and MRC13, respectively (Table 2; Fig. S3, S4, S5). The heritability values for maturity ranged from moderate (0.52) to very high (0.99) (Table 2). The maturity scores showed significant positive correlations across trials for each population (Table S5).

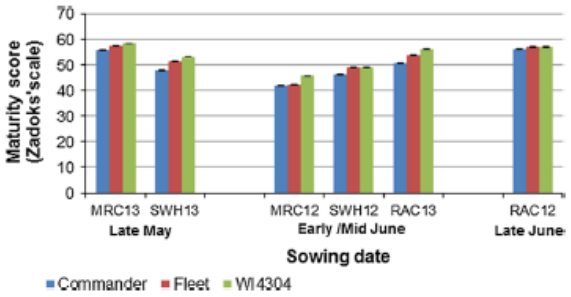

Fig. 1 Variation in maturity scores between the parents at different sowing dates

Variation in early vigour, leaf waxiness, leaf rolling, SPAD and NDVI

Analysis of variance showed significant differences among the parents of the DH populations for leaf waxiness, leaf rolling, NDVI, and SPAD. The parents were not significantly different for early vigour (Table 2). Fleet showed the highest epicuticular wax deposition and WI4304 the least. Fleet had the highest leaf rolling score followed by WI4304, while Commander had the lowest leaf rolling score and the highest NDVI value. WI4304 had significantly lower chlorophyll content than either Commander or Fleet (Table 2). The FW population was relatively more variable for SPAD with a range of 30.5 units, followed by the CF population with a range of 28.4 units and the least

Table 3 QTL detected for maturity in the CF, CW, and FW populations grown in multi-environment trials

QTL	Significant marker[b]	Chromosome	Position (cM)	LOD	PVE (%)	QTL × environment additive effects[a]					
						MRC12	MRC13	RAC12	RAC13	SWH12	SWH13
QMat.CF-2H.1	TP9969	2H	120.29	3.8	2.5–3.7	–	0.42^{F}	–	–	0.35^{C}	–
QMat.CF-2H.2	TP52375	2H	168.53	3.9	4.2–6.5	–	0.45^{F}	–	0.44^{F}	0.56^{F}	0.56^{F}
QMat.CF-4H	TP40082	4H	73.88	4.2	5.8	–	–	–	–	–	0.52^{C}
QMat.CF-5H.1[c]	TP99523	5H	52.78	5.8	3.4–8.7	0.42^{F}	0.42^{F}	0.42^{F}	0.42^{F}	0.42^{F}	0.42^{F}
QMat.CF-5H.2	TP61282	5H	153.65	8.9	3.6–7.7	0.59^{F}	0.42^{C}	0.39^{C}	–	–	–
QMat.CW-4H	TP89118	4H	68.00	6.9	1.9–11.3	–	0.74^{W}	0.34^{W}	–	0.36^{W}	–
QMat.CW-5H	TP59199	5H	170.10	4.6	3.1–6.1		0.55^{C}	0.28^{C}	–	–	–
QMat.CW-6H	TP47818	6H	61.90	6.3	1.9–10.9	0.77^{W}	–	0.26^{W}	0.52^{W}	0.66^{W}	0.23^{W}
QMat.CW-7H	TP81322-HvCO1	7H	70.53	4.3	2.9–13.9	0.61^{W}	0.82^{W}	0.27^{W}	–	–	–
QMat.FW-1H	TP85889	1H	145.90	3.7	3.1–4.6	0.74^{W}	0.56^{W}	–	–	–	0.39^{W}
QMat.FW-2H	TP6364-TP89065	2H	205.85	7.0	1.4–6.5	–	0.80^{F}	–	0.39^{F}	0.46^{F}	0.33^{F}
QMat.FW-3H	TP34075	3H	177.00	5.0	2.9–9.3	0.61^{F}	0.71^{F}	–	0.45^{F}	0.56^{F}	0.82^{F}
QMat.FW-4H	TP69415	4H	100.2.	7.2	2.9–9.3	0.59^{W}	0.79^{W}	0.22^{W}	0.56^{W}	0.84^{W}	0.71^{W}

"–" under the QTL × environment additive effects columns indicates that the QTL is not significant in that environment

[a] The superscript letters C, F and W represent the source of high value allele for that particular QTL (C = Commander, F = Fleet and W = WI4304)

[b] The closest markers from both sides are given when the QTL falls within an interval between two known markers, PVE = percent of variance explained by the QTL

[c] This QTL showed no QTL × environment interaction

variable was the CW population with a range of 24.6 units. The range for NDVI values in the DH populations is similar for CF and FW (Table 2). All the three populations showed wide variations for early vigour, leaf waxiness and leaf rolling suggesting transgressive segregation (Fig. S6, S7, S8). The broad sense heritability values ranged from low (0.32) for early vigour in the CF population to high (0.94) for leaf waxiness in the FW population (Table 2).

Genetic analysis

Thirteen QTL were detected for maturity across the three DH populations and were distributed on all of the seven chromosomes (Table 3). Five QTL were detected in the CF population, and four in both the CW and FW populations. The proportion of variation explained by each of these QTL ranged from 2.5 to 8.7 % in CF, from 1.9 to 13.9 % in CW, and from 1.3 to 9.3 % in FW population (Table 3).

A QTL in the CF population on chromosome 5H (*QMat.CF-5H.1*), with the high value allele contributed by Fleet was detected in all of the six environments and did not show QTL × environment interaction. Another QTL in the FW population on chromosome 4H (*QMat.FW-4H*), with the high value allele contributed by WI4304, was also detected in all of the six environments though its effects were not of the same magnitude providing evidence of QTL × environment interaction. All the other QTL were detected in one to five environments (Table 3). Of the 13 maturity QTL detected in this study, *QMat.CF-2H.2*, *QMat.CF-5H.1*, *QMat.CF-5H.2*, *QMat.CW-4H*, *QMat.CW-5H*, *QMat.CW-7H* and *QMat.FW-2H* were co-located with known phenology genes *HvAP2*, *HvTFL1*, *HvPhyC*, *HvPhyB*, *HvPhyC*, *HvCO1*, and *HvAP2*, respectively (Fig. 2) and explained up to 31.3 % of the variance (Table 3). *QMat.FW-2H* is only 2.75 cM away from the *HvAP2* locus in the FW population.

A total of 18 significant QTL were detected for the other developmental and adaptive traits across the three populations. Three QTL were detected for early vigour at Roseworthy, *QEv.CW-2H* in the CW population on 2H, and QTL *QEv.FW-2H.1* and *QEv.FW-2H.2* in FW, both on chromosome 2H. These QTL explained from 5.1 to 7.8 % of the total phenotypic variation for early vigour (Table 4). Three QTL were detected for leaf rolling at Swan Hill, one QTL (*QLrol.CF-2H*) in CF population on chromosome 2H and two QTL (*QLrol.FW-2H* and *QLrol.FW-3H*) in FW population on chromosomes 2H and 3H, respectively. These QTL explained from 7.7 to 8.4 % of the total phenotypic variation for leaf rolling (Table 4). Alleles for *QLrol.CF-2H* and *QLrol.FW-3H* were contributed from Fleet while WI4304 contributed allele for *QLrol.FW-2H*. A leaf rolling QTL, *QLrol.CF-2H* is co-located with maturity QTL on chromosome 2H at a position 120.3 cM, *QMat.CF-2H.1*, in CF population (Fig. 1).

A total of six QTL were detected for leaf waxiness at Minnipa, including two QTL (*QLwax.CF-1H* and *QLwax.CF-2H*) in the CF population on chromosomes 1H and

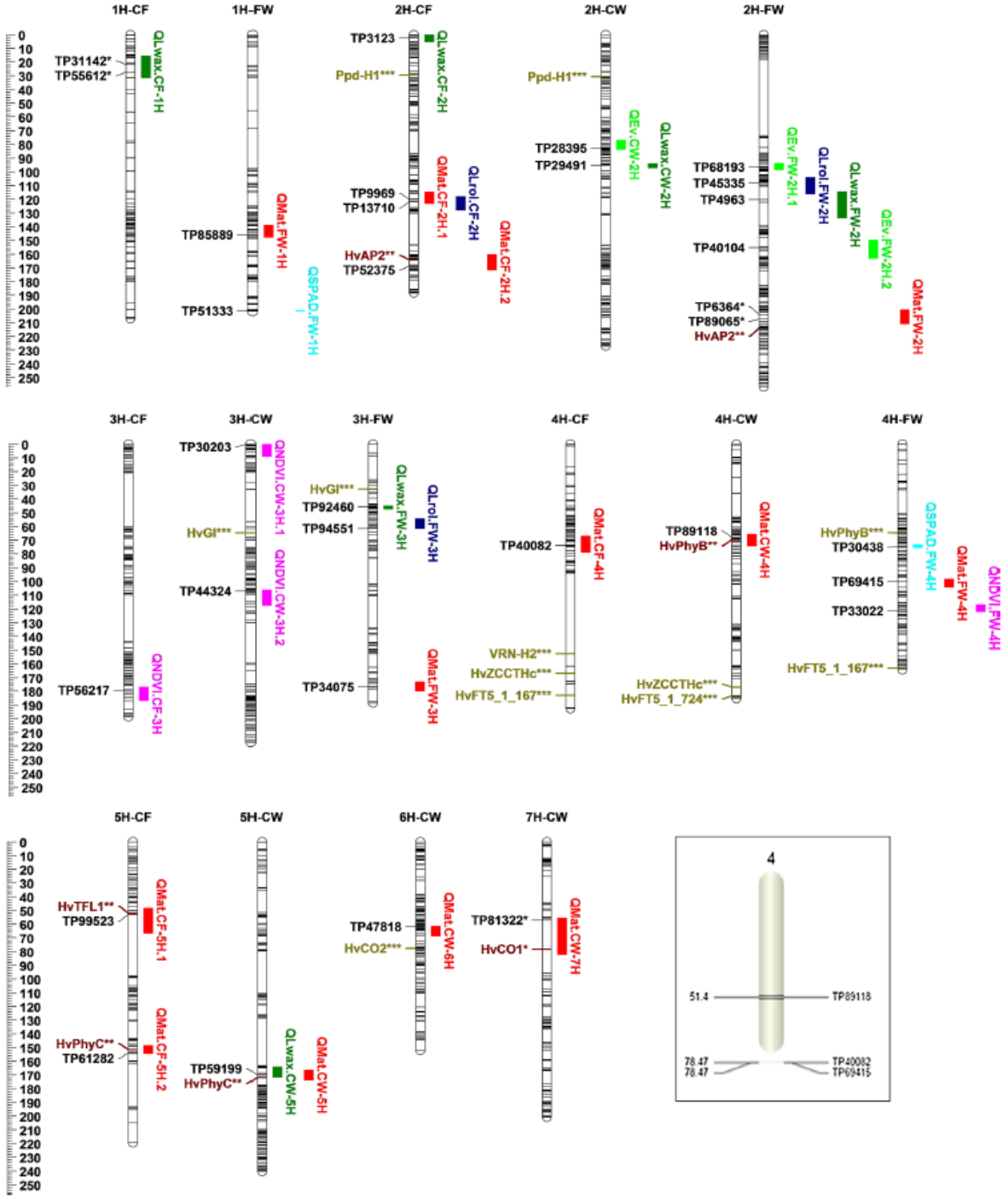

Fig. 2 QTL positions for maturity and other developmental traits in the CF, CW and FW DH populations. The vertical QTL bars represent the 1.5-LOD QTL interval. Only significant markers at the QTL peak are shown on the maps. *Asterisk* indicates the closest markers from both sides of the QTL peak are given when the QTL peak falls in the interval between known markers. *Double asterisk* indicates known phenology genes that are co-located with the QTL, *triple asterisk* indicates phenology genes that are outside of the QTL range. The *small figures* shown below the 4H QTL charts show the alignment of the maturity QTL *QMat.CF-4H* (marker TP40082) and *QMat.FW-4H* (marker TP69415) in CF and FW, respectively, based on POPSEQ barley physical maps. The two markers are exactly at the same position (78.47 cM), hence, *QMat.CF-4H* and *QMat.FW-4H* are the same

Table 4 QTL detected for early vigour, leaf rolling, leaf waxiness, NDVI and SPAD

QTL	Significant marker	Chromosome	Position (cM)	LOD	PVE (%)	Additive effect	HVA	Environment
QEv.CW-2H	TP28395	2H	82.7	4.5	7.8	1.05	Commander	RAC13
QEv.FW-2H.1	TP68193	2H	96.6	5.1	7.1	1.11	Fleet	RAC13
QEv.FW-2H.2	TP40104	2H	155.3	3.8	5.1	1.09	WI4304	RAC13
QLrol.CF-2H	TP13710	2H	121.8	4.1	7.7	1.08	Fleet	SWH13
QLrol.FW-2H	TP45335	2H	108.3	5.5	7.7	1.70	WI4304	SWH13
QLrol.FW-3H	TP94551	3H	61.5	6.2	8.4	1.74	Fleet	SWH13
QLwax.CF-1H[a]	TP31142-TP55612	1H	23.5	3.8	6.6	1.41	Fleet	MRC13
QLwax.CF-2H	TP3123	2H	2.64	3.7	5.7	1.38	Commander	MRC13
QLwax.CW-2H	TP29491	2H	95.2	11.4	20.0	1.07	Commander	MRC13
QLwax.CW-5H	TP59199	5H	170.1	3.7	5.3	1.05	WI4304	MRC13
QLwax.FW-2H	TP4963	2H	120.3	8.1	12.3	1.41	Fleet	MRC13
QLwax.FW-3H	TP92460	3H	45.8	6.6	8.4	1.05	Fleet	MRC13
QNDVI.CF-3H	TP56217	3H	179.3	3.9	7.0	0.02	Commander	RAC13
QNDVI.CW-3H.1	TP30203	3H	0.0	5.0	8.4	0.02	Commander	RAC13
QNDVI.CW-3H.2	TP44324	3H	107.1	3.7	6.0	0.02	Commander	RAC13
QNDVI.FW-4H	TP33022	4H	121.6	7.5	11.1	0.02	Fleet	RAC13
QSPAD.FW-1H	TP51333	1H	201.5	3.8	4.9	1.04	WI4304	RAC13
QSPAD.FW-4H	TP30438	4H	74.9	5.2	7.2	1.05	Fleet	RAC13

PVE percent of variance explained by the QTL, *HVA* high value allele

[a] The left and the right closest markers are given when the QTL falls within an interval between two markers

2H, respectively; two QTL in the CW population (*QLwax.CW-2H* and *QLwax.CW-5H*) on 2H and 5H, respectively, and two QTL in the FW population (*QLwax.FW-2H* and *QLwax.FW-3H*) on 2H and 3H, respectively (Table 4). These QTL explained from 5.3 to 20.0 % of the total phenotypic variation for leaf waxiness (Table 4). The leaf waxiness QTL, *QLwax.CW-5H*, is co-located with a maturity QTL, *QMat.CW-5H*, on chromosome 5H at a position 170.1 cM in the CW population.

Four QTL were detected for NDVI at physiological maturity, including one QTL in the CF population on 3H (*QNDVI.CF-3H*), two QTL in CW (*QNDVI.CW-3H.1* and *QNDVI.CW-3H.2*) both on 3H, and one QTL in the FW population (*QNDVI.FW-4H*) on 4H. These QTL explained from 6.0 to 11.1 % of the total phenotypic variation for NDVI (Table 4). Two QTL, *QSPAD.FW-1H* and *QSPAD.FW-4H*, were detected for chlorophyll content in the FW population on 1H and 4H, respectively, and explained from 4.9 to 7.2 % of the phenotypic variation (Table 4).

Discussion

QTL for adaptive traits to drought-prone environments

The field trials were conducted under different environmental conditions due to different sowing dates (Table 1), rainfall and temperature patterns (Table 1; Fig. S1), photoperiod, and differences in soil physio-chemical properties (Table S1). In the sites where this study was conducted, the temperature usually decreases continuously from sowing to late winter, after which it increases progressively from early spring through to hot and long day summers. Photoperiod shortens until 21st June and then increases. The rainfall received during the cropping season (May–October) in 2012 was only 82, 77, and 59 % of the long-term average rainfall at Minnipa, Roseworthy and Swan Hill, respectively. In 2013, the rainfall in the same months was 7, 2 and 5 % more than the long-term average at Minnipa, Roseworthy and Swan Hill, respectively (Table 1). This rainfall pattern translated into lower soil moisture in Swan Hill in 2012 and 2013 than the other trials (Fig. S2). Consequently, the genotypes were exposed to different temperature, photoperiod, and rainfall distribution during their development due to the differences in the sowing dates (Table 1).

In drought-prone environments, early vigour enables early resource acquisition (Maydup et al. 2012; Tiyagi et al. 2011) and reduce evapotranspiration of water from the soil surface (Kosová et al. 2014), leaving more water available for the crop. In the current study, the three parents had similar early vigour while the populations showed transgressive segregation. The identification of QTL for this trait in Australian elite barley germplasm is an important step

towards improving the trait through molecular breeding. The two early vigour QTL identified in CW (*QEv.CW-2H*) and FW (*QEv-FW-2H.1*) seem to be the related. As no QTL was detected for early vigour in the CF population around this region, both Commander and Fleet might carry the same allele. In previous studies, an early vigour QTL on 2H, explaining 8.5 % of the variation was reported in a DH population derived from a cross Henni × Meltan (Borràs-Gelonch et al. 2010).

The variation in NDVI values reflects the relative differences in the degree of senescence at the whole plot level while the SPAD values are only for the flag leaves. Probably because of this difference, we did not find any colocation between QTL for SPAD and NDVI. In previous studies in barley, QTL for flag leaf chlorophyll content have been reported on chromosomes 2H, 3H and 6H (Xue et al. 2008), and on 2H and 7H (Liu et al. 2015). The QTL *QSPAD.FW-1H*, *QSPAD.FW-4H* and *QNDVI.FW-4H* described here on chromosomes 1H and 4H are therefore new loci. In the absence of common markers between Xue et al. (2008) genetic map and ours, we cannot verify if the QTL they found on 3H is the same than *QNDVI.CW-3H.1*.

Leaf rolling, caused by abiotic factors such as water deficit, high air temperature and intense sunlight (Kadioglu and Terrzi 2007) can be beneficial to plants by reducing transpiration rates through the creation of a favourable microclimate (O'Toole and Cruth 1980). In this study, the leaf rolling trait was measured in the deep sandy soils of Swan Hill. This soil type permits fast water percolation beyond the root zone, and is assumed to be the cause for the observed leaf rolling at Swan Hill during the unevenly distributed few rainy days in September 2013 (Fig. S1C). Two of the three QTL detected for leaf rolling (*QLrol.CF-2H* and *QLrol.FW-2H*) were located on 2H, the former being co-located with the maturity QTL (*QMat.CF-2H.1*). However, *QMat.CF-2H.1* was detected in different environments to the leaf rolling QTL and may not be related (Table 4; Fig. 2). To our knowledge, this is the first report of QTL for leaf rolling per se in barley.

Leaf waxiness was measured at Minnipa in 2013 where the rainfall was relatively high (Table 1; Fig. S1). In previous studies, epicuticular wax has been reported to have positive correlation with grain yield under stress conditions in barley (González and Ayerbe 2009). Similarly, a relationship between leaf waxiness and drought tolerance has been proposed (Febrero et al. 1998). In wheat, glaucousness is controlled by *W1* and *W2* genes and their corresponding inhibitors of wax 1 and 2 (*Iw1* and *Iw2*) which are located on chromosome arms 2BS and 2DS, respectively (Tsunewaki and Ebana 1999). The QTL *QLwax.CF-2H* found in this study on chromosome 2H may be the barley ortholog of the wheat *Iw1* locus which was fine-mapped to three candidate genes (Adamski et al. 2013). Similarly, the QTL *QLwax.FW-3H* identified on chromosome 3H could be orthologous to the wheat glaucousness QTL identified on chromosome 3A in Australian wheat germplasm (Bennett et al. 2012). However, further investigation is required to establish the syntenic relationship between the barley and wheat leaf waxiness QTL/genes. Moreover, further trials and measurements will be needed to establish the link between leaf waxiness and drought tolerance in Australian barley germplasm. It is also possible that the level and composition of the waxes changes in response to different environmental signals and this could be elucidated by further study under different environmental conditions.

Maturity QTL

QTL mapping detected 13 loci significantly associated with variation in maturity across the three populations. Most of these QTL have low additive effects and show QTL × environment interaction, except a QTL on 5H (*QMat.CF-5H.1*). While the QTL × environment interaction could be due to differences in the seeding dates at the different environments (Table 1), the specific environmental variables that trigger the expression of each QTL need further investigation under controlled environments.

Eight of the maturity QTL co-locate with known phenology genes as found in other studies (Wang et al. 2010; Alqudah et al. 2014). *QMat.CF-2H.2* and *QMat.FW-2H* co-locate with the phenology gene *HvAP2* (Houston et al. 2013; Nair et al. 2010) (Fig. 2), which is a candidate for *Flt-2L*. *Flt-2L* has been reported to have an effect on flowering time, spike density and plant height in the Amagi Nijo × WI2585 (Chen et al. 2009).

QMat.CW-4H is co-located with the *HvPhyB* gene (Fig. 2) that plays a role in mediating photoperiodic induction of flowering (Hanumappa et al. 1999). Alqudah et al. (2014) identified two QTL at the same genomic location, one for tipping and the other for heading date. *QMat.CF-4H* and *QMat.FW-4H* are likely to represent the same QTL, with the corresponding linked markers TP40082 and TP69415 mapped at exactly the same position (78.5 cM) on the barley POPSEQ physical map (Fig. 2).

QMat.CF-5H.1 is the only maturity QTL detected in this study that did not show QTL × environment interaction and had the same additive effects at all of the six environments (Table 3). This QTL is co-located with, and could be the direct effect of, *HvTFL1* that plays a role in regulating flowering time and in maintaining the fate of the inflorescence meristem (Mimida et al. 2001). This suggests that *HvTFL1* controls maturity independently of the environmental conditions, which will make it a useful target for modifying phenology consistently across Australian regions. Six different QTL controlling various pre-anthesis stages (awn primordia, tipping or awn emergence, heading,

anther extrusion, awn primordia to tipping, and tipping to heading) of barley have been reported in this genomic region (Alqudah et al. 2014). Other studies have also reported maturity QTL around this position (Laurie et al. 1995; Marquez-Cedillo et al. 2001; Pillen et al. 2003).

QMat.CF-5H.2 in the CF and *QMat.CW-5H* in the CW populations (Fig. 2) could be the same QTL as both co-locate with *HvPHYC*, a candidate gene for the early maturity 5 (*eam5*) locus that interacts with *Ppd-H1* to accelerate flowering under non-inductive short days (Pankin et al. 2014). *QMat.CF-5H.2* and *QMat.CW-5H* could be similar to the five QTL controlling different pre-anthesis development stages of barley reported in Alqudah et al. (2014) as all are co-located with *HvPhyC*.

QMat.CW-6H was detected in five of the six environments and is coincident with *HvCO2*. Alqudah et al. (2014) detected three QTL at this position that were expressed between awn primordia formation and anther extrusion. Boyd et al. (2003) also reported QTL for anthesis date under different day lengths in Australian mapping populations. The maturity QTL *QMat.CW-7H* is co-located with *HvCO1*, whose over-expression accelerates flowering by up-regulation of *HvFT1* under long-day conditions (Campoli et al. 2012). QTL for heading date around the approximate location of *QMat.CW-7H* have also been reported in other populations (Bezant et al. 1996; Long et al. 2003).

QMat.FW-3H seems to be related to the maturity genes *eps3I/eam10*, which co-located with the *HvLUX* gene at the proximal end of chromosome 3H. *Eam10* causes circadian defects and interacts with *Ppd-H1* to accelerate flowering under long and short days. Maturity QTL in this region has also been reported in other populations including Alexis × Sloop, Halcyon × Sloop, Tallon × Kaputar, and Arapiles × Franklin (Boyd et al. 2003).

The effect of major developmental genes were studied in a population derived from a wide cross between the elite spring barley (Scarlet) and a wild barley (Wang et al. 2010) and in a world-wide spring barley collections comprising photoperiod-sensitive and reduced photoperiod sensitivity accessions (Alqudah et al. 2014). In both approaches the developmental genes played a major role in developmental variation. Since the populations studied here were derived from locally adapted elite × elite Australian germplasm, confounding effects of the major genes are expected to have been minimized enabling us to identify variation for adaptation to local environment. Some genes that showed polymorphism between our parental lines were mapped but did not show any overlap with maturity QTL: *Ppd-H1*, *HvGI*, *Vrn-H2*, *HvZCCTHc* and *HvFT5*. Most other studies using Australian mapping populations have found that the *Ppd-H1* and *eps2* loci have the largest effect on phenology and agronomic performance in barley (Boyd et al. 2003; Coventry et al. 2003). Interestingly, we failed to find any significant QTL associated with *Ppd-H1* on chromosome 2H. This could explain the narrow range of maturities between the adapted parental germplasm in our populations. This also means that the QTL we identified are relevant to crop improvement through fine-tuning flowering time for specific target environments.

Interestingly we found five maturity QTL (*QMat.FW-1H*, *QMat.CF-2H.1*, *QMat.FW-3H*, *QMat.CF-4H*, and *QMat.FW-4H*)—that do not match known phenology genes, suggesting that there are still genes controlling maturity to be discovered in barley. In FW population these QTL explained most of the maturity variance while the known phenology genes explained only 0–6.5 % of the variance. *QMat-FW-1H* and *QMat.CF-2H.1* could be similar with the QTL that mapped to the same approximate regions for different stages of pre-anthesis development phases in a set of world-wide spring barley collections (Alqudah et al. 2014). *QMat.CF-2H.1* may also be similar with the QTL reported for early flowering reported in the Alexis × Sloop population at the marker Xabg14 (Barr et al. 2003). The remaining three maturity QTL that did not match known phenology genes (*QMat.FW-3H*, *QMat.CF-4H* and *QMat-FW-4H*) could be novel. This suggests that new flowering genes could be discovered in barley by positional cloning the five maturity QTL described here using the large populations of 7000 recombinant inbred lines already available from the crosses C × F, C × W and F × W.

Conclusions

The three interlinked populations with high-density linkage maps described in this study are a significant resource for examining the genetic basis for adaptation in low-to-medium rainfall Mediterranean type environments. The parental lines exhibit a relatively narrow range of phenology and plant architecture typical of elite Australian varieties. The major developmental genes *Ppd-H1*, *Vrn-H1* and *Vrn-H2* that delineate macro-scale germplasm pools are not associated with maturity variation in these populations. However, the populations do exhibit transgressive segregation for maturity within a range appropriate to examine and define the molecular basis for elite regional adaptation. The QTL for relative maturity were coincident with known developmental genes which provides an opportunity to identify sequence variants relevant to routine breeding and to define allele combinations which form the basis of a molecular ideotype for regional adaptation. Each population was developed from reciprocal crosses, and while the direction of the cross was not significantly associated with variation for any traits in the current study, this does provide a platform for testing maternal effects. Physiological traits such as early vigour, leaf chlorophyll content,

leaf rolling and epicuticular wax have long been proposed as target traits to improve adaptation, particularly in lower rainfall environments. The populations derived from Commander, Fleet and WI4304 exhibit variation for these traits generally independent of plant architecture and phenology, and their association with adaptation can be formally tested in elite germplasm.

Author contribution statement BTO collected and analysed data in field, KASP genotyped the SNP, analysed the QTL and wrote the manuscript with input from JE, SC, PL and DF. SC and JE generated the populations, designed and conducted the field trials. TM generated and analysed the genotyping-by-sequencing data. DF, JE and PL conceived the project. DF supervised BTO and the overall experiments. All authors reviewed and approved this submission.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Chapter 5: Mapping of yield and grain plumpness QTL in three doubled haploid populations of barley (*Hordeum vulgare* L.)

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Contribution to the Paper	Collected and analysed phenotypic data, KASP genotyped the SNP, analysed the QTL and wrote the manuscript		
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By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
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Mapping of yield and grain plumpness QTL in three doubled haploid populations of barley (*Hordeum vulgare* L.)

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5.1 Abstract

Three interconnected genetic populations Commander x Fleet (CF), Commander x WI4304 (CW), and Fleet x WI4304 (FW) developed from crossing of Australian elite barley genotypes were used to map QTL controlling yield and grain plumpness. Genetic linkage maps were constructed using genotyping-by-sequencing and major phenology genes. Field trials were conducted at three drought prone environments for two growing seasons. Seventeen QTL were detected for grain plumpness. Eighteen yield QTL explaining from 1.2% to 25.0% of phenotypic variation were found across populations and environments. Significant QTL x environment interaction was observed for all grain plumpness and yield QTL except *QPlum.FW-4H.1* and *QYld.FW-2H.1*. The major phenology genes *Ppd-H1, Vrn-H1* and *Vrn-H3* were not associated with grain plumpness and yield QTL in this study, and adjustment for maturity effect through co-variance analysis had no major effect on yield QTL. Adjustment for phenology genes identified six yield *per se* QTL that are independent of phenology genes and six new yield QTL with major effects and stable expression across environments. The six new QTL are located in close proximity to known phenology genes, with individual QTL explaining up to 57.4% of phenotypic variance at a single environment. A yield QTL on chromosome 2H coincident with the *Hv CENTRORADIALIS/EARLINESS PER SE 2* (*HvCEN*) locus was expressed in CW and FW populations. Sequencing of *HvCEN* in the parental lines revealed new SNP in this gene. Genotyping of HvCEN showed that the gene is located in the yield QTL on chromosome 2H *(QYld.CW-2H.1* and *QYld.FW-2H.1)*. Further study is required to verify whether the yield QTL found on chromosome 2H is related to *HvCEN* itself or whether a novel haplotype closely linked to the *HvCEN* locus is responsible for the observed yield variation. The close proximity to phenology genes of the major-effect new yield QTL warrant further investigation to verify whether they are related to different haplotypes or novel alleles of the phenology genes.

Key message

QTL mapping identified 17 grain plumpness and 18 yield QTL in three interconnected populations. A yield QTL coincident with the *HvCEN/EPS2* locus was found in two populations. Adjustment of yield for phenology genes effects identified yield QTL that are independent of phenology genes, and new major-effect yield QTL that were not detected before the adjustment.

5.2 Introduction

The average yield of Australian barley is 2.05 t/ha (ABARES 2014), which is below the world average of 2.91 t/ha (FAOSTAT 2014). Barley production in southern Australia is particularly constrained by cyclic and terminal drought in addition to a number of biotic, abiotic and physiochemical subsoil stresses. Yield is a complex quantitative trait whose expression is highly influenced by the environment and agronomic management. This makes phenotype-based selection slow and unreliable, especially under environments where multiple abiotic stresses prevail. Developing barley varieties with improved and stable yield in such environments is expected to be more challenging with ongoing climate change, thus requiring substantial changes in agronomic practices and crop improvement approaches (Tester and Langridge 2010).

Additionally to yield, barley varieties need to meet minimum grain plumpness standards to be marketed to different end users. Grain plumpness is the minimum retention (% by weight) of grain above a 2.5 mm slotted screen, the specifications for the MALT1, MALT2 and MALT3 grades being 70%, 62% and 58% respectively (GTA 2014). Increased grain plumpness is associated with important quality attributes of brewing barley such as higher malt extract and moderate grain protein (Burger and LaBerge 1985). Grain plumpness is affected by the genotype and the environment (Coventry et al. 2003) and is highly heritable with values of 88% to 96% under variable environments (Fox et al. 2006), indicating the potential for improvement. Grain plumpness is determined by pre-anthesis plant development related traits that affect assimilate accumulation and post-anthesis physiological traits affecting assimilate supply to the developing grain (Coventry et al. 2003). Farmers aim to maximise yield and grain plumpness agronomically by optimising pre-anthesis biomass production and flowering time for their environment. Genetics can be used to achieve this through improved water use efficiency, biomass production and partitioning to the grain, by selecting for abiotic and biotic stress tolerance.

Quantitative trait loci (QTL) mapping is an important step towards development of reliable markers for marker assisted selection. QTL mapping studies for yield and other agronomic traits have been conducted under different environments using different genetic backgrounds in barley (Baum et al. 2003; Cuesta-Marcos et al. 2009; Eshghi et al. 2013; Kalladan et al. 2013; Kandemir et al. 2000; Kraakman et al. 2004; Mansour et al. 2014; Rollins et al. 2013; Shahinnia et al. 2014; Swamy et al. 2011; Talamé et al. 2004; Teulat et al. 2001; Tondelli et

al. 2014; Walker et al. 2013; Wang et al. 2014; Xue et al. 2010). Nevertheless, most of the reported yield-related QTL are associated with the major phenology genes such as the vernalization requirement genes (*Vrn-H1, Vrn-H2,* and *Vrn-H3*)(Cockram et al. 2007), the photoperiod response genes (*Ppd-H1* and *Ppd-H2*) and the earliness *per se* (*EPS2*) locus (Laurie et al. 1995; Tondelli et al. 2014). QTL have been mapped for different aspects of grain size including grain weight, grain length, grain width, and grain width to length ratio (Kalladan et al. 2013; Walker et al. 2013). Genomic regions affecting barley grain weight and size in different International and Australian mapping populations have been summarized in Coventry et al. (2003); most of these QTL were associated with loci influencing plant development, mainly with *Ppd-H1*, the *Eps2*, and the semi-dwarfing gene *Denso* (*sdw1*).
Ppd-H1 and *Vrn-H1* are the two major genes affecting flowering time in barley and have significant effects on agronomic traits including yield components (Karsai et al. 1999). An important gene family called *FLOWERING LOCUS T* (*FT*) induces or represses flowering in plants; this includes the barley genes *HvFT1/Vrn-H3* (Turner et al. 2005), *TERMINAL FLOWER 1* (*HvTFL1*) (Kikuchi et al 2009) and *CENTRORADIALIS* (*HvCEN*) gene, which is the candidate gene for *EPS2* (Comadran et al. 2012). *EPS2* affects flowering time and other agronomic traits including tiller biomass, tiller grain weight, ear grain number, and plant height (Laurie et al. 1994). Other phenology genes are associated with circadian expression such as the barley *CONSTANS* gene (*HvCO1* and *HvCO2;* (Deng et al. 2015), the *GIGANTEA* (*HvGI*) (Dunford et al. 2005) and *ZCCT* genes (Trevaskis et al. 2006), and the red/far-red light *PHYTOCHROMES* with the barley genes, *HvPhyA*, *HvPhyB* and *HvPhyC* (Szucs et al. 2006), and the *APETALA2* (*HvAP2*) gene that control inflorescence development (Houston et al. 2013).

Identifying yield and grain plumpness QTL that are independent of developmental variation or phenology is of paramount importance for developing widely adapted and stable varieties through the application of marker assisted selection. The current study was designed to dissect the genetic basis of yield performance and grain plumpness in southern Australia using three doubled haploid (DH) populations developed from crosses between adapted materials that are similar in maturity and overall plant development.

5.3 Materials and methods

5.3.1 Plant material and phenotyping

Three DH populations of barley and environments used for this study have been described in (Obsa et al. 2016). The populations include 229 lines from the Commander x Fleet (CF), 228 lines from the Commander x WI4304 (CW), and 299 lines from the Fleet x WI4304 (FW). The environments were in South Australia at Minnipa research centre (MRC), Roseworthy agricultural college (RAC), and Swan Hill (SWA) in 2012 and 2013 cropping seasons. The grain was machine harvested at physiological maturity from standard breeder's plots and yields were converted into tonnes per hectare. Grain plumpness was obtained from the plot yields using seed cleaning machine with a 2.5 mm slotted screen, and expressed as retention (% by weight) based on the specifications given by the Grain Trade Australia (GTA 2014).

5.3.2 Statistical analysis of phenotypic data

A multi-stage analysis was performed using the regular grid spatial design and Residual Maximum Likelihood (REML) variance components model in GenStat version 17 (VSN International Ltd, UK). Spatial models (random and linear row and column effects) were fitted for each experiment using plotted variograms to identify spatial co-variance structures (Gilmour et al. 1997). Best Linear Unbiased Predictors (BLUPs) were generated using genotype random effects to estimate the generalized heritability (Cullis et al. 2006).

Best Linear Unbiased Estimates (BLUEs) were generated using genotype fixed effects (Smith et al. 2001) for QTL and multi-environment analysis since BLUPs are inappropriately scaled by their individual environment heritability and variance estimates (Mathews et al. 2008). The inverse of the variance matrix of means from each environment was used to generate unit errors for use as weights in the multi-environment analysis to account for variance heterogeneity. The best model for comparison of across environment covariation was selected based on Schwarz Information Criteria (Schwarz 1978). Genetic correlations among environments for yield were generated from the multi-environment model using the variance-covariance matrix of the selected best model (Mathews et al. 2008). Pearson's correlation coefficients (r) were computed to assess the association of yield and grain plumpness with maturity as reported in Obsa et al. (2016). Two separate covariance analyses were performed using the BLUEs of yield as variate and (1) maturity scores (Zadoks), and (2) phenology genes scores as covariates. The 'AA' and 'BB' genotype scores of phenology genes were

converted to numerical values of 1 and 0, respectively to use the as covariates. The covariance analysis was conducted using analysis of variance by REML model in GenStat, from which BLUEs adjusted for maturity and for phenology genes were generated for each environment.

5.3.3 QTL analysis

QTL analysis of yield and grain plumpness for each population was performed using the generated BLUEs and genetic linkage maps of 2178 GBS markers and 7 phenology genes in CF, 2892 GBS markers and 8 phenology genes in CW, and 2252 GBS markers and 5 phenology genes in the FW population. The polymorphic phenology genes mapped in the three populations were *Ppd-H1*, *Vrn-H2*, *HvAP2*, *HvFT5, HvTFL1*, *HvCO2*, *HvCO1, HvGI*, *HvPhyB* and *HvPhyC*. The sequences of GBS markers were aligned to the barley physical map databases (Cantalapiedra et al. 2015) (http://floresta.eead.csic.es/barleymap/) to assign markers to the correct chromosomal locations and orientations. The details of the GBS maps and the mapped phenology genes are available in Obsa et al. (2016) and in Chapter 3 of this thesis. Three sets of QTL analyses were performed for yield using (a) the original unadjusted BLUEs, (b) BLUEs adjusted for maturity using Zadoks score as covariate in the analysis of variance by REML model, and (c) BLUEs adjusted for phenology genes scores as described above. The phenology genes used as covariates include *PpdH1*, *HvAP2*, *VrnH2*, *HvFT5_1_167*, *HvTFL1*, and *HvPhyC* in CF; *PpdH1*, *HvGI*, *HvPhyB*, *HvZCCTHc, HvFT5_1_724*, *HvPhyC*, *HvCO2*, and *HvCO1* in CW, and *HvAP2*, *HvGI*, *HvPhyB*, *HvFT5_1_167*, and *HvCO2* in FW populations.

The best variance-covariance model selected in the phenotypic analysis step was used for multi-environment QTL analysis. A genome wide scan to detect candidate QTL positions was performed using Simple Interval Mapping (SIM) (Lander and Botstein 1989) followed by Composite Interval Mapping (CIM) (Zeng 1994), in which the QTL detected by SIM were used as cofactors. A genome-wide significance level of $\alpha = 0.05$ was used as a threshold to reject the null hypothesis of no QTL effect based on the method of (Li and Ji 2005).

Genetic predictors were estimated with a step size of 2 cM interval and the minimum distances for cofactor proximity and for declaring independent QTL were set to 30 cM and 15 cM, respectively. Repeated iterations of CIM were performed until no further change in the significant QTL was observed (Mansour et al. 2014). QTL main effects, QTL x Environment interaction effects, percent of variance explained by the QTL (PVE) as a range over

environments and the source of high value allele at each environment were determined for all significant QTL remained in the final model.

QTL analysis was also performed using the consensus genetic map constructed from the individual linkage maps (see Chapter 3) to identify consensus QTL across populations. QTL positions on the chromosomes were plotted using MapChart 2.2 (Voorrips 2002) for each population. As each population has genotypic data only for the markers mapped in that particular population, it is not possible to use the whole consensus map directly for QTL analysis. Therefore, markers corresponding to the available genotypic data in each population were searched from the consensus map along with their consensus positions using the 'VLOOKUP' function in Excel. In this way, three subsets of the consensus map were constituted, one for each population, and these were used for QTL analysis in the respective populations. Then, the QTL identified in each population with their new positions were plotted on the main consensus map using MapChart 2.2 (Voorrips 2002).

5.3.4 Meristem development study

A controlled environment experiment investigating meristem development was initiated after a yield QTL coincident on chromosome 2H was identified in the three populations with independent effects from the field phenotyped Zadoks scores. Its suspected location coincident with the EPS2 locus (HvCEN) was confirmed by aligning markers within the QTL interval with the *HvCEN* gene on barley physical map (POPSEQ) (Fig. 4.2). Two genotypes from each population were selected, contrasting for yield in the 2H QTL region but fixed for all mapped phenology genes. The low and high yielding respective genotypes selected were FC-DH064 and FC-DH216 from CF, WC-DH042 and WC-DH216 from CW, and WF-DH155 and FW-DH220 from the FW populations. The genotypes were grown under short day (8hr light/16hr dark) and long day (16hr light/ 8hr dark) light regimes with day and night temperatures of 20^{O}C and 15^{O}C, respectively. The same genotypes were planted in the field to monitor plant development and maturity under natural conditions. Three plants were sampled for meristem development study at every three days interval starting from 46 days after emergence under the short day, and 26 days after emergence under the long day. Nikon SMZ2S stereomicroscope was used for microscopic study of the development of shoot apical meristem.

5.4 Results

5.4.1 Variations in grain yield and grain plumpness

Highly significant (P<0.001) yield differences were observed among the parents of the DH lines in five environments (MRC12, MRC13, RAC13, SWH12 and SWH13), while it was not significant in RAC12 (Table 5.1). Commander and Fleet yielded equally and more than WI4304 except at RAC13 where WI4304 yield was higher (Table 5.1). The DH lines showed transgressive segregation for yield in all of the three populations (Table 5.1 and supplemental Fig. 5.1, 5.2 and 5.3) with heritability values ranging from low (0.39) to high (0.86).

MRC12 was the lowest yielding environment with mean yield of 1.26 t/ha in CF population, 1.00 t/ha in CW population, and 1.07 t/ha in FW population. MRC13 was the highest yielding environment for CF and FW populations, the mean yields of both populations being 3.44 t/ha. RAC12 was the highest yielding environment for CW population with a mean yield of 3.53 t/ha (Table 5.1). The genetic correlations among environments for grain yield were positive for all populations and range from a weak correlation between RAC13 and SWH13 in CF population (r = 0.14) to a moderate correlation between RAC12 and SWH12 in FW population (r = 0.73) (Table S5.1).

Highly significant differences (P<0.001) were observed among the parental genotypes for grain plumpness in all environments except in RAC12 (Table 5.1). Fleet had higher proportion of plump grains than Commander and WI4304 in all environments. Commander had more plump grains than WI4304 in MRC13 and SWH12 while the reverse was true in RAC13, but they did not differ significantly in SWA13 (Table 5.1). The DH lines in each population showed moderate to high heritability and a wide range of variation for grain plumpness (Table 5.1).

Table 5.1 Summary statistics based on BLUPs for yield and grain plumpness for three populations and variability of their parents in six different environments

	Yield (t/ha)						Grain plumpness (% >2.5 mm)				
	MRC12	MRC13	RAC12	RAC13	SWA12	SWA13	MRC13	RAC12	RAC13	SWH12	SWH13
Commander	1.31a	3.83a	3.45	3.11b	2.81a	2.61a	82.3b	80.6	85.4c	83.9b	92.6b
Fleet	1.34a	3.92a	3.32	3.33b	2.82a	2.59a	86.4a	82.3	93.6a	90.1a	95.9a
WI4304	1.00b	3.54b	3.41	3.73a	2.34b	2.15b	78.7c	81	91.2b	77.2c	91.5b
F-probability	**<0.001**	**<0.001**	**0.54^{ns}**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.80^{ns}**	**<0.001**	**<0.001**	**<0.001**
CF mean	1.26	3.44	3.20	2.91	2.69	2.65	82.8	83.4	93.7	77.6	89.6
CF minimum	0.70	2.62	1.80	1.55	1.82	0.56	57.8	56.3	82.5	40.4	67.4
CF maximum	1.62	4.12	4.06	4.17	3.25	3.72	96.4	96.5	98.5	97.5	97.8
s.d.	0.15	0.26	0.40	0.46	0.24	0.38	7.2	7.7	3.1	11.5	5.3
Heritability	**0.53**	**0.51**	**0.68**	**0.59**	**0.7**	**0.39**	**0.71**	**0.78**	**0.66**	**0.85**	**0.71**
CW mean	1.10	3.37	3.53	3.06	2.49	2.30	78.9	73.8	91.3	79	86.7
CW minimum	0.56	2.56	2.54	1.35	1.58	1.46	48.2	38.3	76.7	38.9	53.2
CW maximum	1.58	4.04	4.40	4.41	3.52	3.50	96.2	94.3	97.9	98.7	98.2
s.d.	0.19	0.28	0.36	0.51	0.32	0.36	9.0	11.1	3.4	11.6	7.2
Heritability	**0.71**	**0.76**	**0.86**	**0.59**	**0.82**	**0.65**	**0.83**	**0.82**	**0.76**	**0.85**	**0.78**
FW mean	1.07	3.44	3.13	3.08	2.45	2.07	83.7	84.9	89.4	74.7	90.9
FW minimum	0.25	1.25	0.50	0.68	0.58	0.37	65.3	53.3	60.6	39.2	71.9
FW maximum	1.56	4.16	4.25	4.10	3.28	2.77	96.7	97.9	98.0	96.9	98.9
s.d.	0.21	0.36	0.40	0.39	0.34	0.29	6.9	7.9	5.82	12.6	4.2
Heritability	**0.74**	**0.75**	**0.77**	**0.65**	**0.57**	**0.67**	**0.64**	**0.87**	**0.65**	**0.79**	**0.81**

s.d. = standard deviation; MRC12= Minnipa 2012, MRC13= Minnipa 2013, RAC12= Roseworthy 2012, RAC13=Roseworthy 2013, SWH2= Swan Hill 2012, SWH13=Swan Hill 2013; CF =Commander x Fleet, CW= Commander x WI4304, FW= Fleet x WI4304.

5.4.2 Yield QTL based on unadjusted yield data

Eighteen QTL were detected for yield across the three populations. All QTL except one on chromosome 2H had significant QTL x environment interaction (Table 5.2). Yield QTL common between two populations were found on chromosomes 2H (Fig. 5.2) and 7H (Fig.5.1), while a yield QTL on 6H (Fig.5.1) was detected in all of the three populations as judged based on common markers.

Four QTL were detected in the CF population on chromosomes 2H, 4H, 6H and 7H. Commander contributed the high value allele for all of these QTL except *QYld.CF-6H* in which the allele was contributed by Fleet. *QYld.CF-2H* and *QYld.CF-6H* were expressed in two environments while *QYld.CF-4H* and *QYld.CF-7H* were expressed at three environments, all showing QTL x environment interaction. The QTL *QYld.CF-6H* with the significant marker at the peak position being TP88355 explained 25% and 6.1% of the total phenotypic variance for yield in RAC13 and MRC13, respectively (Table 5.2). In terms of the actual allele effect on phenotypic value, the Fleet allele has increased yield by 3.5% and 16.6%, respectively at MRC13 and RAC13.

Six QTL were detected in the CW population on 2H, 5H, 6H and 7H. Commander contributed the high value allele for *QYld.CW-2H.1* and *QYld.CW-7H* while WI4304 was the high value allele for *QYld.CW-2H.2*, *QYld.CW-6H.1* and *QYld.CW-6H.2*. The QTL *QYld.CW-5H* was co-located with the phenology gene *HvPhyC* (Fig. 5.1). *QYld.CW-2H.*1 had the highest LOD score of 15.3 and was expressed in four environments (MRC12, RAC12, SWH12 and SWH13) explaining from 4.6% to 24.4% of the phenotypic variance for yield. *QYld.CW-6H.2* on chromosome 6H is co-located with the phenology gene *HvCO2*. The QTL on 7H, *QYld.CW-7H*, was expressed in five of the six environments with 2.6% to 6.0% of explained phenotypic variance (Table 5.2).

Eight QTL were detected in the FW population on 1H, 2H, 4H, 5H and 6H. The high value alleles for five of these QTL (*QYld.FW-2H.1*, *QYld.FW-4H*, *QYld.FW-5H*, *QYld.FW-6H.1* and *QYld.FW-6H.2*) were from Fleet while WI4304 contributed the high value allele for *QYld.FW-2H.2*. Both Fleet and WI4304 contributed high value alleles for *QYld.FW-1H* and *QYld.FW-2H.3* at different environments. The QTL *QYld.FW-2H.1* at 108.6 cM on 2H, with a LOD score of 6.0, was expressed in all of the six environments with no QTL x environment interaction and explained between 2.6% to 9.3% of the total phenotypic variation for yield.

Table 5.2 Yield QTL in three doubled haploid populations of barley at six environments in southern Australia

QTL	Significant marker	Chr.	Position (cM)	LOD	QTL x E	PVE (%)	QTL additive effects (t/ha)**					
							MRC12	MRC13	RAC12	RAC13	SWH12	SWH13
QYld.CF-2H	TP10554	2H	105.9	4.2	yes	2.6-8.2	-	-	0.11^{C}	-	-	0.06^{C}
QYld.CF-4H	TP15526	4H	67.1	4.2	yes	1.8-9.1	-	0.08^{C}	-	-	0.05^{C}	0.05^{C}
QYld.CF-6H	TP88355	6H	58.1	14.7	yes	6.1-25.0	-	0.06^{F}	-	0.23^{F}	-	-
QYld.CF-7H	TP81322	7H	50.2	4.0	yes	1.5-8.5	-	-	-	0.06^{C}	0.07^{C}	0.08^{C}
QYld.CW-2H.1	TP23249	2H	84.2	15.3	yes	4.6-24.4	0.04^{C}	-	0.10^{C}	-	0.16^{C}	0.08^{C}
QYld.CW-2H.2	TP43335	2H	164.6	6.9	yes	4.2-9.6	0.06^{W}	0.06^{W}	-	-	-	-
QYld.CW-5H	TP91995-TP83176 #	5H	173.9	3.7	yes	2.0-5.3	0.04^{C}	-	-	0.07^{W}	-	-
QYld.CW-6H.1	TP24121	6H	62.7	2.9	yes	8.8	-	-	-	-	-	0.11^{W}
QYld.CW-6H.2	TP77911	6H	83.0	2.9	yes	4.0-6.0	-	0.06^{W}	-	0.13^{W}	-	-
QYld.CW-7H	TP41903- TP89783 #	7H	40.7	4.2	yes	2.7-6.0	-	0.06^{C}	0.06^{C}	0.09^{C}	0.08^{C}	0.07^{C}
QYld.FW-1H	TP43397	1H	144.5	4.8	yes	4.9-5.5	-	0.08^{F}	-	-	-	0.07^{F}
QYld.FW-2H.1	TP60114	2H	108.6	6.0	no	2.6-9.3	0.06^{F}	0.06^{F}	0.06^{F}	0.06^{F}	0.06^{F}	0.06^{F}
QYld.FW-2H.2	TP34123-TP7819 #	2H	131.8	3.8	yes	7.8	-	-	0.11^{W}	-	-	-
QYld.FW-2H.3	TP78288-TP88727 #	2H	203.3	7.7	yes	2.2-4.0	-	-	0.06^{W}	-	0.07^{F}	0.05^{W}
QYld.FW-4H	TP17370	4H	53.7	5.3	yes	1.2-5.1	-	-	-	0.09^{F}	-	0.03^{F}
QYld.FW-5H	TP21942	5H	162.3	3.5	yes	4.8	0.05^{F}	-	-	-	-	-
QYld.FW-6H.1	TP58326	6H	5.8	5.3	yes	2.4-7.7	-	0.08^{F}	0.06^{F}	-	-	0.08^{F}
QYld.FW-6H.2	TP35346-TP21790 #	6H	60.6	9.1	yes	10.3	-	0.11^{F}	-	-	-	-

the QTL peak is between the indicated markers; Chr. = chromosome; LOD = logarithm of the odds; QTL x E = QTL x environment interaction; PVE= percent of variance explained by the QTL and are given ranges (smallest and highest) over the environments where the QTL were significant. ** the "-" under the QTL additive effects columns show that no significant QTL was detected in that environment, and the superscript letters associated with the values of additive effects represent the source of the high value allele (C= Commander, F= Fleet, W= WI4304).

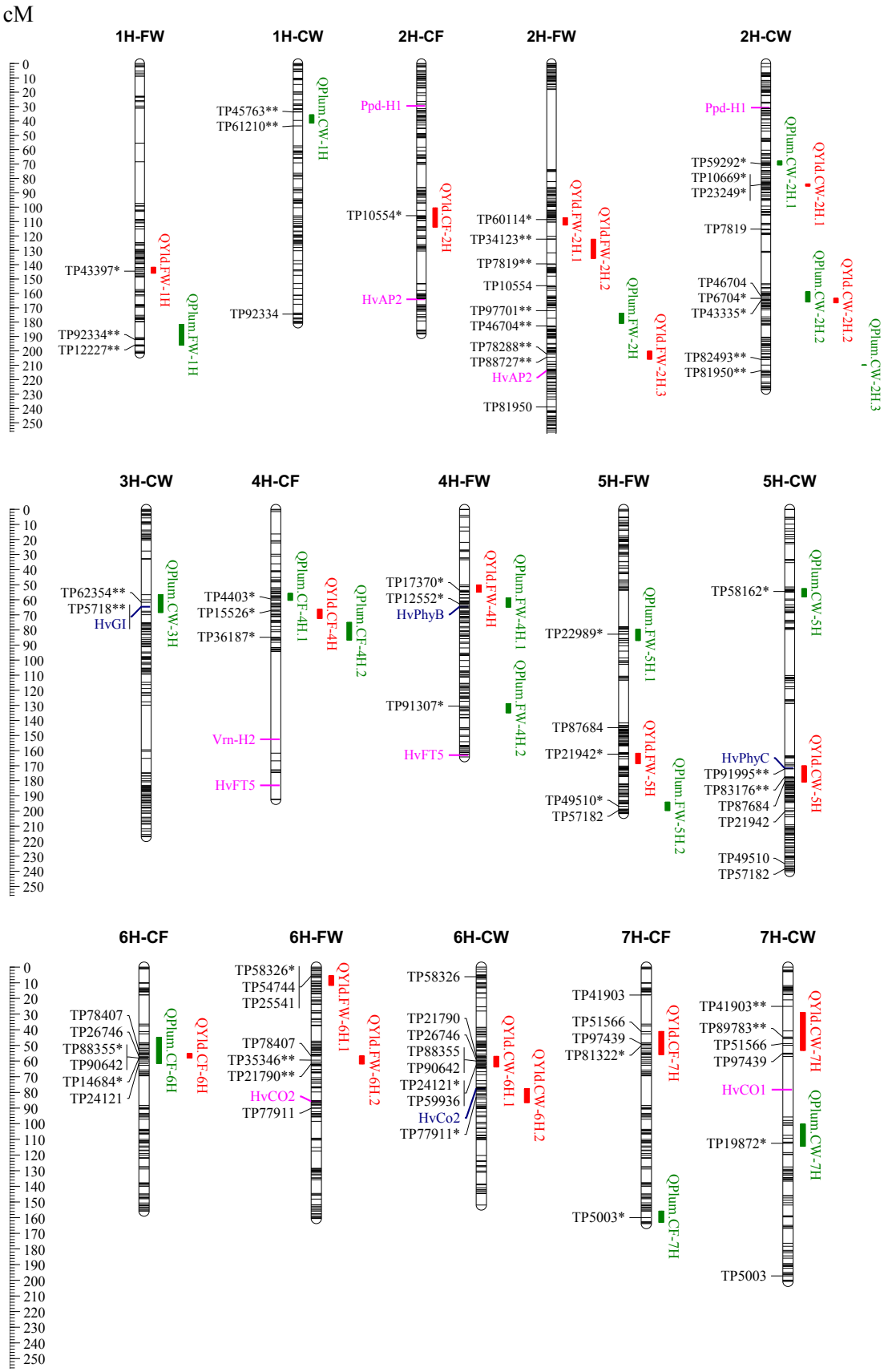


Fig. 5.1 Grain yield and grain plumpness QTL positions in CF, CW and FW populations

* = most significant marker at the QTL peak. **= markers flanking a QTL peak. Known phenology genes outside of the QTL interval are shown with pink colour, while those co-located with a QTL are shown in blue colour.

5.4.3 Consensus QTL

Analysis of QTL for each population identified Yield QTL that are presumably common between two or among the three populations based on shared markers. Examples are yield QTL detected on chromosome 2H in CW and FW populations (*QYld.CW-2H.1* and *QYld.FW-2H.1*), and on chromosome 6H (*QYld.CF-6H*, *QYld.CW-6H.1*, and *QYld.FW-6H.2*) in all of the three populations (see section 5.4.2 above).

Re-analysis of QTL with the consensus map, as described in the Materials and Methods section above, confirmed that *QYld.CW-2H.1* and *QYld.FW-2H.1* represent the same QTL as they were mapped at the same position on the consensus map of 2H, and are related to the grain plumpness QTL (*QPlum.CW-2H.1*) (Fig. 5.2). The significant markers corresponding to these QTL are co-located with the *EARLENESS PER SE 2* (*EPS2/HvCEN*) gene based on alignment on the barley physical map (Cantalapiedra et al. 2015) (Fig. 5.3b). This was further elucidated by genotyping and mapping of the *HvCEN* gene in CW and FW populations, details on this is available in Obsa et al. (2016b). QTL analysis using the genetic maps involving *HvCEN* confirmed that QYld.CW-*2H.1* and *QYld.FW-2H.1* are highly associated with *HvCEN* (Fig.5.3b).

Similarly, the three yield QTL detected in CF, CW, and FW (*QYld.CF-6H*, *QYld.CW-6H.1*, and *QYld.FW-6H.2)* are confirmed to be related based on their common position on the consensus map, although the interval lengths of the three QTL are different (Fig. 5.2). Yield QTL that are co-located with grain plumpness QTL were identified on the consensus map, including *QPlum.CW-2H.1*, which is already mentioned above. Others include *QYld.CW-2H.2* and *QPlum.CW-2H.2* that are co-located and share a portion of *QPlum.FW-2H.1*, while *QPlum.FW-2H.2* falls within the interval of *QYld.FW-2H.3*. *QYld.CW-6H.1* and *QYld.FW-6H.2* on 6H are also co-located with *QPlum.CF-6H* on the consensus map (Fig.5.2).

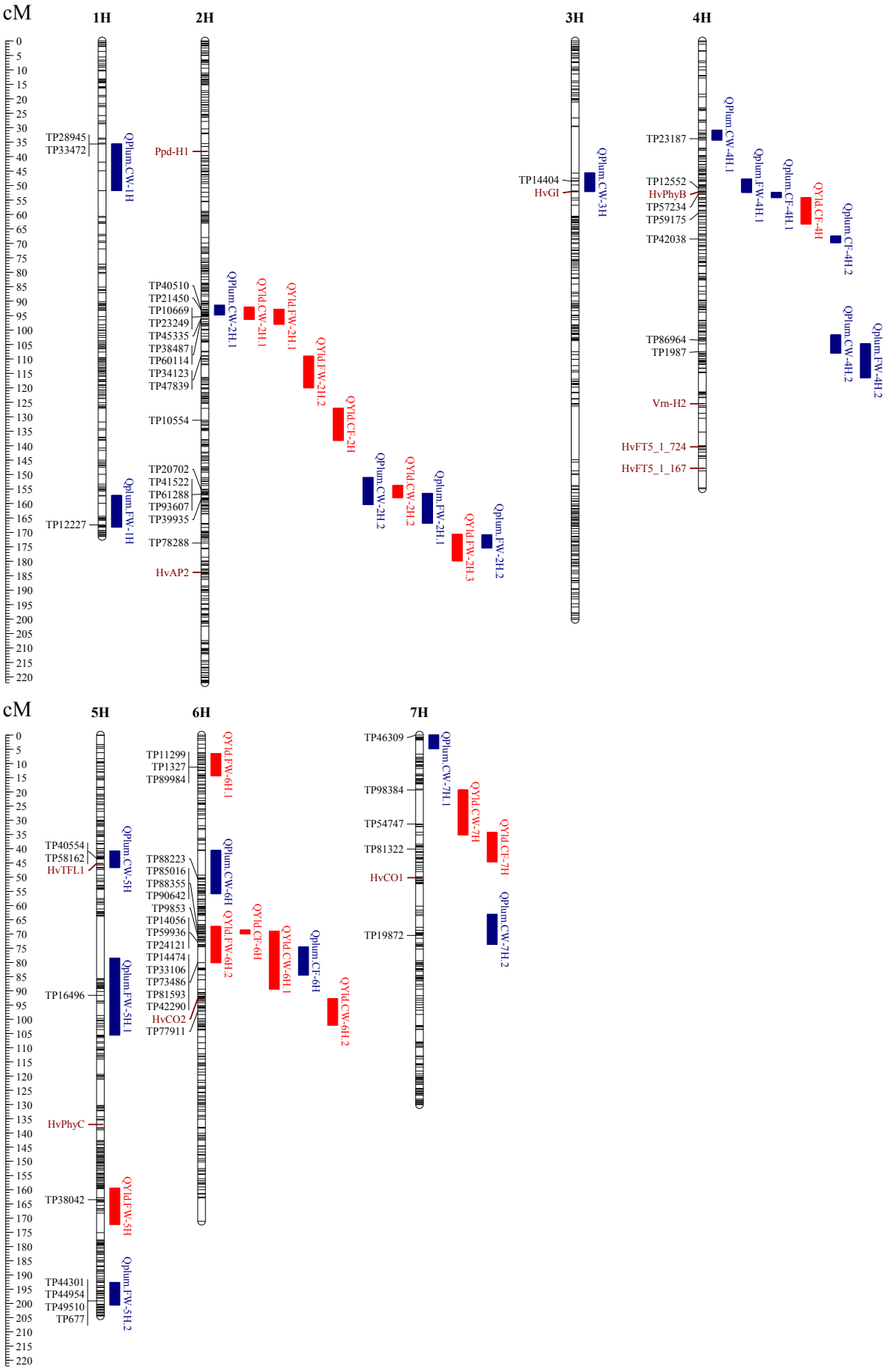


Fig. 5.2 Consensus QTL graphs for grain yield (red) and grain plumpness (blue) across the three populations (CF, CW and FW). Known phenology genes are indicated in brown colour.

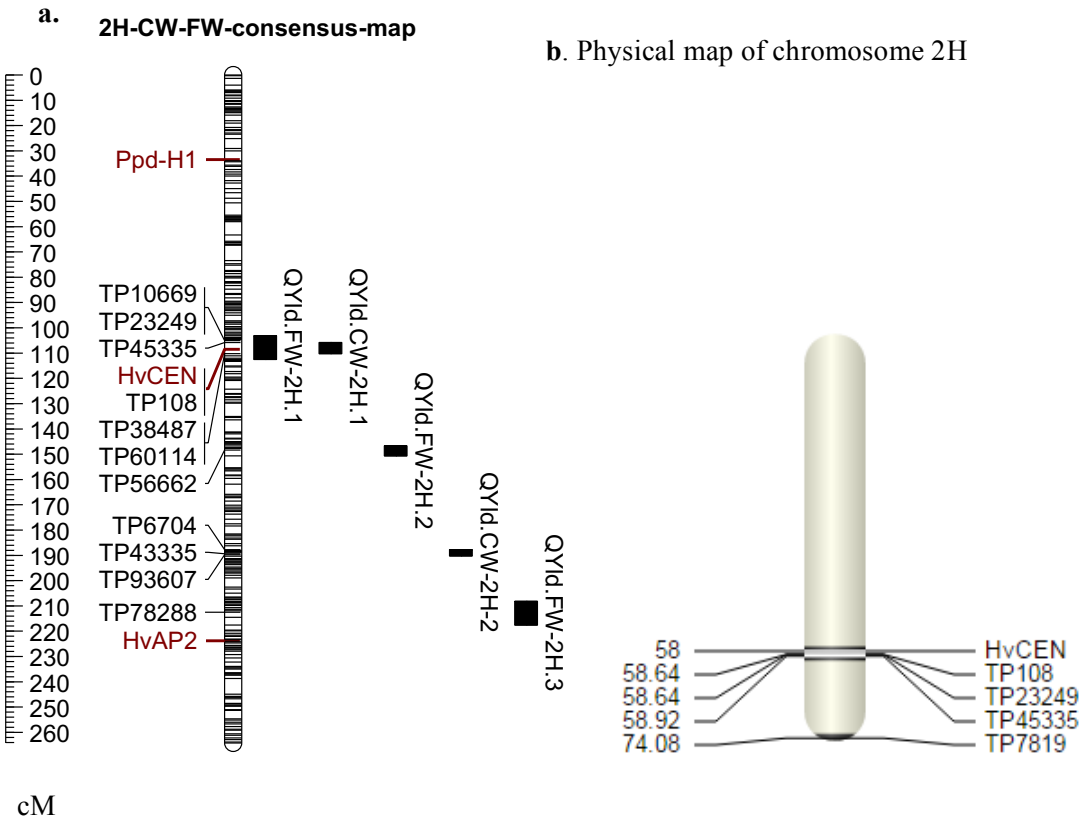


Fig. 5.3 Integrated linkage map of chromosome 2H showing consensus yield QTL between CW and FW populations (QYld.CW-2H.1 and QYld.FW-2H-1) collocated with HvCEN. The chart on the right (B) is the physical map of chromosome 2H showing the co-location with HvCEN of markers (TP108, TP23249 and TP45335) associated to the consensus yield QTL in CW and FW populations as shown on 2H-CW-FW-consensus map (A). Comparison the two charts (A and B) confirms agreement between the genetic map and the physical map with respect to the consensus yield QTL between CW and FW populations. Detailed procedure used to map HvCEN in CW and FW is available in Obsa et al. (2016b).

5.4.4 Maturity effect on yield QTL

Significant correlations between yield and maturity were observed in some trials (Table S5.2). Adjustment of the yield QTL for maturity effect through covariance analysis did not significantly change the QTL as shown in Table S5.3.

Minor changes due to the adjustment in CF include a slight shift of QTL position and associated change of significant marker for *QYld.CF-4H*; slight decreases in LOD scores of *QYld.CF-2H*, *QYld.CF-4H* and *QYld.CF-6H*; a slight increase in LOD score of *QYld.CF-7H*, and a slight increase or decrease of the PVE values in different environments. Moreover, *QYld.CF-4H* was detected in one more environment while *QYld.CF-6H* was detected in two more environments after correction for maturity (Table S5.3).

In CW population, a slight shift in the QTL position was observed for the two QTL on 2H, *QYld.CW-2H.1* and *QYld.CW-2H.2*, by 1.9 cM and 0.4 cM, respectively with slight increase in LOD for the latter from 6.9 to 7.5. The QTL positions and the significant markers did not change for the two QTL on chromosome 6H, *QYld.CW-6H.1* and *QYld.CW-6H.2,* and for the QTL on chromosome 7H, *QYld.CW-7H*. The PVE values showed both an increase and a decrease in different environments, though the changes were small. *QYld.CW-6H.1* appeared in one additional environment (MRC12) after correction for maturity (Table S5.3). A significant change was the disappearance of the QTL on 5H, *QYld.CW-5H* after the adjustment showing its dependency on maturity.

In FW population, one more QTL on 6H (*QYld.FW-6H.3*) appeared after correction for maturity effect (Table S5.3), and slight shifts in QTL positions were observed for the *QYld.FW-1H*, *QYld.FW-2H.2*, and *QYld.FW-6H.1*. The LOD scores were increased for *QYld.FW-1H*, *QYld.FW-4H*, *QYld.FW-5H*, *QYld.FW-6H.*1 and *QYld.FW-6H.2*, but slightly decreased or remained unchanged for all the other QTL (Table S5.3). A significant increase in PVE from 10.3% (before correction) to 18.9% (after correction for maturity) was obtained for *QYld.FW-6H.2*. *QYld.FW-1H* and *QYld.FW-6H.1* appeared in two more environments, while *QYld.FW-5H* appeared in one additional environment after correction (Table S5.3).

5.4.5 Effects of phenology genes on yield QTL

Adjustment of yield data for phenology genes through covariance analysis caused significant changes in QTL detection. In CF population, two of the four QTL previously detected with the unadjusted data (Table 5.2), *QYld.CF-4H* and *QYld.CF-7H,* disappeared after adjustment for phenology genes while *QYld.CF-2H* and *QYld.CF-6H* remained unchanged (Table 5.3).

In CW, two of the previously detected QTL with the unadjusted data (*QYld.CW-6H.1* and *QYld.CW-7H)* (Table 5.2) disappeared after adjustment for phenology genes while four QTL (*QYld.CW-2H.1*,*QYld.CW-2H.2*, *QYld.CW-5H*, and *QYld.CW-6H.2*) remained unchanged; except slight shift in QTL positions, increased or decreased LOD scores and PVE values and expression in more environments than before the adjustment (Table 5.3 and Fig. S5.5). Interestingly, four new loci were detected on chromosomes 3H (58.3 cM), 4H (69.5 cM), 4H (166.6 cM), and 7H (84.03 cM), designated as *QYld.CW-3H*, *QYld.CW-4H.1*, *QYld.CW-4H.2*, and *QYld.CW-7H*, after adjusting yield data for phenology genes. These new loci had stable expression with the same additive effects in all of the six environments, except the 7H locus

that was expressed in four environments (Table 5.3). *QYld.CW-3H*, *QYld.CW-4H.1*, and *QYld.CW-7H* had LOD scores of 27.1, 33.6, and 15.7, respectively, and associated PVE values ranging from 4.4-16.5%, 5.3-20%, and 2.4-22.4%, respectively (Table 5.3 and Table S5.4).The high yield allele for the three QTL was from WI4304 while Commander contributed the allele for *QYld.CW-4H.2*.

In FW, four previously detected QTL with the unadjusted data (*QYld.FW-1H*, *QYld.FW-2H.3*, *QYld.FW-4H*, and *QYld.FW-6H.2*) (Table 5.2) disappeared while four QTL (*QYld.FW-2H.1*, *QYld.FW-2H.2*, *QYld.FW-5H*, and *QYld.FW-6H.1*) remained unchanged, except minor changes in positions, LOD scores, and PVE values (Table 5.3 and Fig. S5.5). More interestingly, three new QTL, designated as *QYld.FW-2H.3*, *QYld.FW-6H.2*, and *QYld.FW-6H-3*, were detected on chromosomes 2H (214 cM), 6H (51.6 cM), and 6H (98.5 cM). These new QTL were expressed in all of the six environments with LOD scores of 45.5, 79.8, and 5.4, respectively, and the associated PVE values ranging from 4.4-11.6%, 36.8-57.4%, and 2.1-3.1%, respectively. The high yield allele for these new loci was contributed from WI4304 and they had the same additive effects in all environments (Table 5.3 and Table S5.5).

Table 5.3 LOD scores, QTL x E, percent of variance explained (PVE %) and additive effects of phenology adjusted yield QTL in CF, CW and FW populations over six environments

QTL	Significant Marker	Chr.	Pos.	LOD	QTL x E	PVE (%)	QTL additive effects at each environment***					
							MRC12	MRC13	RAC12	RAC13	SWA12	SWA13
QYld.CF-2H	TP10554	2H	105.9	3.8	yes	3.0-9.2	-		0.126^{C}	-	-	0.068^{C}
QYld.CF-6H	TP14684	6H	58.4	10.6	yes	5.4-22.2	-	0.06^{F}	-	0.204^{F}	-	-
QYld.CW-2H.1	TP58367	2H	86.1	9.6	yes	0.9-15.8	0.026^{C}	-	0.083^{C}	-	0.148^{C}	0.059^{C}
QYld.CW-2H.1	TP23323	2H	159.6	6.5	yes	1.8-5.2	0.064^{W}	0.045^{W}	-	-	-	-
QYld.CW-3H	TP29580 -TP62354*	3H	58.3	27.1	no	4.4-16.5	0.115^{W}	0.115^{W}	0.115^{W}	0.115^{W}	0.115^{W}	0.115^{W}
QYld.CW-4H.1	TP61189	4H	69.5	33.6	no	5.3-20	0.127^{W}	0.127^{W}	0.127^{W}	0.127^{W}	0.127^{W}	0.127^{W}
QYld.CW-4H.2	TP73004	4H	166.6	7.8	no	1.1-4.2	0.058^{C}	0.058^{C}	0.058^{C}	0.058^{C}	0.058^{C}	0.058^{C}
QYld.CW-5H	TP68883	5H	171.6	7.4	yes	1.2-10.2	0.03^{W}	0.105^{W}	0.075^{W}	0.128^{W}	0.072^{W}	-
QYld.CW-6H	TP77911	6H	83.0	7.5	no	1.1-4.1	0.057^{W}	0.057^{W}	0.057^{W}	0.057^{W}	0.057^{W}	0.057^{W}
QYld.CW-7H	HvCO1 - TP34872**	7H	84.0	15.7	yes	2.4-22.4	0.134^{W}	0.051^{W}	0.071^{W}	-	0.073^{W}	-
QYld.FW-2H.1	TP33039	2H	107.1	4.2	no	0.7-1.1	0.064^{F}	0.064^{F}	0.064^{F}	0.064^{F}	0.064^{F}	0.064^{F}
QYld.FW-2H.2	TP34123 - TP6042*	2H	133.7	4.4	yes	2.8	-	-	0.126^{W}	-	-	-
QYld.FW-2H.3	TP75824	2H	214.0	45.5	yes	4.4-11.6	0.157^{W}	0.198^{W}	0.243^{W}	0.231^{W}	0.137^{W}	0.22^{W}
QYld.FW-5H	TP38042	5H	161.6	3.2	yes	0.4	0.042^{F}	-	-	-	-	-
QYld.FW-6H.1	TP89984	6H	9.6	3.6	yes	0.9-1.3	-	0.068^{F}	0.069^{F}	-	-	0.074^{F}
QYld.FW-6H.2	TP2594 - TP5400**	6H	81.6	79.8	yes	36.8-57.4	0.472^{W}	0.372^{W}	0.498^{W}	0.422^{W}	0.448^{W}	0.472^{W}
QYld.FW-6H.3	TP46163	6H	98.5	5.4	no	2.1-3.1	0.108^{W}	0.108^{W}	0.108^{W}	0.108^{W}	0.108^{W}	0.108^{W}

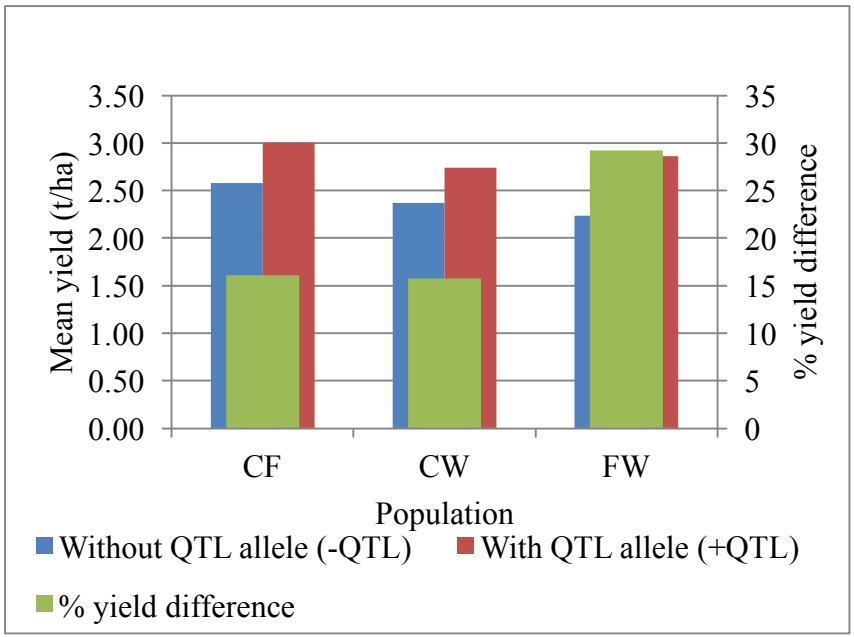
*The QTL is found at estimated genetic predictor position between the two markers, the two markers representing the lower and the upper boundaries of the QTL interval, **=The QTL is found at estimated genetic predictor position between the two shown markers but the two markers are outside of the calculated QTL interval based on 1.5-LOD interval. Chr. = chromosome, Pos. = position, LOD= logarithm of the odds, and QTL x E= QTL by environment interaction. ***=superscript letters shown with QTL additive effects represent the source of high value allele (C= Commander, W= WI4304), and "-" indicate that the QTL was not significant at that environment. The PVE values are given as ranges over the environments where the QTL were significant. The PVE values for each environment are given in **Table S5.4**.

5.4.6 Developmental variation between contrasting pairs of genotypes selected from the consensus yield QTL region on chromosome 2H: meristem development study

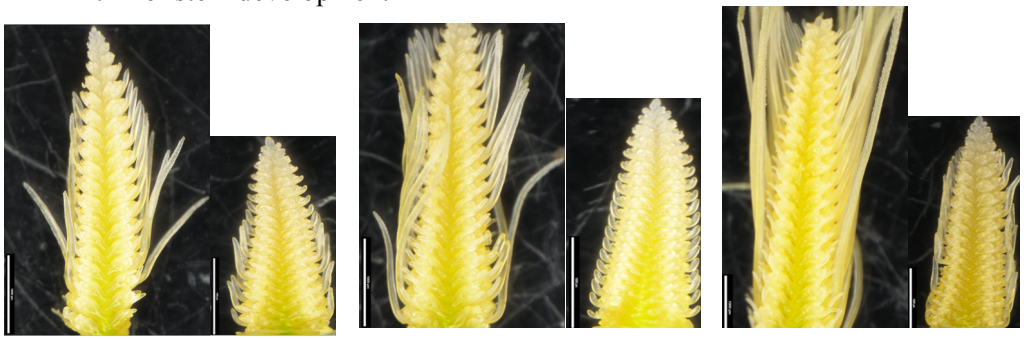
This controlled environment study was designed to verify whether the consensus yield QTL found in CW and FW on chromosome 2H is related to developmental variation. The mean yields across environments of contrasting genotypes selected for the controlled experiment and the percentage yield increase due to the QTL allele is given along with the meristem pictures in Fig. 5.4.

Meristem dissection study showed slight differences between the pairs of contrasting genotypes selected from the three populations. In each population, the high yielding genotype (+QTL) was at advanced stage of awn development compared to the low yielding genotype (-QTL) at 60 days after emergence under short day condition. All of the genotypes studied had rapid growth rate under the long day condition, and passed the meristematic stage when sampling was done. Hence, only variation in floret development was studied under the long day condition (data not shown). The number of days from seedling emergence to anthesis stage was not significantly different between the pairs of genotypes from each population under both light regimes (short day and long day) (***see*** the small table under Fig. 5.4).

A. Yield



B. Meristem development



C.

		FC-DH216	FC-DH064	WC-DH216	WC-DH042	FW-DH220	WF-DH155
Days to anthesis	SD	96[a]	98[a]	94[a]	96[a]	98[a]	100[a]
	LD	30[a]	32[a]	28[a]	31[a]	33[a]	35[a]

SD= Short day, LD= Long day, pairs of means followed by the same letter in the SD and LD row are not significantly different from each other

Fig. 5.4. Effects of yield QTL detected on chromosome 2H on yield and reproductive development in CF, CW and FW populations. The chart on the left (A) shows the effect of the QTL allele on grain yield (yields on the left axis are averages of six environments).

The meristem pictures on the left (B) were taken on day 60 after seedling emergence under short day condition and show differences in awn development stages between pair of contrasting (with and without the QTL allele) genotypes in each population. +QTL and –QTL represent the contrasting genotypes selected from the QTL region (+QTL= genotype with high yield and carrying the high value allele, -QTL= genotype with low yield and carrying the opposite allele). The table below the meristem pictures (C) shows data for days to anthesis under short day (SD) and long day (LD) conditions.

5.4.7 Grain plumpness QTL

Seventeen grain plumpness QTL were detected across the three populations: four QTL in CF, seven QTL in CW and six QTL in FW (Table 5.4 and Fig. 5.1). All QTL except one on 4H in FW showed significant QTL x environment interaction (Table 5.4).

Two QTL (*QPlum.CF-4H.1* and *QPlum.CF-4H.2*) were detected on 4H in CF population. *QPlum.CF-4H.1* was detected only at RAC13 with the high value allele from Fleet and explaining 13.8% of the phenotypic variance for grain plumpness. The high value allele for *QPlum.CF-4H.2*, explaining 5.7% and 9.8% of phenotypic variance at RAC13 and SWH12, respectively was contribute from Commander. *QPlum.CF-6H* was detected in all environments except SWH12 with the high value allele from Fleet and explaining from 2.5% to 10.9% of the phenotypic variance. *QPlum.CF-7H* was detected in all environments with the high value allele from Fleet, increasing the percentage of plump grain by 0.63 to 2.55 (Table 5.4).

Grain plumpness QTL in CW population were detected on chromosomes 1H, 2H, 3H, 5H and 7H. *QPlum.CW-1H*, explaining 3.1% to 8.4% of the phenotypic variance, was detected in all environments except in RAC12 and the high value allele was from Commander. Three grain plumpness QTL were detected on 2H (*QPlum.CW-2H.1*, *QPlum.CW-2H.2* and *QPlum.CW-2H.3*) explaining from 3.0% to 8.2% of the phenotypic variance (Table 5.4). Commander contributed the high value alleles for *QPlum.CW-2H.1* and *QPlum.CW-2H.3,* while WI4304 contributed high value allele for *QPlum.CW-2H.2* (Table 5.4). *QPlum.CW-3H* is co-located with the phenology gene *HvGI* (Fig. 5.1), explaining 1.6% and 9.8% of phenotypic variance at RAC13 and SWH12, respectively. *QPlum.CW-5H* was detected in MRC13 and RAC12, explaining 2.2% and 6.8% of phenotypic variance, respectively. *QPlum.CW-7H*, high value allele from Commander, was detected in MRC13, RAC12 and RAC13, explaining 1.8% to 3.5% of the phenotypic variance (Table 5.4).

QTL for grain plumpness in FW population were detected on chromosomes 1H, 2H, 4H, and 5H, explaining from 3.1% (*QPlum.FW-4H.1*) to 11.1% (*QPlum.FW-2H*) of the phenotypic variance. *QPlum.FW-4H.1* and *QPlum.FW-4H.2* were detected in all of the five environments. *QPlum.FW-4H.1*is co-located with the phenology gene *HvPhyB* and had the same additive effects across the five environments with no QTL x Environment interaction. Fleet contributed the high value allele for all QTL detected in the FW population except *QPlum.FW-4H.2* (Table 5.4).

Table 5.4 QTL for grain plumpness in three doubled haploid populations of barley at six environments in southern Australia

QTL	Significant marker	Chr.	Position (cM)	LOD	QTL x E	PVE (%)	QTL additive effects (% >2.5mm)				
							MRC13	RAC12	RAC13	SWH12	SWH13
QPlum.CF-4H.1	TP4403	4H	59.5	8.3	yes	13.8	-	-	1.98^{F}	-	-
QPlum.CF-4H.2	TP36187	4H	84.8	4.8	yes	5.7-9.8	-	-	1.27^{C}	2.41^{C}	-
QPlum.CF-6H	TP14684	6H	58.4	5.5	yes	2.5-10.9	1.14^{F}	2.24^{F}	1.76^{F}	-	0.54^{F}
QPlum.CF-7H	TP5003	7H	160.0	4.3	yes	2.0-10.9	1.47^{F}	2.55^{F}	0.76^{F}	2.53^{F}	0.63^{F}
QPlum.CW-1H	TP45763-TP36876 [#]	1H	39.6	4.1	yes	3.1-8.4	2.62^{C}	-	1.67^{C}	2.23^{C}	0.60^{C}
QPlum.CW-2H.1	TP59292	2H	69.8	14.2	yes	3.0-8.2	1.82^{W}	2.11^{W}	2.05^{W}	-	0.60^{C}
QPlum.CW-2H.2	TP6704	2H	163.4	5.3	yes	3.1-8.2	2.58^{C}	2.76^{C}	2.06^{C}	2.65^{C}	0.60^{C}
QPlum.CW-2H.3	TP82493-TP81950 [#]	2H	209.8	4.2	yes	7.4	-	-	-	3.02^{W}	-
QPlum.CW-3H	TP62354-TP5718 [#]	3H	63.1	5.0	yes	1.6-9.8	-	-	0.91^{C}	3.48^{C}	-
QPlum.CW-5H	TP58162	5H	54.5	2.8	yes	2.2-6.8	2.35^{C}	1.72^{C}	-	-	-
QPlum.CW-7H	TP19872	7H	112.5	2.5	yes	1.8-3.5	1.20^{C}	2.19^{C}	1.20^{C}	-	-
QPlum.FW-1H	TP92334-TP12227 [#]	1H	194.0	4.1	yes	1.5-7.5	1.03^{F}	1.83^{F}	-	0.95^{F}	1.60^{F}
QPlum.FW-2H	TP97701-TP46704 [#]	2H	177.3	11.1	yes	7.0-10.8	2.28^{F}	-	1.19^{F}	2.08^{F}	1.81^{F}
QPlum.FW-4H.1	TP12552	4H	62.7	3.1	no	0.3-2.8	0.70^{F}	0.70^{F}	0.70^{F}	0.70^{F}	0.70^{F}
QPlum.FW-4H.2	TP91307	4H	130.5	10.8	yes	2.2-13.7	1.28^{W}	3.48^{W}	0.62^{W}	2.58^{W}	2.15^{W}
QPlum.FW-5H.1	TP22989	5H	82.8	4.4	yes	3.5-5.7	1.65^{F}	2.35^{F}	0.92^{F}	-	-
QPlum.FW-5H.2	TP49510	5H	196.9	5.9	yes	1.9-7.4	1.69^{F}	1.76^{F}	0.88^{F}	2.15^{F}	-

[#] the QTL peak is between the indicated markers; Chr. = chromosome; LOD = logarithm of the odds; QTL x E = QTL x environment interaction; PVE = percentage of variance explained by the QTL and are given as ranges (smallest and highest) over the environments where the QTL were significant. The "-" shows that no significant QTL was detected in that environment, and the superscript letters represent the source of the high value allele (C= Commander, F= Fleet, W= WI4304).

5.5 Discussion

5.5.1 Environment effects on yield and grain plumpness

The parents of the three populations have been selected based on their long-term yield performances in southern Australia. Commander and Fleet had stable yields across a range of environments, while WI4304 had low yields under drought-affected environments. In this study, Commander and Fleet had similar yields, significantly higher than WI4304 with the exception of RAC13 where the rankings were reversed (Table 5.1). The environments showed substantial variation for yield, which could be attributed mainly to the rainfall patterns (amount and distribution), and other climatic and edaphic factors (Obsa et al. 2016). The wide variations observed in yield and grain plumpness in all of the three populations was expected for such quantitative traits due to transgressive segregation. Except one QTL for grain plumpness (*QPlum.FW-4H.1*) and one QTL for yield (*QYld.FW-2H.1*), which were consistent across environments, all QTL for the two traits had significant QTL x environment interactions. One QTL on chromosome 2H in CW, and one QTL on chromosome 6H in CF populations had the strongest effects, though their effects are environment specific.

5.5.2 Yield and grain plumpness QTL related to maturity

None of the major developmental genes, including *Ppd-H1*, *Vrn-H1* and *Vrn-H2,* that drive barley adaption have significantly affected grain plumpness and yield in this study. This could be due to the nature of the population, which were derived from elite x elite crosses aimed at discovering QTL that could be deployed in breeding programs under Mediterranean type environments. The lack of significant effects on yield QTL after correction for maturity is also consistent with the nature of the population as stated above. However, we found a number of QTL for yield or grain plumpness that are co-located with maturity QTL and some with phenology genes suggesting pleiotropic effects. The yield QTL (*QYld.FW-1H*) on chromosome 1H in the FW population is co-located with the maturity QTL *QMat.FW-1H*, while the yield QTL *QYld.FW-2H.3* on chromosome 2H in the FW population is co-located with the maturity QTL (*QMat.FW-2H*) as reported in Obsa et al. (2016). The peak markers for the yield QTL *QYld.FW-4H* (TP17370), the grain plumpness QTL *QPlum.CF-4H.1* (TP4403) and *QPlum.FW-4H.1* (TP12552) on chromosome 4H are located closer to each other on the physical map (Fig. S5.6). These markers are also co-located with TP89118 for the maturity QTL (*QMat.CW-4H*) reported in Obsa *et al.* (2016). This suggests that this QTL

might have pleiotropic effects on yield, grain plumpness and maturity. Tondelli et al. (2014) have reported QTL for plant height, thousand-grain weight, spikes per square metre, and spike morphology approximately around the same genomic region in the Nure x Tremois population.

The yield QTL *QYld.CW-5H* on chromosome 5 disappeared after adjustment for maturity effect showing its dependency on maturity. This QTL is co-located with the maturity QTL (*QMat.CW-5H*) and leaf waxiness QTL (*QLwax.CW-5H*) (Obsa et al. 2016), and aligned on the physical map with the maturity QTL (*QMat.CF-5H.2*) in the CF population. In a previous study, different QTL that control reproductive development stages from awn primordia formation to anther extrusion have been mapped to this region (Alqudah et al. 2014). Thus, it appears that this yield QTL is related to the direct effect of maturity. *QYld.CW-5H* is closely linked to *HvPhyC* locus that has a direct role in promoting long day flowering in barley (Nishida et al. 2013).

Four QTL on chromosome 6H (*QPlum.CF-6H*, *QYld.CF-6H*, *QYld.FW-6H.2*, and *QYld.CW-6H.1*) shared common markers with each other (Fig. 5.1), and overlap a maturity QTL (*QMat.CW-6H*) previously reported in Obsa et al. (2016). Adjustment for maturity effect in the QTL analysis increased PVE from 10.3% to 18.9% for the *QYld.FW-6H.2* showing its dependency on flowering time. *QYld.CW-6H.2* is located around the same position of previously reported yield QTL (Comadran et al. 2008; Tondelli et al. 2014) and heading date QTL (Alqudah et al. 2014), and is also co-located with the phenology gene *HvCO2* (Fig. 5.1) and could be related to the direct effect of this gene.

The two yield QTL detected on 7H (*QYld.CF-7H* and *QYld.CW-7H*) have common markers (TP51566 and TP97439) on the genetic map (Fig. 5.1), though they are clearly separated on the physical map (Fig. S5.6). The positions of these two QTL appear to be around the *Vrn-H3* locus, where (Walker et al. 2013) have reported QTL for yield and flowering date. Other previous studies have also reported yield QTL in the same genomic region (Comadran et al. 2008; Xue et al. 2010).

5.5.3 Yield QTL co-located with phenology genes without affecting maturity

We identified some QTL for yield or grain plumpness that are co-located with phenology genes but not with a maturity QTL, either in the same population (Obsa et al. 2016) (Fig. S5.6) or in the literature. We hypothesize that either these phenology genes underneath a

QTL affect the inflorescence development with an impact on yield or grain plumpness without changing the flowering time, or another genes in the vicinity of these phenology genes might be responsible. Such examples were found on chromosomes 2H, 3H and 7H.

The grain plumpness QTL (*QPlum.CW-2H.2* and *QPlum.FW-2H*) and the yield QTL *QYld.CW-2H.2* have a common marker TP46704 (Fig. 5.1) that is coincident with the phenology gene *HvFT5* on the physical map (Fig. S5.6), thus these QTL are assumed the same. On chromosome 3H, *QPlum.CW-3H* is co-located with *HvGI*, the barley homologue of an Arabidopsis photoperiod pathway (Dunford et al. 2005). *QPlum.CF-7H* in CF is near the *eps7HL* locus on chromosome 7H reported in Harrington x Morex population (Coventry et al. 2003).

QTL analysis using yield data adjusted for phenology genes further elucidated the relationships between the yield QTL and the phenology genes mapped in this study. This resulted in disappearance of eight previously identified QTL with the unadjusted data; 10 QTL that were expressed in both the unadjusted and adjusted analysis, and seven new QTL across the populations (Table 5.3, Table S5.4, and Fig. S5.5).

From the QTL that disappeared after adjusting for phenology, *QYld.CW-6H.1* was within 15 cM from *HvCO2*, while *QYld.FW-2H.3*, *QYld.CW-FW.4H*, and *QYld.FW-6H.2* were about 10 cM from *HvAP2*, *HvPhyB*, and *HvCO2*, respectively. As these phenology genes were used as covariates during the adjustment, it may seem reasonable to deduce that the QTL that disappeared due to the adjustment are related to these phenology genes, if the genes could exert their effects over 10 or 15 cM distances. It should be noted that the reported co-location of QTL with phenology genes in the original (unadjusted) analysis (Fig. 5.1) was based on co-location within the 1.5-LOD QTL interval. None of the QTL that disappeared after the adjustment were located within this QTL interval and were not reported considered co-located with the phenology genes. However, these genes may exert their effects beyond this interval, which might explain the reason for disappearance of the QTL after adjusting for the effects of these genes.

The disappearance of *QYld.CF-4H*, *QYld.CF-7H*, and *QYld.FW-1H* is unclear, as these QTL were not related to any of the mapped phenology genes and *QYld.CW-7 H* was 38.2 cM away from *HvCO1* locus. One possible reason may be related to the effects of changes in the other regions of the genome due to epistatic interactions.

More interestingly, six of the seven new QTL identified after adjustment for phenology genes (*QYld.CW-3H*, *QYld.CW-4H.1*, *QYld.CW-4H.2*, *QYld.CW-7H*, *QYld.FW-2H.3*, and *QYld.FW-6H.2*) are within a maximum of 10 cM distance from *HvGI*, *HvPhyB*, *HvZCCTHc*, *HvCO1*, *HvAP2*, and *HvCO2*, respectively. All of these QTL with the exception of *QYld.CW-4H-2* had stable expression in all of the six environments with major effects in some environments. *QYld.FW-6H.2* had the most pronounced effect in all environments with **LOD score of 79.8** and **PVE values ranging from 36.8% to 57.4%** over the six environments (Table S5.4). As the yield data were adjusted for the differences in the phenology genes listed above and others, the close proximity of these QTL to the phenology genes is not expected to imply direct relationship between the QTL and the genes. We rather hypothesize that different haplotypes or novel alleles of the genes may underlie these QTL. This needs further study aiming at fine mapping and positional cloning of these genomic regions. None of these new loci were detected when QTL analysis was done using data adjusted for maturity effect (Table S5.3).

Two of the 10 QTL that were not affected by the adjustment (*QYld.CW-5H* and *QYld.CW-6H.2*) were co-located with *HvPhyC* and *HvCO2*, respectively. This is surprising, as the adjustment should have suppressed these QTL assuming that they are related based on their co-location. We again hypothesize that *QYld.CW-5H* and *QYld.CW-6H.2* may be related to different haplotypes or novel alleles of *HvPhyC* and *HvCO2*, respectively. The six remaining QTL that were not affected by the adjustment (*QYld.CW-2H.1*, *QYld.CW-2H.2*, *QYld.FW-2H.1*, *QYld.FW-2H-2*, *QYld.FW-5H*, and *QYld.FW-6H-1*) were not related to maturity in the unadjusted analysis and the lack of effect of the adjustment is an expected result. This is a significant confirmatory result that enables us conclude that these QTL are yield *per se* QTL that are independent of phenology genes. As discussed earlier, *QYld.CW-2H.1* and *QYld.FW-2H.1* are same QTL common between the CW and FW populations and are co-located with *HvCEN* on barley physical map. *HvCEN* is the gene for *EPS2* (Comadran et al. 2012), which influences flowering time independently of vernalization and photoperiod (Laurie et al. 1995). *HvCEN* is associated with phenology QTL and coincide with yield and grain size QTL by association of correlated phenotypes (Coventry et al. 2003; Mansour et al. 2014; Tondelli et al. 2014; Xue et al. 2010).

QYld.CW-2H.1 and *QYld.FW-2H.1* were further confirmed to be the same QTL after re-analysis of QTL using the consensus genetic map construction by integration of the three individual linkage maps. The co-location of these QTL was also confirmed by using new

maps of CW and FW that involve the mapped HvCEN gene (Obsa et al. 2016b). Comadran et al. (2012) found 13 haplotypes of *HvCEN* in a collection of 1,143 barley accessions, which included only two Australian accessions. The *QYld.CW-2H.1* and *QYld.FW-2H.1* could be either (1) a new haplotype of *HvCEN* that may have a positive effect on yield independently of flowering time, or (2) another gene closely linked to *HvCEN*. Further work is underway to test these hypotheses.

5.5.4 Yield QTL independent of maturity or phenology genes

Fourteen QTL controlled yield or grain plumpness without being affected by maturity, be collocated neither with maturity QTL nor with phenology genes. Some of these QTL seem to correspond to QTL described in other populations. *QPlum.FW-1H*, located around the proximal end of 1H, was in a similar position in the Galleon x Haruna Nijo barley population (Karakousis et al. 2003). *QPlum.CW-1H*, which is on a different region than *QPlum.FW-1H*, is around the distal end of chromosome 1H where Coventry et al. (2003) have reported grain plumpness QTL in Blenheim x E224/3, Harrington x Morex, and Chebec x Harrington populations. The location of the other grain plumpness QTL on chromosome 2H in CW (*QPlum.CW-2H.3*) seems to coincide with the screenings QTL reported in Sloop x Alexis population, and thousand grain weight QTL found in Blenheim x E224/3 populations (Coventry et al. 2003). The grain plumpness QTL detected in CW and FW populations on 5H seems to be at a similar position with the QTL for grain plumpness and screenings in Chebec x Harrington population (Barr et al. 2003). Some QTL were not reported in other populations and are therefore new in our populations: *QYld.CF-2H*, *QYld.FW-5H,* and *QPlum.CW-2H.1*.

Interestingly some overlapping QTL were found for both yield and grain plumpness without an effect on maturity: *QYld.CW-2H.2* and *QPlum.CW-2H.2* in the CW population on chromosome 2H, and *QPlum.CF-6H* and *QYld.CF-6H*, in the CF population on chromosome 6H. This indicates that the QTL effect on grain plumpness is eventually translated into a yield effect, or the QTL may have pleiotropic effect on both traits.

Regarding the meristem development study, even though the genotype with the positive QTL allele was slightly at an advanced stage of awn development (Fig 5.4) in each of the three populations, there was no statistically significant difference with respect to the number of days from emergence to anthesis under the same light regime (SD and LD). It is worth noting that the QTL effect on yield and development as depicted in Fig. 5.4 is the combined effects

of all the yield QTL detected on chromosome 2H. This is because; the selected genotypes for the study had contrasting alleles across the genomic region spanning the entire yield QTL detected on chromosome 2H. It is therefore possible that the slight developmental differences observed between the genotypes could be due to the effects of the yield QTL that are associated with maturity.

5.5.5 Conclusions

Genetic analysis based on elite x elite Australian cultivars produced significant results that that could be used as platform for future barley breeding in Mediterranean type environment of southern Australia. The study identified yield and grain plumpness QTL and elucidated their relationship between phenology genes. Adjustment for phenology genes have confirmed that *QYld.CW-2H.1*, *QYld.CW-2H.2*, *QYld.FW-2H.1*, *QYld.FW-2H-2*, *QYld.FW-5H*, and *QYld.FW-6H-1* are independent of phenology genes, and could be considered as yield *per se* QTL. Adjustment for phenology genes has also enabled identification of new loci wit stable expression and major effects across environments, explaining up to 57.4% of phenotypic variance. However, the close proximity of these QTL to phenology genes warrant further investigation to verify whether they are related to different haplotypes or novel alleles of the genes. QTL that are common between two or among the three populations have been identified on chromosomes 2H, 6H and 7H. Such QTL segregating in different genetic backgrounds could be reliable to use for marker-assisted selection. However, these QTL had their largest effects only at specific environments, which could limit their application for breeding widely adapted varieties. Marker-assisted pyramiding of the significant QTL into a common genetic background may be a useful breeding strategy to develop varieties adapted to the south Australian environment. Moreover, the QTL on 2H (*QYld.CW-2H.1* and *QYld.FW-2H.1*), which is common between the CW and FW populations, needs further in-depth investigation, which is already started, to verify whether it is related to the direct effect of *HvCEN/EPS2* or whether a novel haplotype closely linked to the *HvCEN* locus is responsible for the observed yield variation.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Chapter 6: General discussion

6.1 The genetic basis of barley adaptation

Adaptation to a particular environment is decisive for the success of any crop variety. Cultivated crops have been selected for traits that enable them to thrive under prevailing environments throughout the course of evolution, resulting in variations in global distributions of crop species and varieties. Deliberate intervention by humans in the process of plant breeding has further facilitated fine-tuning of crop variety selections to specific agricultural environments.

Temperature, day length and rainfall are important climatic factors that dictate crop adaptation and distribution. Photoperiod response and vernalization requirement are the major determinants of adaptation in barley and other cereals like wheat and major genes controlling these traits have been identified. These genes have pleiotropic effects on heading date, plant architecture, yield and other important traits and exert large effects that differentiate the different types of germplasm such as spring versus winter types. These genes tend not to vary across locally adapted elite germplasm; thus breeding for a particular local environment requires deliberate selection of germplasm that when crossed would create new allelic combinations leading to superior high yielding genotypes for the target environment. The work described in this thesis was designed with a similar purpose in mind, specifically to identify novel alleles that control yield and adaptive traits of barley in the Mediterranean-type environment of South Australia.

The three mapping populations used for this study were developed from inter-crossing well-characterized elite Australian genotypes. The lack of association of the major phenology genes (*Ppd-H1*, *Ppd-H2*, *Vrn-H1*, *Vrn-H2* and *Vrn-H3*/*HvFT1*) with any of the traits evaluated in this study (see Chapters 4 and 5) supports the objective for which the parents were selected. Only two populations based on elite x elite crosses have been used previously in Australia, namely the Mundah x Keel and Tallon x Kaputar populations (see Chapter 2), but these suffered from the limitations of small population size and were not targeted for studying yield and adaptation.

6.2 The link between mapped QTL and known phenology genes

The high-density genetic linkage maps constructed using the three inter-connected populations were comprised of GBS markers and markers for the phenology genes, and were the first such map developed for Australian germplasm. The genetic linkage maps previously constructed were based on the populations reviewed in Chapter 2 of this thesis and were of low resolution due to small population size and low marker coverage. The incorporation of the known phenology genes in the maps constructed in this study has helped as a reference to compare QTL positions with previously reported QTL in which these known genes were also mapped. Within this study, mapping of known phenology genes has helped to ascertain whether a QTL identified for a trait in the three populations were related to known genes or not. Examples include the maturity QTL *QMat.CF-2H.2* and *QMat.FW-2H*, identified in the CF and FW populations, respectively, which were found to be the same based on their co-segregation with *APETALA2* (*HvAP2*), and *Qmat.CF-5H.2* and *QMat.CW-5H* in CF and CW populations which were found to be the same based on their coincidence with the *Phytochrome C* (*HvPhyC*) gene.

The availability of the barley physical maps (IBSC 2012 and POPSEQ) has helped not only to assign the GBS markers to the correct chromosomes and to orient the chromosomes, but also to align the QTL markers on the physical map. Accordingly, significant markers for QTL identified for different traits in the three populations were found to be co-located on the physical map (see supplemental Fig. 5.6, chapter 5). The following are examples of these types of co-located QTL:

- Two early vigour QTL (*QEv.FW-2H.1* and *QEv.CW-2H*), a leaf rolling QTL (*QLrol.FW-2H*), and a yield QTL (*QYld.CW.2H.1*) were co-located and were coincident with the *HvCEN*/*EPS2* locus.
- A maturity QTL *QMat.FW-2H* and a yield QTL *QYld.FW-2H.3*.
- Two grain plumpness QTL *QPlum.CF-4H.1* and *QPlum.FW-4H.1*, a maturity QTL *QMat.CW-4H*, and a yield QTL *QYld.FW-4H*.
- A maturity QTL *QMat.CW-5H* and leaf waxiness QTL *QWax.CW-5H*.
- A maturity QTL *QMat.CW-6H* and a yield QTL *QYld.CF-6H*.

- A maturity QTL *QMat.CW-7H* and a yield QTL *QYld.CF-7H*.

The co-locations of QTL for different traits suggest possible pleiotropic effects of the QTL on the traits. The physical map was also helpful in identifying QTL related markers that are coincident with the known phenology genes, a good example being the *HvCEN*/*EPS2* locus that is mentioned above.

6.3 QTL x Environment interaction and its implications for future breeding strategy

Genotype x environment interaction (G x E) complicate improvement of complex quantitative traits through phenotype-based selection. G x E can be decomposed into QTL x Environment interaction components that can be used for marker-assisted selection in crop improvement (Wang et al. 1999).

All the maturity, yield and grain plumpness QTL detected in this study, with the exception of *QMat.CF-5H.1, QYld.FW-2H.1* and *QPlum.FW-4H.1*, showed significant QTL x E interaction. Two types of QTL x E interactions were observed. The first one was the type where the QTL showed different additive effects across different environments but the changes were only of quantitative nature. This means only the magnitude of the effects differed while the high value alleles remain the same at all environments. The second type of QTL x environment interaction was the crossover interaction where different alleles were expressed in different environments (see tables in Chapters 4 & 5).

The QTL with non-cross over type interactions could be directly utilized in MAS to develop widely adapted and stable varieties. Marker assisted pyramiding of these QTL could increase their cumulative phenotypic effects. Two approaches could be pursued to utilize the QTL with the crossover type of interaction.

1. The QTL exhibiting major effects at specific environments could be directly used in marker-assisted selection to develop varieties with specific adaptation to that environment. However, this may not be feasible for efficient resource utilization and commercial use.
2. The most reliable option would be to undertake further pre-breeding work to pyramid the QTL alleles into common genetic backgrounds using the linked molecular markers to develop widely adapted varieties. This approach matches with the prime goal of most breeding programs, it is most efficient in resource utilization, and the varieties developed

will be widely adapted and commercially sustainable. Several examples of successful QTL pyramiding are described in the literature. For example, QTL pyramiding has been applied for stripe rust resistance in barley (Castro et al. 2003; Richardson et al. 2006), and for grain quality, heading date and yield in rice (Wang et al. 2011). The marker assisted pyramiding of eight QTL/genes for seven different traits including grain quality traits and resistance to the three rusts in wheat (Tyagi et al. 2014), and QTL pyramiding for durable blast resistance in rice (Fukuoka et al. 2015) are recent examples.

In this study, the detected QTL for all traits had minor to moderate phenotypic effects except *QLwax.CW-2H*, *QYld.CW-2H.1*and *QYld.CF-6H* that accounted for 20%, 24.4% and 25.0%, of the phenotypic variation, respectively, at specific environments. QTL pyramiding could accumulate the minor QTL into a single genotype to increase the overall gains.

6.4 Conclusions

QTL analysis using three interconnected populations and the corresponding dense genetic maps has helped identify the genomic regions underlying variation for yield, grain plumpness, maturity, early vigour, normalized difference vegetation index, chlorophyll content, leaf waxiness and leaf rolling. Incorporation of phenology genes with known genomic positions to the GBS maps has served as a reference against which the relative positions of QTL were compared between populations and with published QTL positions. The availability of the barley physical map was helpful in aligning significant markers across populations and traits.

The analysis identified 66 QTL across eight traits studied in the three populations. Seven out of 13 QTL identified for maturity are related to known phenology genes. The remaining five QTL may indicate new maturity genes that warrant further study. The major phenology genes, including the photoperiod response (*Ppd*) and the vernalization sensitivity (*Vrn*) genes, were associated with some but not all of the traits studied. Only five of the 18 yield QTL were coincident with maturity QTL. A major effect yield QTL that is independent of maturity and explaining up to 24.4% of phenotypic variance was found on chromosome 2H. This QTL is coincident with the *HvCEN*/*EPS2* locus but further study will be required to verify whether this QTL is *EPS2* itself, or a novel allele linked to the *HvCEN*/*EPS2* locus.

The three interlinked populations with high-density linkage maps are a significant resource for examining the genetic basis for barley adaptation in low to medium rainfall in the Mediterranean type environment. The identification of a QTL for increased yield that is not associated with maturity differences provides an opportunity to apply marker-based selection for grain yield. Marker assisted pyramiding of the various minor effects and environment specific yield QTL could be a desirable breeding strategy for developing widely adapted and high yielding barley varieties for Australia.

The identification of the *EARLINESS PER SE 2* (*EPS2*) or a novel allele linked to this locus as a major regulator of barley yield under Australian conditions represents a meaningful contribution to the knowledge of barley genetics.

6.5 References

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Appendices

Supplemental Tables and Figures in Chapter 3

Table S3.1 Programs and protocols used for PCR and HRM genotyping

PCR program for HvZCCTHc				PCR protocol for HvZCCTHc and Vrn-H2	
Step		Temperature (°C)	Time	Component	1 reaction (µl)
1	Denaturation	94°C	10 minutes	10x PCR buffer (-$MgCl_2$)	2.0
2		94°C	1 minute	50mM $MgCl_2$	0.6
3	Annealing	70°C	30 second	2.5 µm dNTP	1.6
4	Extension	72°C	2 minutes	Primer: F (10nm)	0.6
5	Step 2 for 9 more times			Primer: R (10nm)	0.6
6		94°C	1 minute	Platinium Taq polymerase	0.1
7		60°C	30 second	Milli-Q water	12.5
8		72°C	2 minutes	DNA template	2.0
9		Step 6 for 24 more times		**Total**	**20.0µl**
10		72°C	5 minutes	**Protocol used for HRM genotyping**	
11		15°C	forever		
				Component	**1 reaction (µl)**
PCR program for Vrn-H2				Molecular biology grade water	3.95
Step		Temperature (°C)	Time	10x Immolase PCR (1.5mM $MgCl_2$)	1.0
1	Denaturation	94°C	5 minutes	50mM $MgCl_2$	1.2
2		94°C	30 second	2.5µm dNTP	1.0
3	Annealing	50°C	30 second	Primer: F	0.2
4	Extension	72°C	2 minutes	Primer: R	0.2
5	Step 2 for 37 more times			10mg/mL BSA (Bioline) 100x	0.1
6		72°C	10 minutes	5U/ µl Immolase (Bioline)	0.05
7		15°C	forever	50 µm SYTO® 9 dye	0.3
				DNA template (10ng/ µl)	2.0
				Total	**10.0 µl**

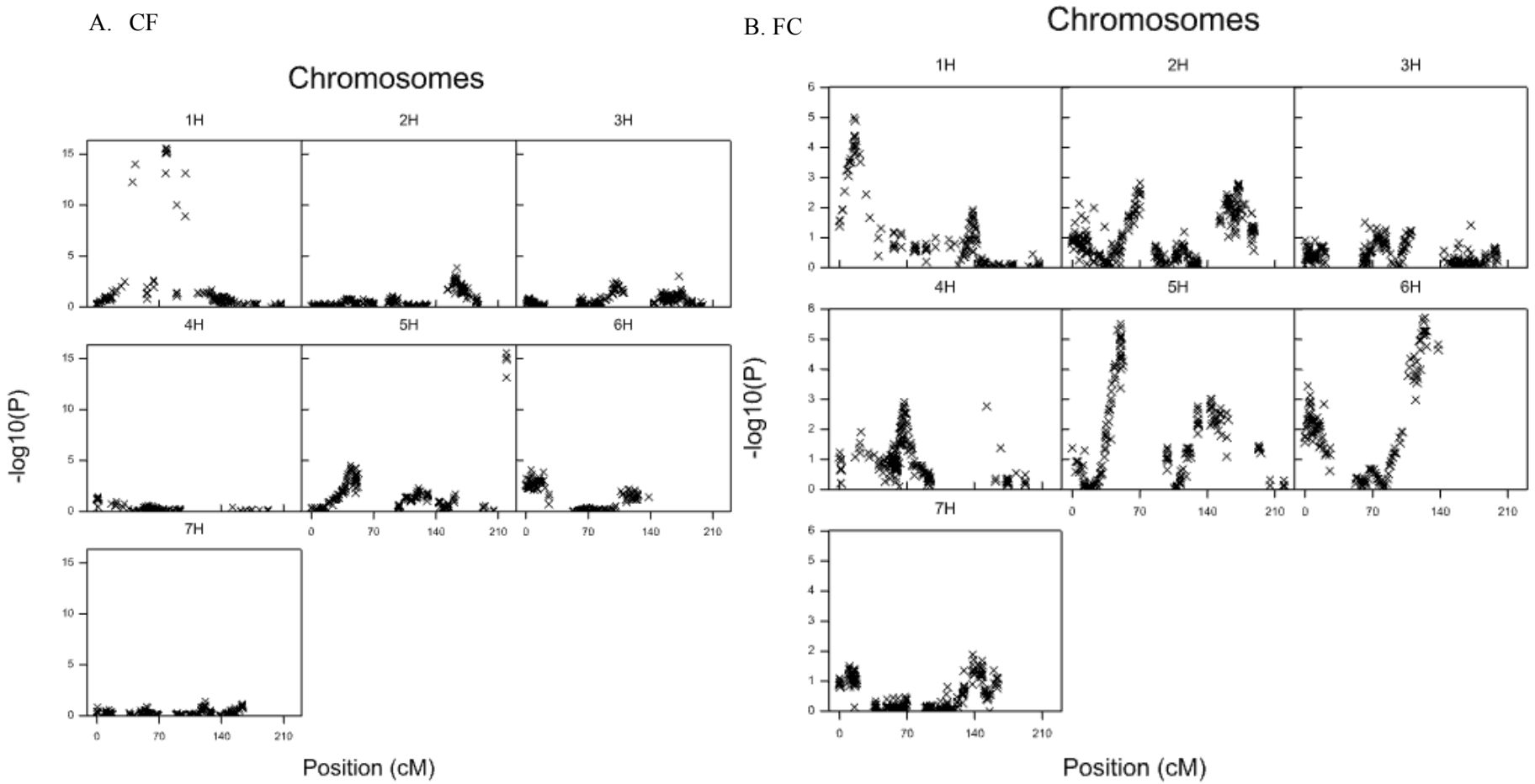


Fig.S3.1 Trellis plot of segregation distortion in reciprocal crosses of CF/FC genetic linkage map (CF (A) and FC (B))

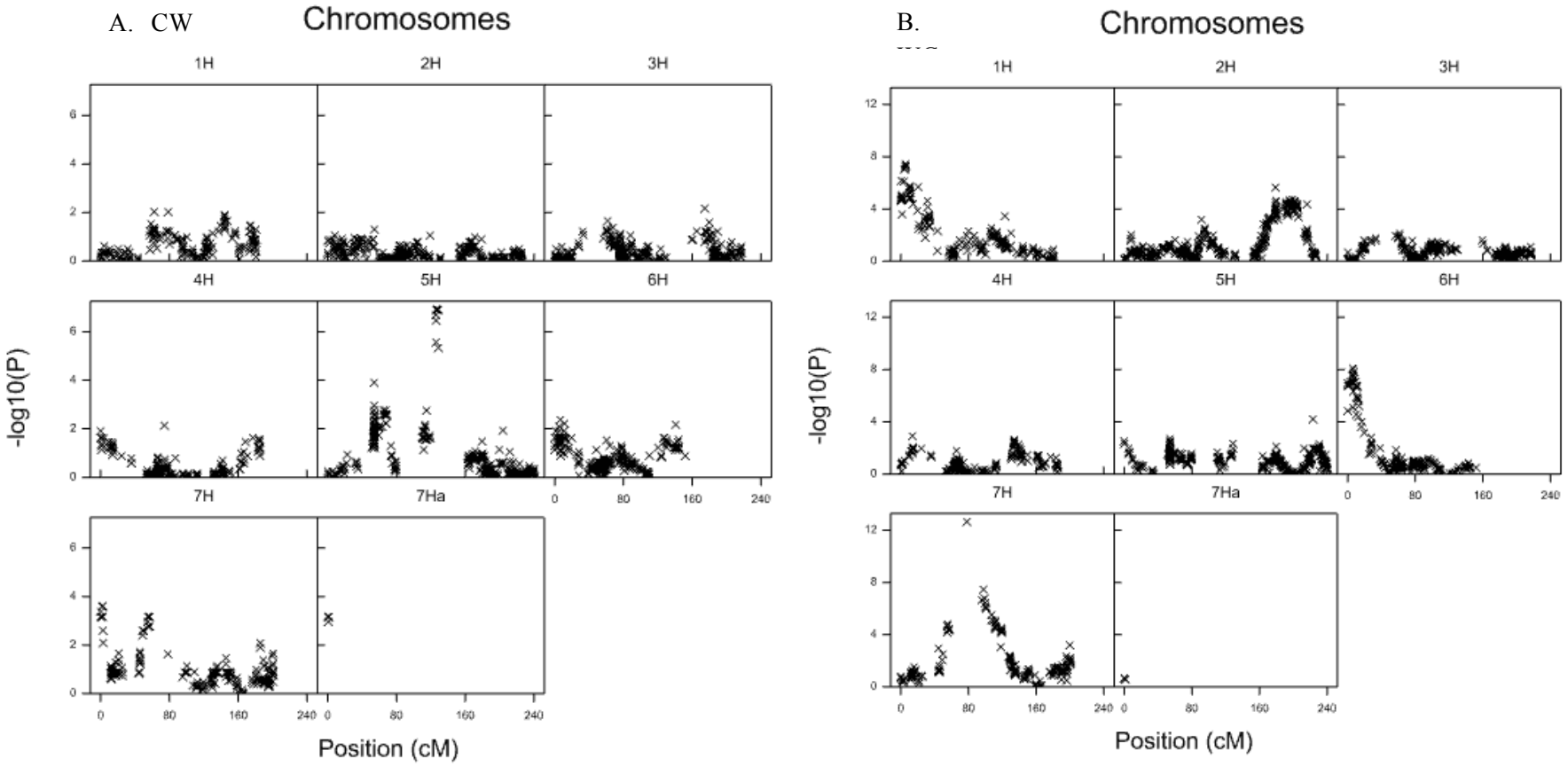


Fig. S3.2 Trellis plot of segregation distortion in reciprocal crosses of CW/WC genetic linkage map (CW (A) and WC (B))

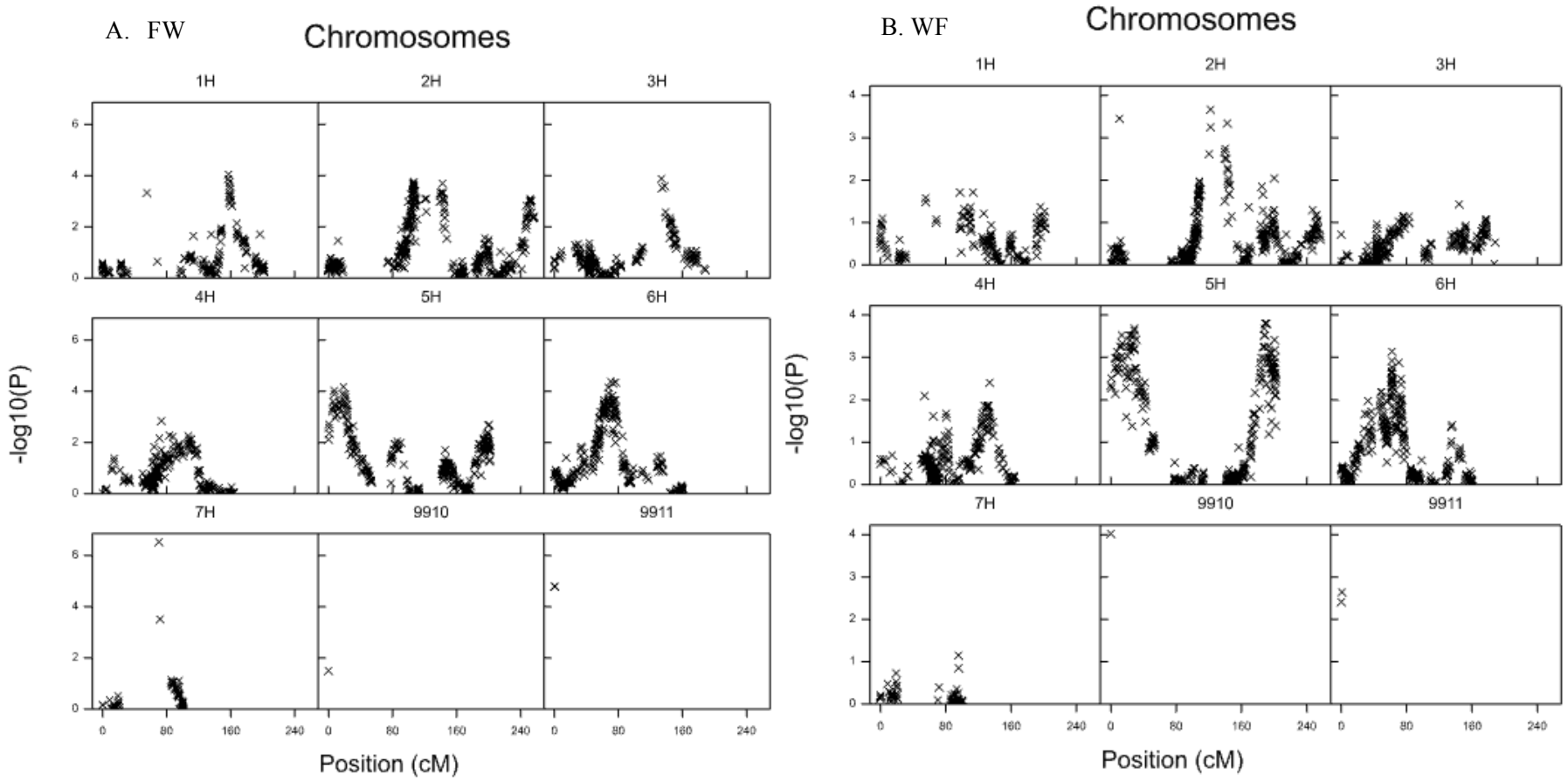


Fig. S3.3 Trellis plot of segregation distortion in reciprocal crosses of FW/WF genetic linkage map (FW (A) and WF (B))

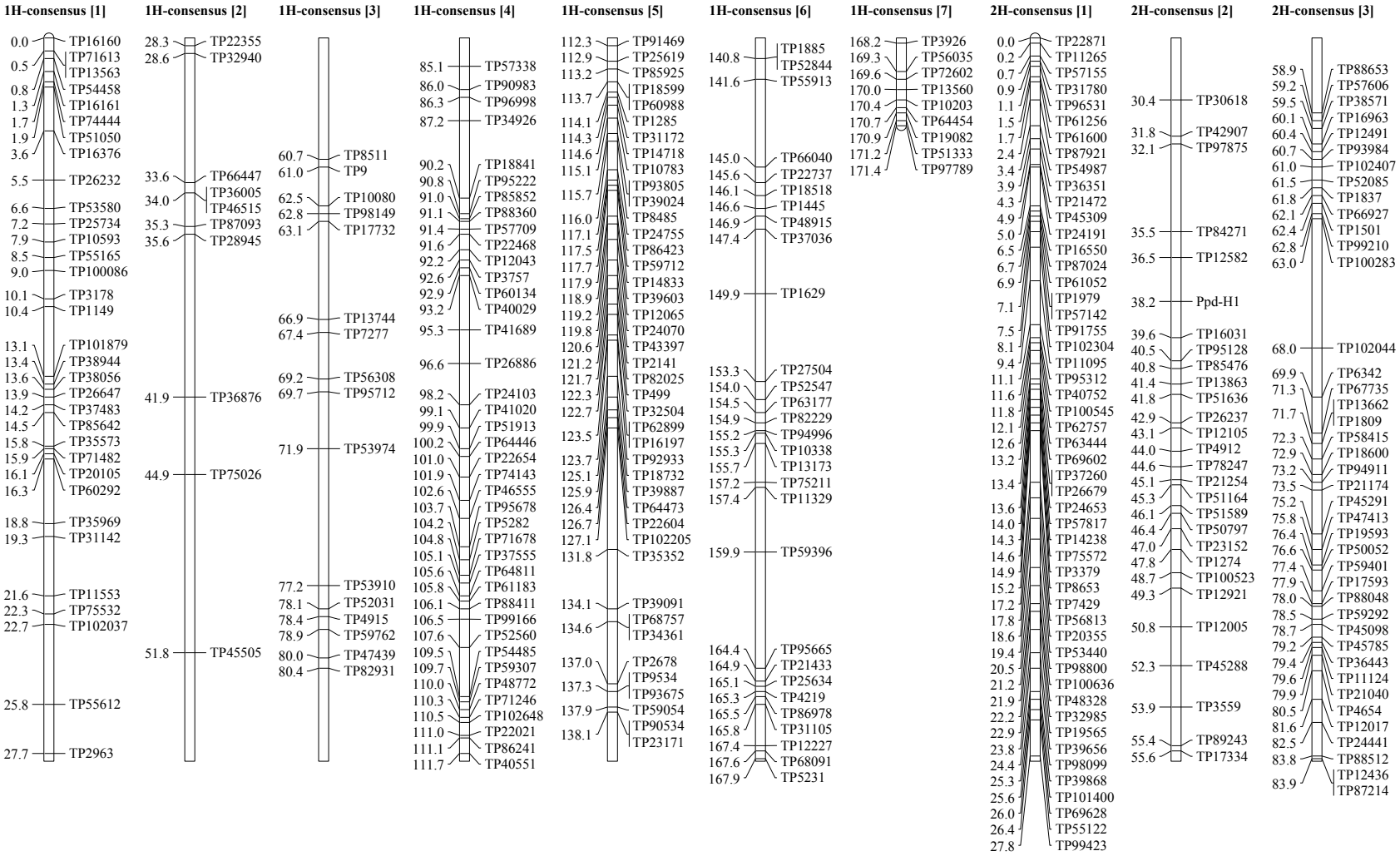


Fig. S3.4 Consensus genetic linkage maps of CF, CW and FW populations. Each linkage group is broken in to multiple charts to fit to the page.

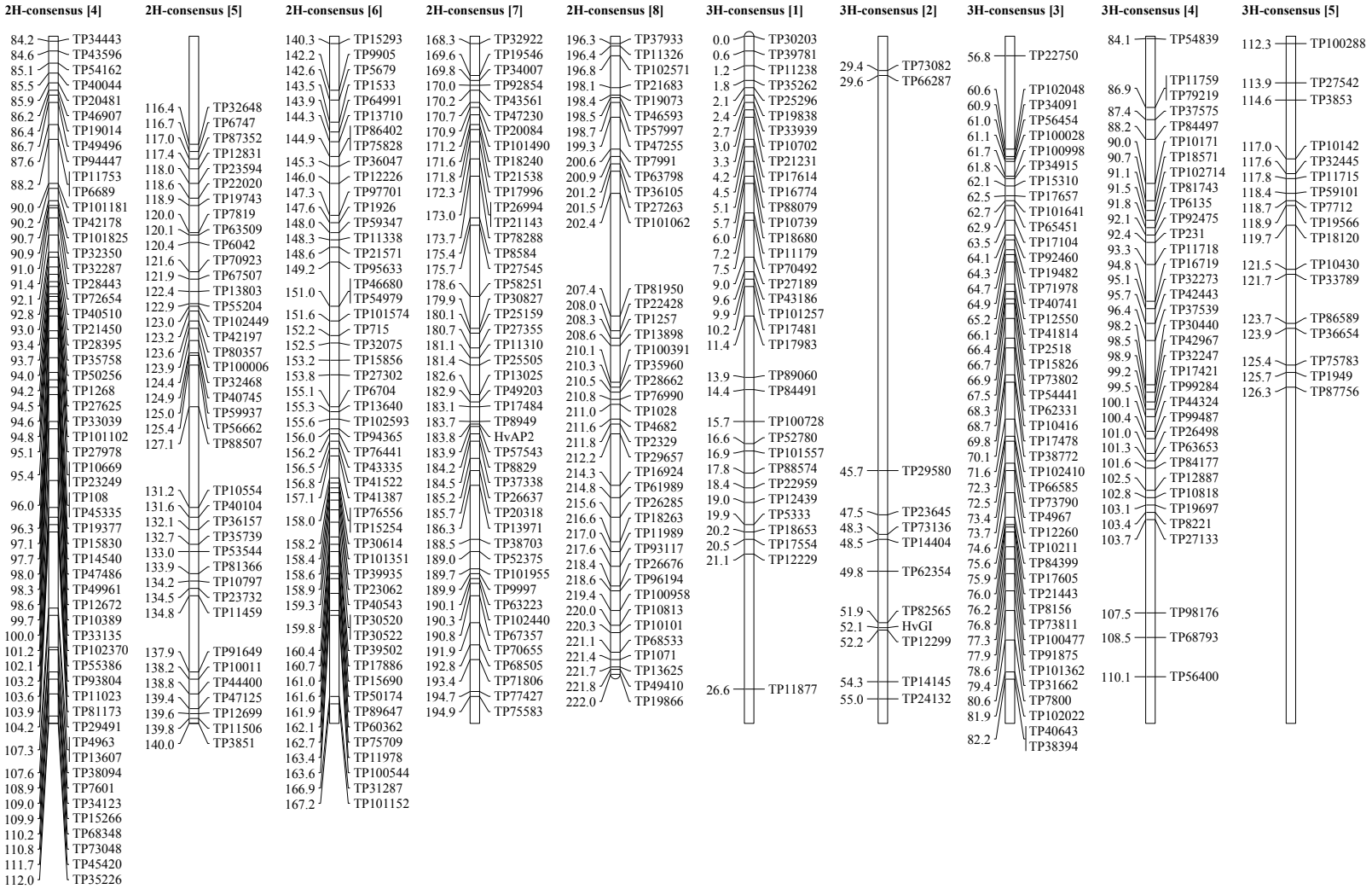


Fig. S3.4 (*continued*)

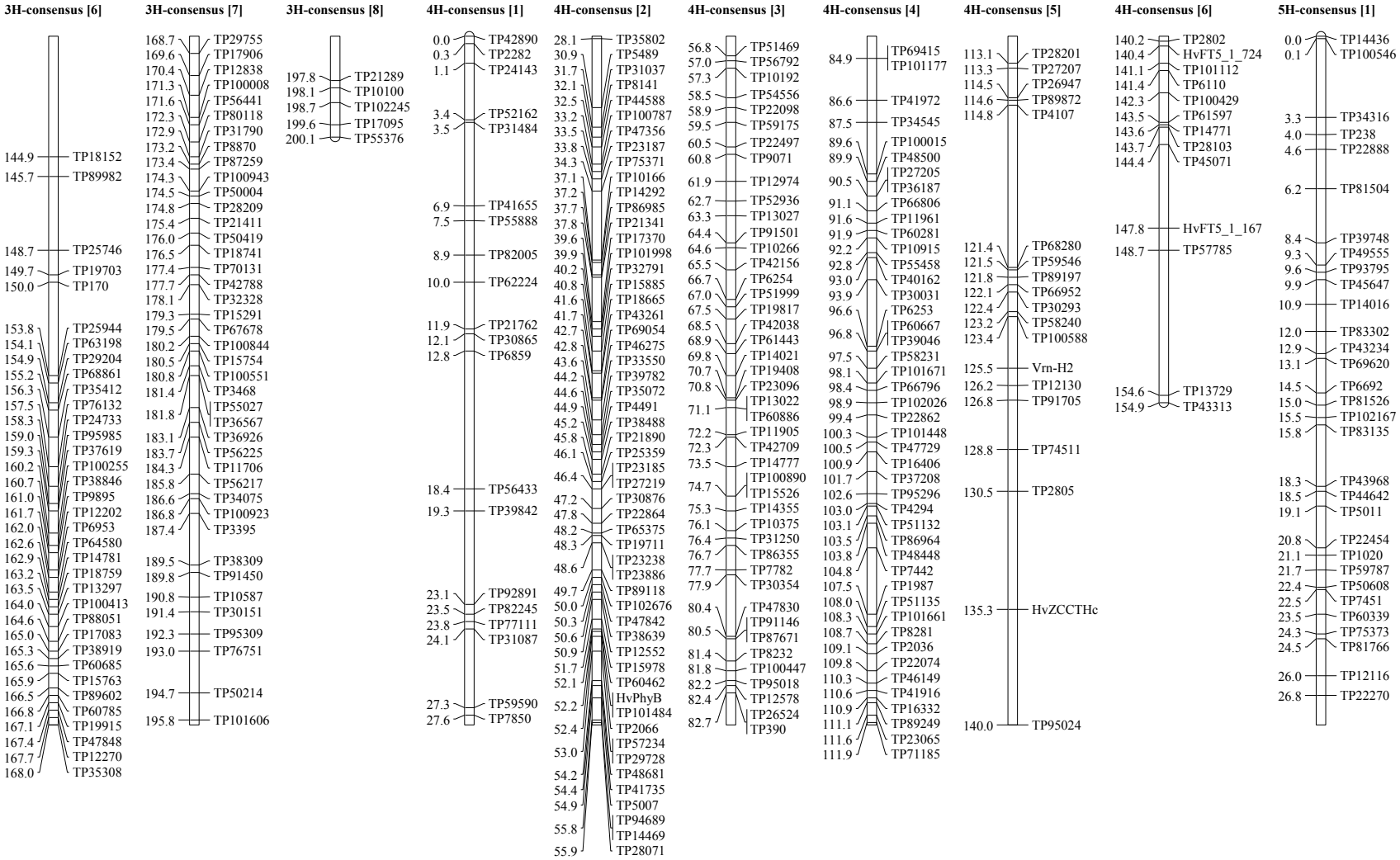


Fig. S3.4 (*continued*)

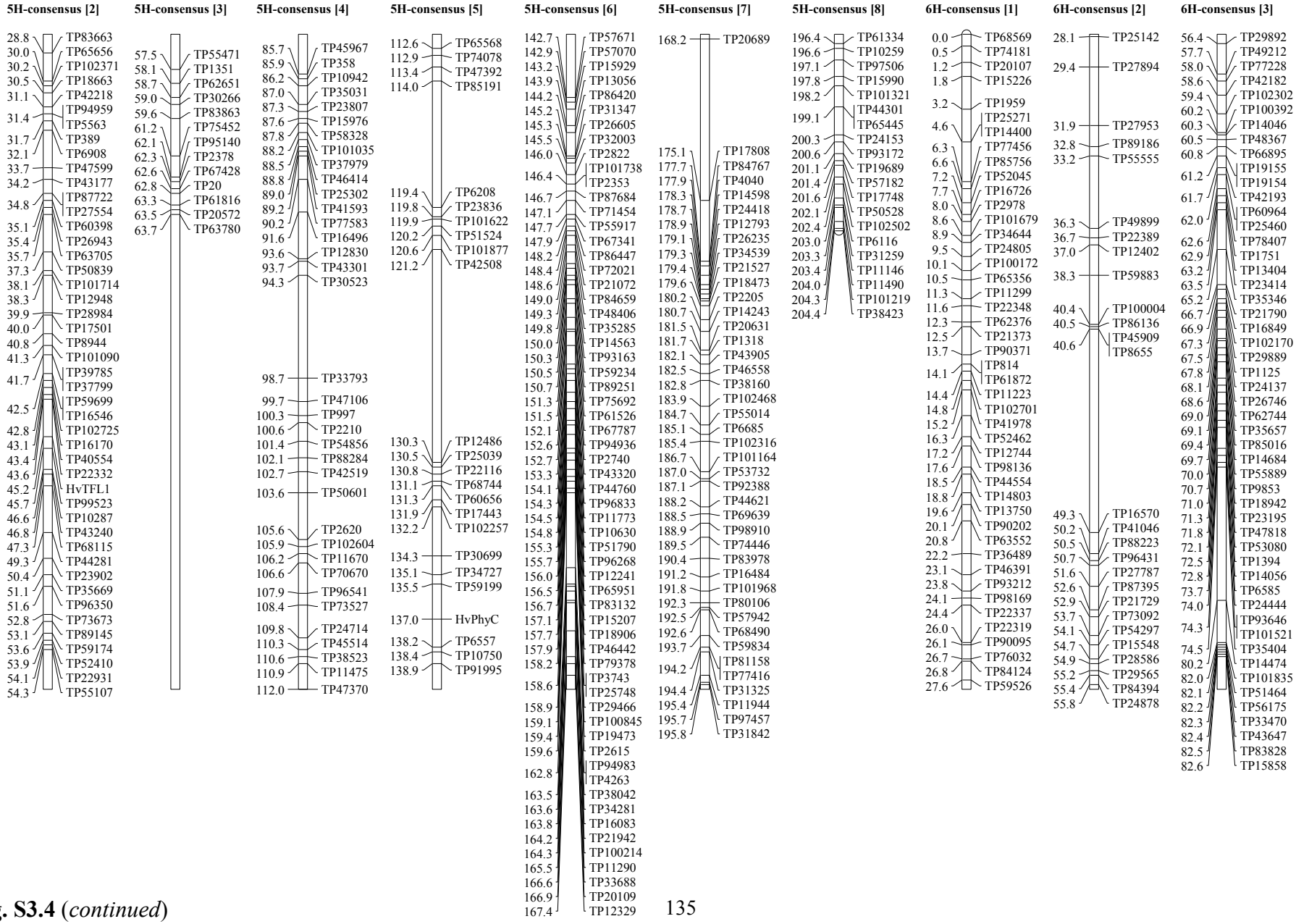


Fig. S3.4 (*continued*)

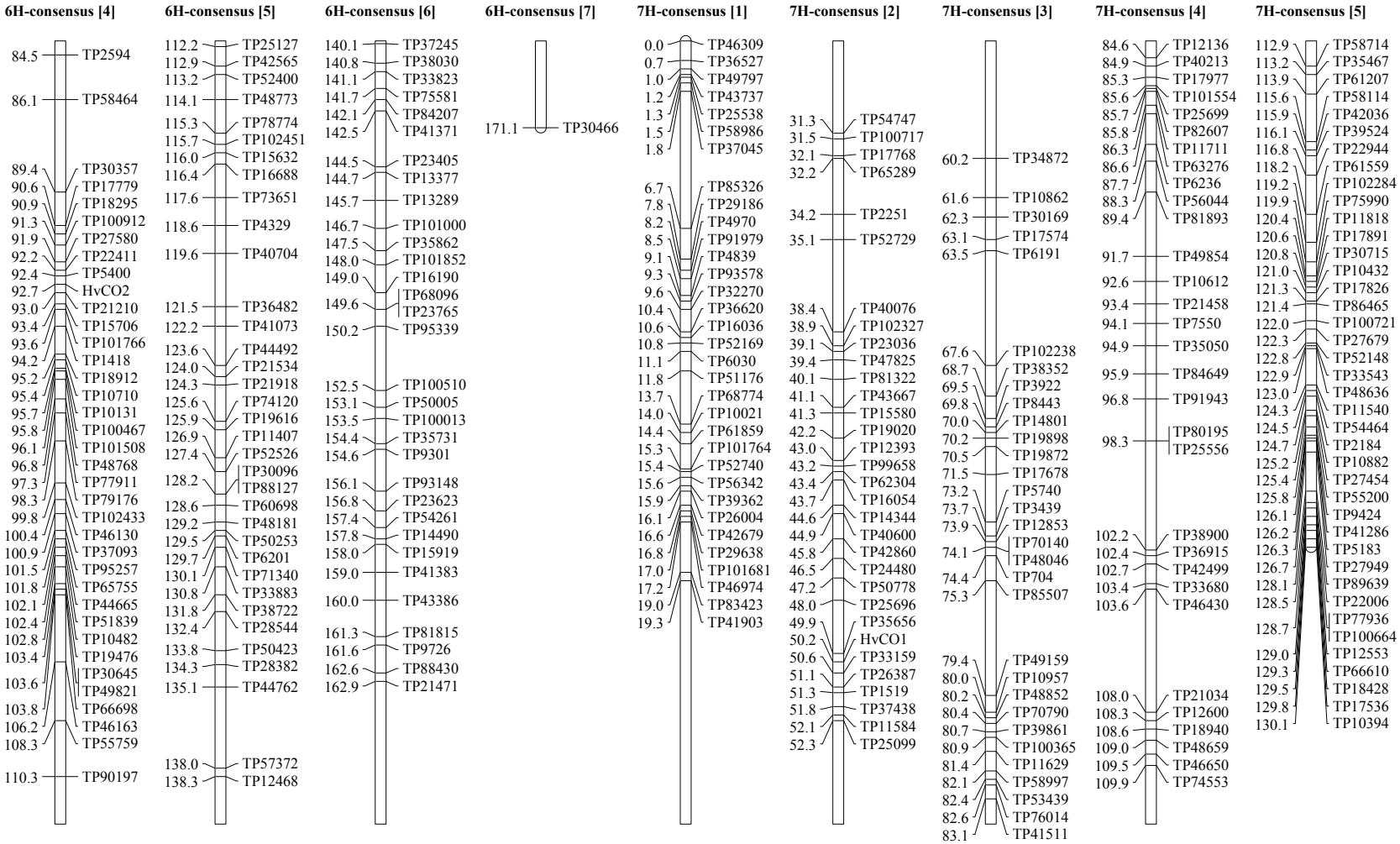


Fig. S3.4 (*continued*)

Supplemental Tables and Figures in Chapter 4

Table S4.1 Soil chemical and physical properties of trial sites (2012)

					mg/Kg									mg/Kg				meq/100g					
Environment	Soil Core (cm)	Colour	Gravel %	Texture	NH_4	$N0_3^-$	P Colwell	K Colwell	S	Organic C %	Conductivity dS/m	pH 1 ($CaCl_2$)	pHl (H_2O)	DTPA Cu	DTPA Fe	DTPA Mn	DTPA Zinc	Exc. Al	Exc. Ca	Exc. Mg	Exc. K	Exc. Na	B Hot $CaCl_2$
RAC12	0-20	BR	0	3	6	12	44	455	8.1	1.13	0.129	6.8	7.4	1.75	18.99	35.35	2.03	0.058	15.82	2.76	1.22	0.21	1.75
	20-40	OR	0	3	7	3	11	528	4.2	0.4	0.127	7.8	8.6	0.98	12.22	5.51	0.15	0.058	12.07	2.62	1.33	0.14	1.29
	40-60	GRPK	0	3	3	19	16	231	7.1	0.54	0.158	7.6	8.6	1.96	10.39	8.45	0.47	0.032	17.7	2.23	0.58	0.31	1.57
SWH12	0-20	LTBR	0	2.5	7	6	35	512	3.7	0.77	0.116	7.4	8	0.68	11.26	9.86	1.03	0.023	7.32	1.35	1.24	0.05	0.59
	20-40	BRWH	0	3	4	16	37	357	5.1	0.99	0.182	7.5	8.3	1.82	15.42	19.01	0.97	0.029	18.49	2.27	0.96	0.25	1.69
	40-60	OR	0	3	11	4	3	529	3.6	0.3	0.136	7.9	8.8	1.16	6.29	4.01	0.24	0.049	12.74	3.99	1.29	0.35	2.11
	60-80	OR	0	3	9	3	4	661	2.9	0.27	0.204	8.2	9.2	1.28	9.35	2.58	0.82	0.046	9.07	5	1.54	1.75	6.51
MRC12	0-40	GRPK	0	3	5.67	7.33	16.00	490.67	37.37	0.73	0.43	8.10	8.90	0.71	14.08	14.36	0.48	0.02	14.70	2.24	1.25	2.47	10.20
	40-80	BRWH	0	3	2.67	17.00	3.67	342.33	196.50	0.39	1.58	8.37	9.33	0.32	12.96	2.04	2.32	0.03	11.85	4.70	0.86	8.52	26.73

Table S4.2 Phenotypic correlation coefficients of Zadok's scores among six environments in Commander x Fleet (CF), Commander x WI4304 (CW) and Fleet x WI4304 (FW) populations

		MRC12	MRC123	RAC12	RAC13	SWH12	SWH13
CF	MRC12	-					
	MRC13	0.23**	-				
	RAC12	0.17*	0.40***	-			
	RAC13	0.23***	0.45***	0.29***	-		
	SWH12	0.36***	0.46***	0.30***	0.33***	-	
	SWH13	0.41***	0.49***	0.28***	0.44***	0.36***	-
CW	MRC12	-					
	MRC13	0.44***	-				
	RAC12	0.37***	0.42***	-			
	RAC13	0.35***	0.43***	0.34***	-		
	SWH12	0.48***	0.41***	0.30***	0.37***	-	
	SWH13	0.30***	0.21**	0.18**	0.24***	0.26***	-
FW	MRC12	-					
	MRC13	0.52***	-				
	RAC12	0.28***	0.41***	-			
	RAC13	0.36***	0.64***	0.36***	-		
	SWH12	0.41***	0.54***	0.31***	0.41***	-	
	SWH13	0.47***	0.62***	0.26***	0.55***	0.39***	-

Environments: MRC12= Minnipa 2012, RAC12= Roseworthy 2012, SWH12= Swan Hill 2012, MRC13= Minnipa 2013, RAC13= Roseworthy 2013, SWH13= Swan Hill 2013

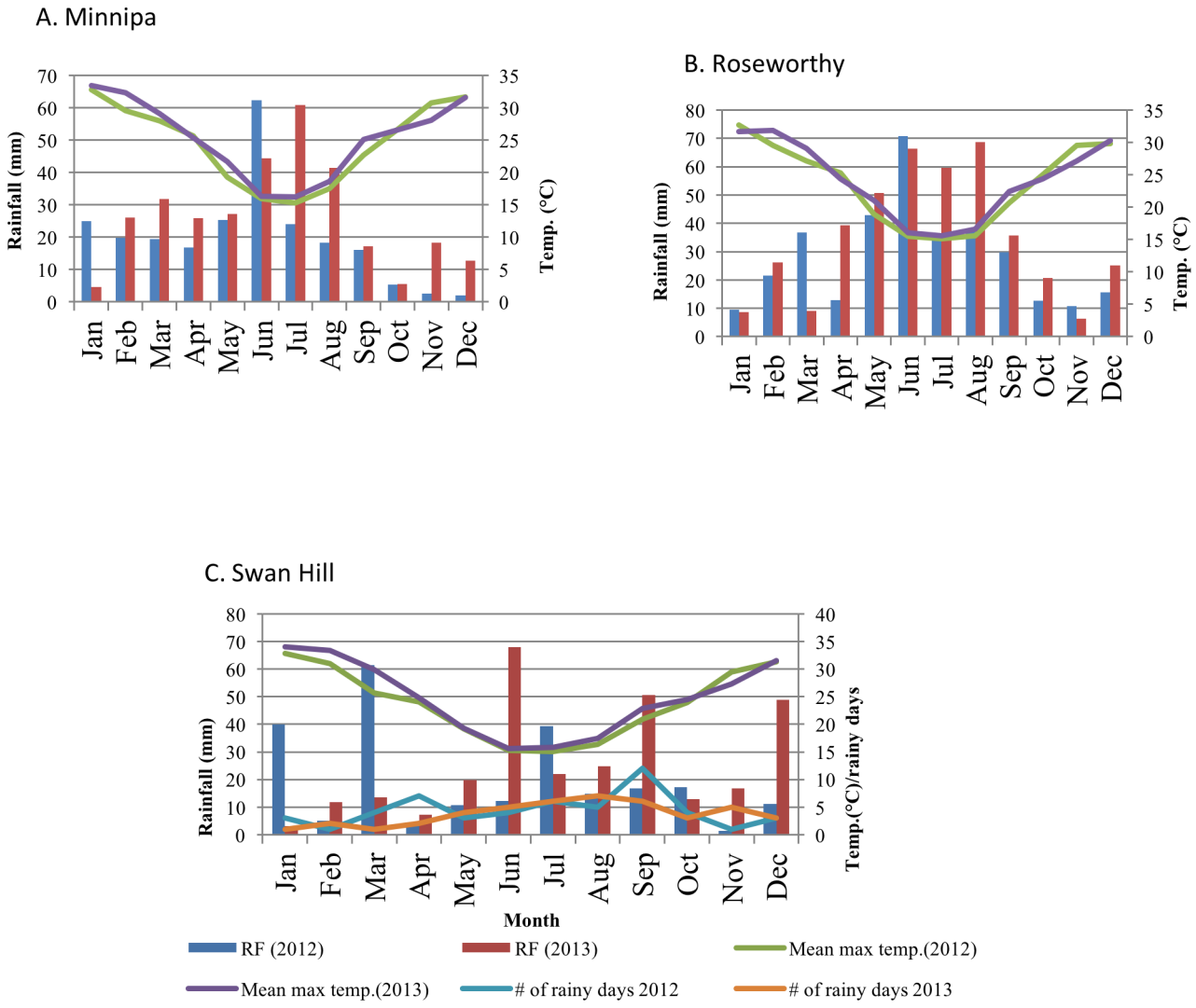
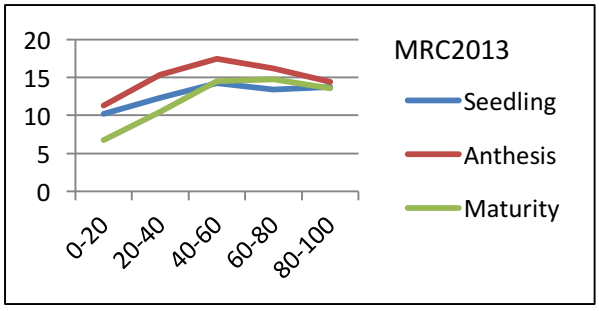
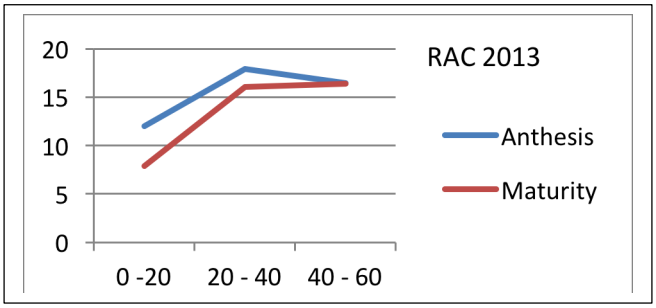
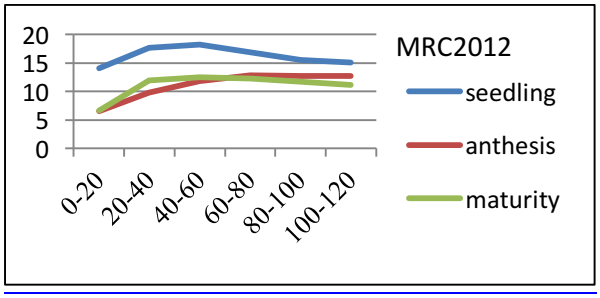
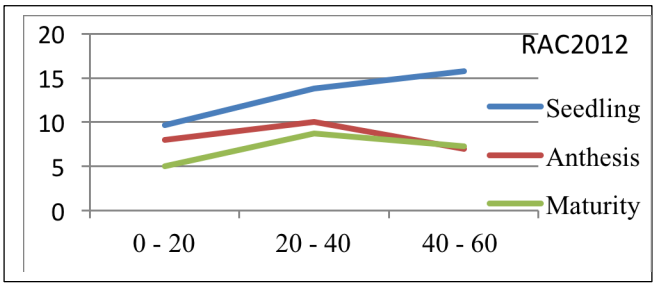


Fig. S4.1 Monthly rainfall (mm) and mean maximum temperature (°C) at Minnipa (A), Roseworthy (B) and Swan Hill (C) during 2012 and 2013 cropping seasons









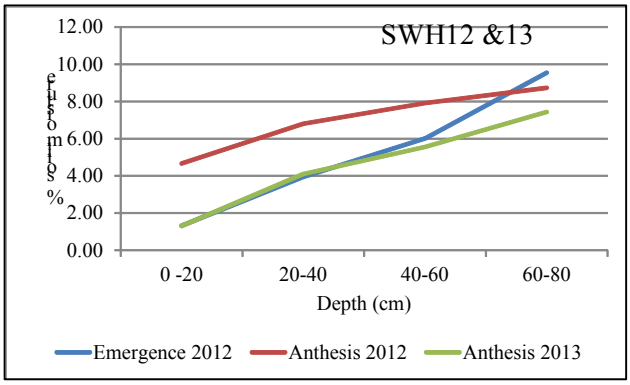


Fig. S4.2 Soil moisture profiles at different developmental stages

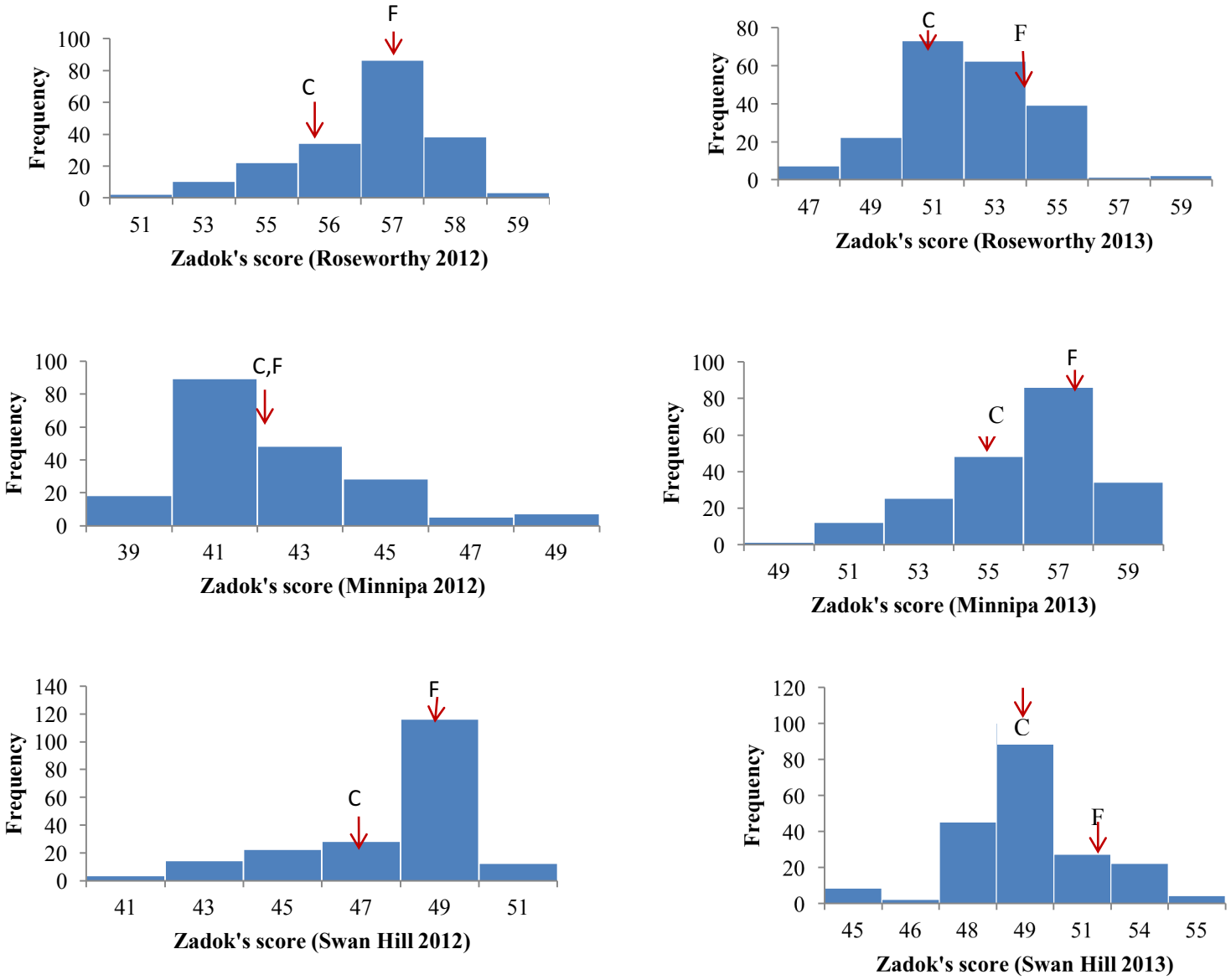


Fig. S4.3 Histograms of Zadok's scores at six environments (three sites and two years) in Commander x Fleet population

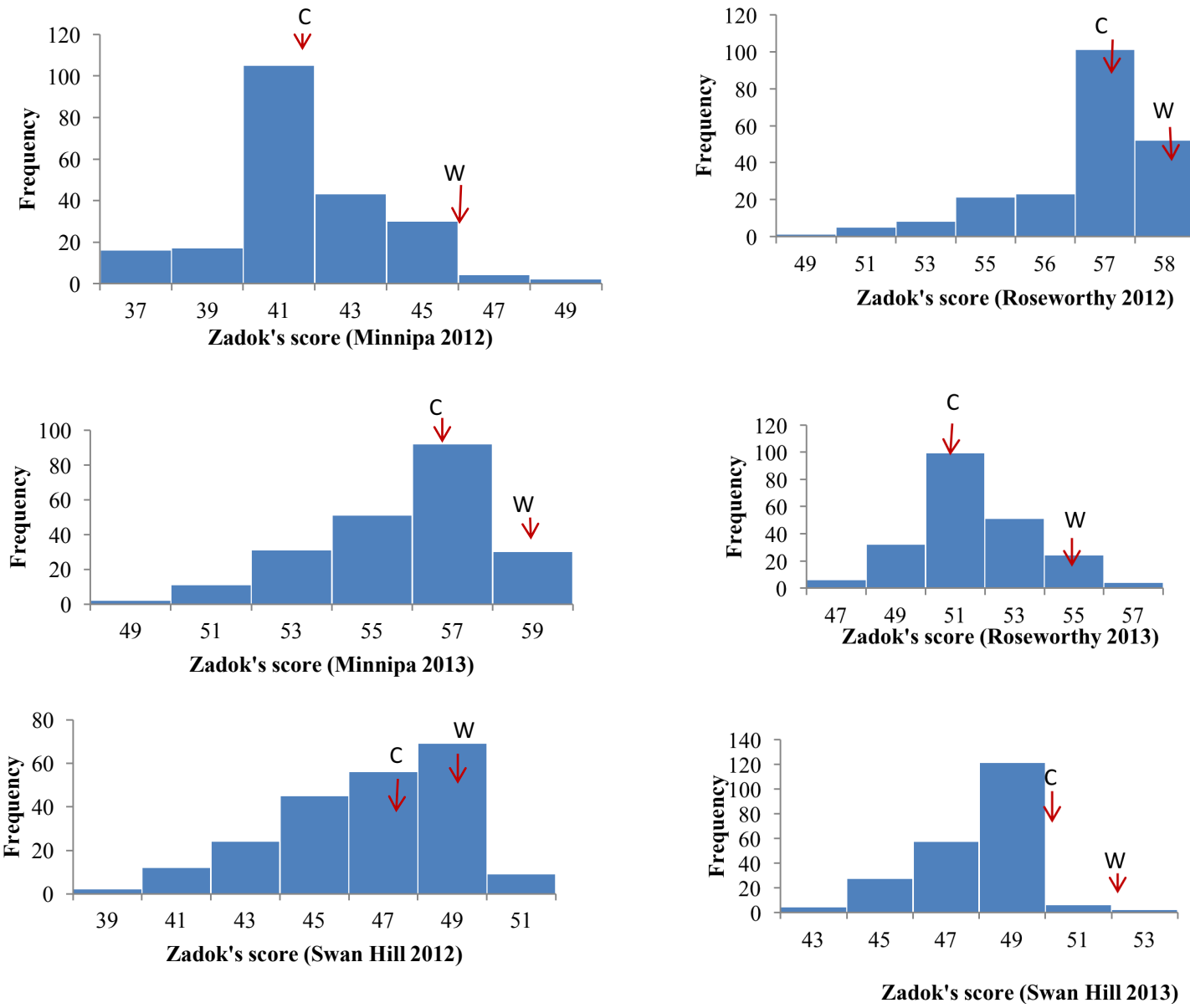


Fig. S4.4 Histograms of Zadok's scores at six environments in Commander x WI4304 DH population

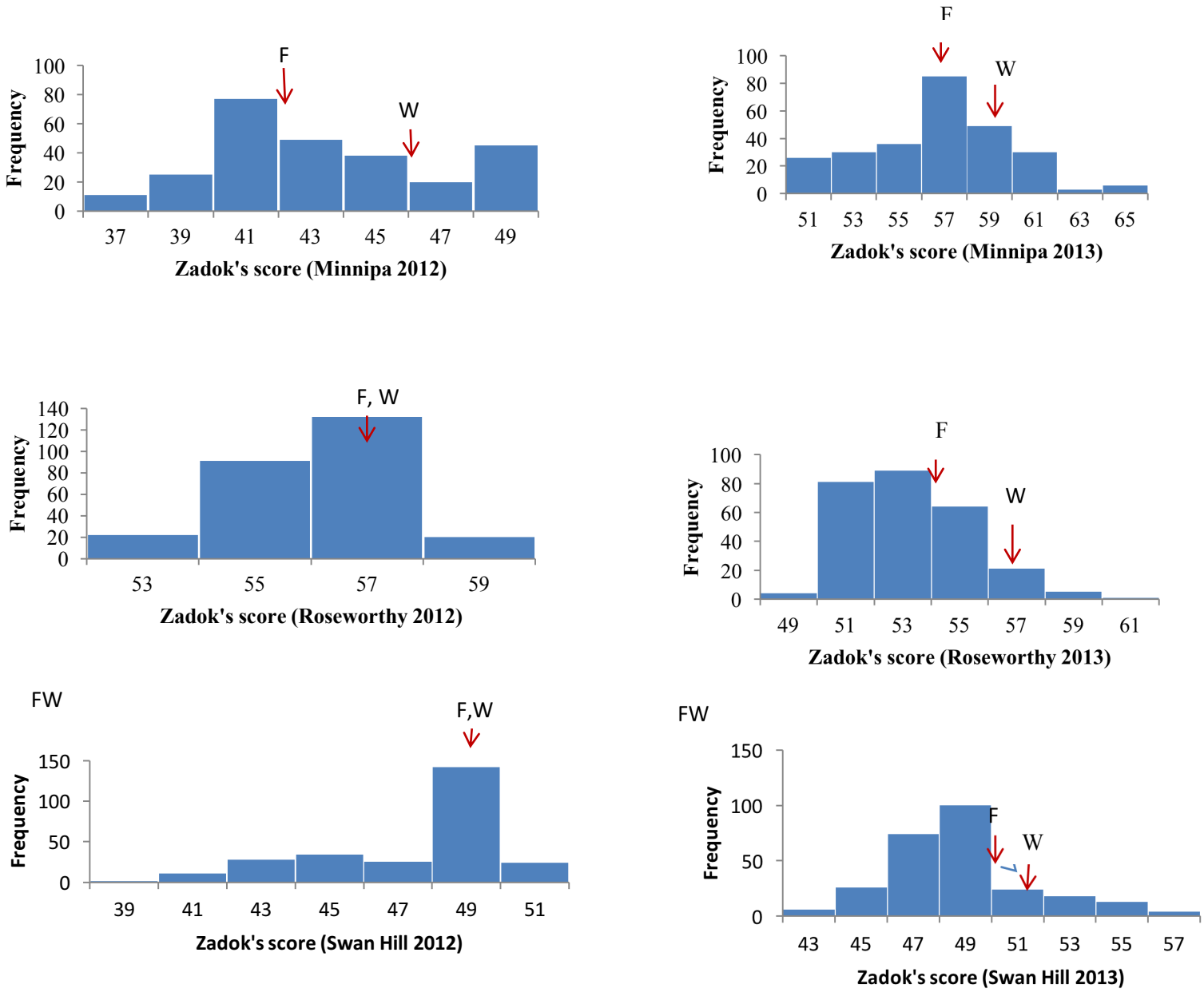


Fig. S4.5 Histograms of Zadok's scores at six environments (three sites and two years) in Fleet x WI4304 DH population

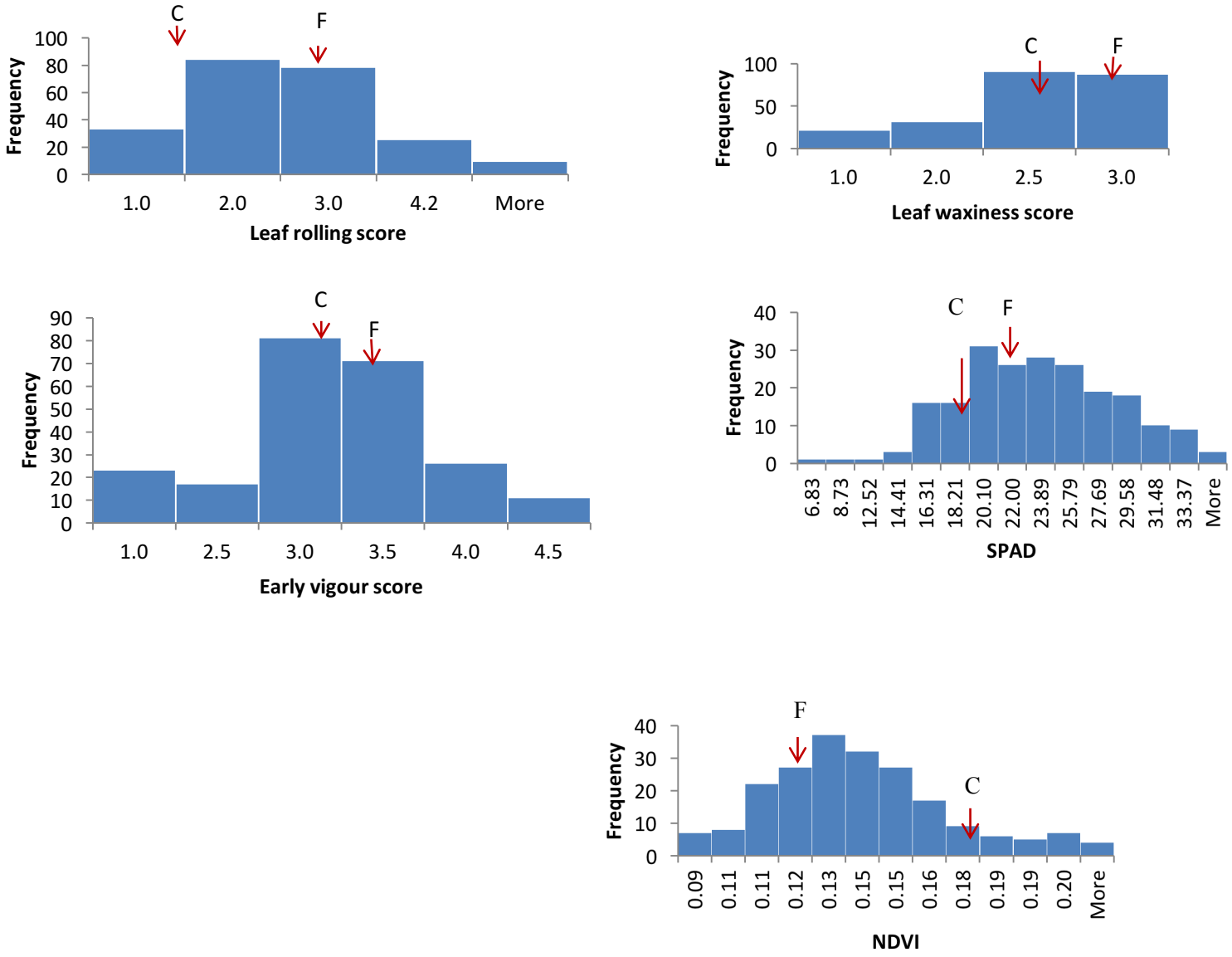


Fig. S4.6 Frequency distributions leaf rolling, leaf waxiness, early vigor, SPAD and NDVI in CF DH population

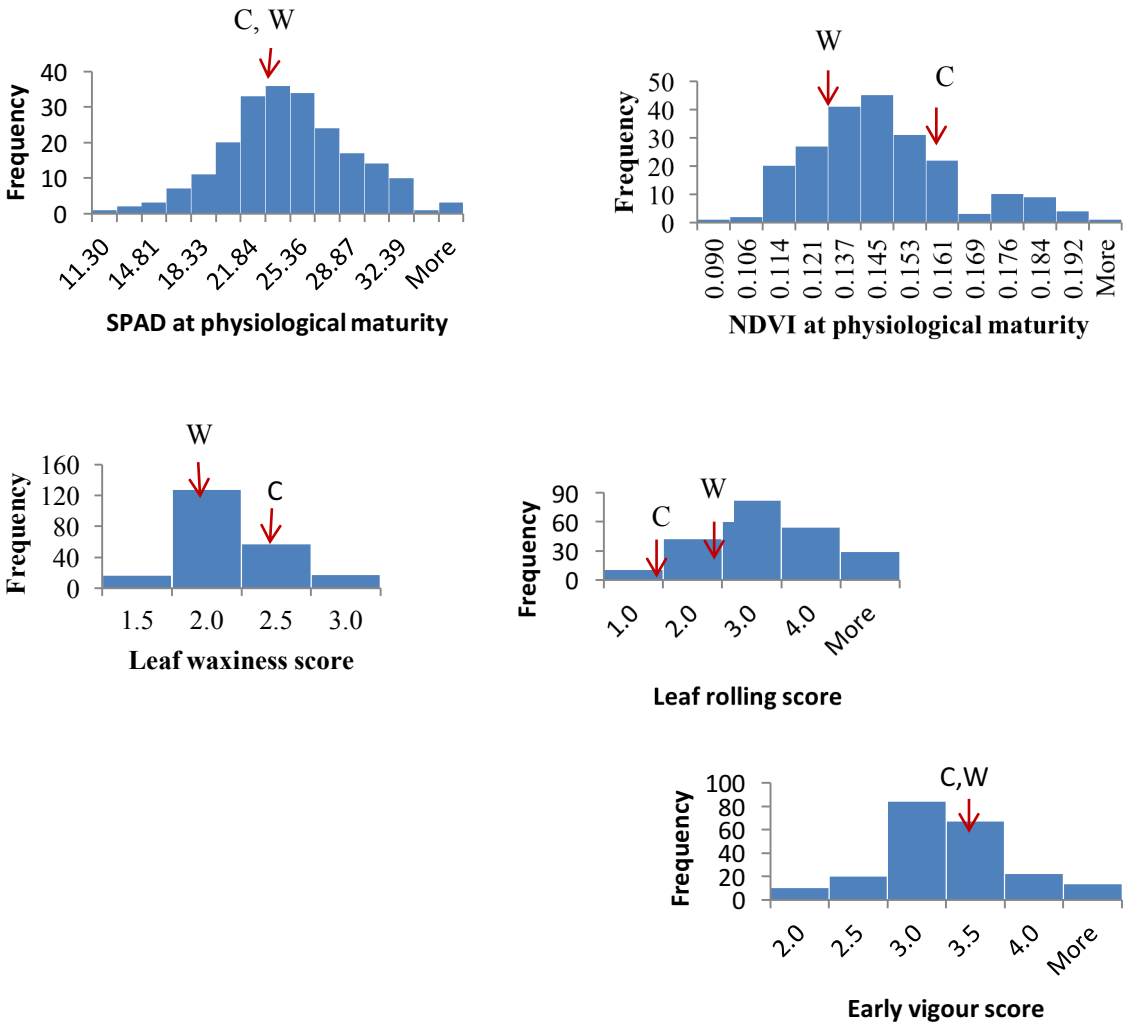


Fig. S4.7 Frequency distributions leaf rolling, leaf waxiness, early vigor, SPAD and NDVI in CW DH population

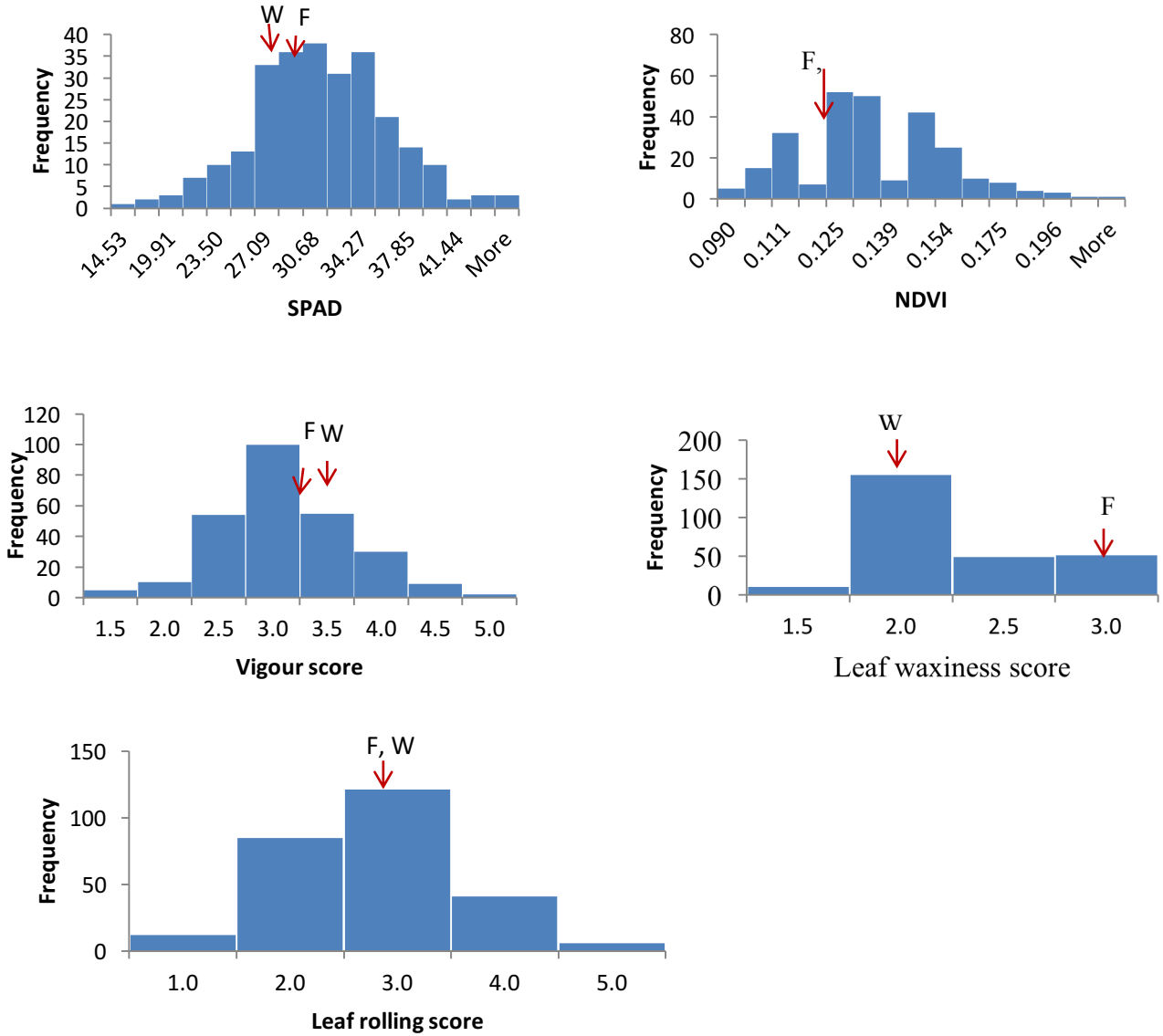


Fig. S4.8 Frequency distributions leaf rolling, leaf waxiness, early vigor, SPAD and NDVI in FW DH population

Supplemental Tables and Figures in Chapter 5

Table S5.1 Genetic correlations among six environments for yield in CF, CW and FW populations

Population	Environment	Genetic correlation (r)					
CF	MRC12	1					
	MRC13	0.45	1				
	RAC12	0.38	0.37	1			
	RAC13	0.21	0.21	0.18	1		
	SWA12	0.52	0.51	0.43	0.24	1	
	SWA13	0.30	0.30	0.25	0.14	0.34	1
CW	MRC12	1					
	MRC13	0.63	1				
	RAC12	0.71	0.65	1			
	RAC13	0.28	0.26	0.29	1		
	SWH12	0.69	0.63	0.72	0.28	1	
	SWH13	0.52	0.48	0.54	0.21	0.53	1
FW	MRC12	1					
	MRC13	0.55	1				
	RAC12	0.60	0.62	1			
	RAC13	0.45	0.66	0.51	1		
	SWH12	0.64	0.67	0.73	0.55	1	
	SWH13	0.51	0.67	0.58	0.61	0.62	1
		MRC12	MRC13	RAC12	RAC13	SWH12	SWH13

Table S5.2 Phenotypic correlations (r) between maturity and grain yield at six environments in CF, CW and FW populations

Population	Environment					
	MRC12	MRC13	RAC12	RAC13	SWH12	SWH13
CF	0.29***	0.21***	0.15*	0.18**	0.13ns	0.18**
CW	0.42***	-0.02ns	0.18**	0.02ns	-0.04ns	0.10ns
FW	0.14*	-0.14*	0.26***	-0.09ns	0.2***	-0.06ns

*= significant (P< 0.05) **= highly significant (P<0.01), ***= very highly significant (P<0.001), ns= non-significant

Table S5.3 Yield QTL after adjustment for maturity score in CF, CW and FW populations

QTL	Significant marker	Chr.	Position	LOD	QTL x E	PVE (%)	QTL additive effects					
							MRC12	MRC13	RAC12	RAC13	SWH12	SWH13
QYld.CF-2H	TP10554	2H	105.9	4.0	yes	2.8-7.4	-	-	0.102^{C}	-	-	0.06^{C}
QYld.CF-4H	TP40024	4H	64.4	3.6	yes	1.9-5.6	-	0.056^{C}	-	0.06^{C}	0.046^{C}	0.051^{C}
QYld.CF-6H	TP88355	6H	58.1	14.0	yes	1.8-21.7	0.022^{F}	0.065^{F}	0.05^{F}	0.202^{F}	-	-
QYld.CF-7H	TP81322	7H	50.2	4.4	yes	3.9-7.3	-	-	-	-	0.062^{C}	0.07^{C}
QYld.CW-2H.1	TP5613	2H	82.3	15.1	yes	4.3-23.0	0.04^{C}	-	0.09^{C}	-	0.15^{C}	0.08^{C}
QYld.CW-2H.2	TP41522	2H	165.0	7.5	yes	4.4-10.0	0.06^{W}	0.06^{W}	-	-	-	-
QYld.CW-6H.1	TP24121	6H	62.7	3.5	yes	3.5-10.2	0.03^{W}	-	-	-	-	0.11^{W}
QYld.CW-6H.2	TP77911	6H	83.0	3.0	yes	3.9-6.4	-	0.06^{W}	-	0.13^{W}	-	-
QYld.CW-7H	TP41903- TP89783 #	7H	40.7	4.5	yes	2.8-6.5	-	0.06^{C}	0.06^{C}	0.09^{C}	0.08^{C}	0.07^{C}
QYld.FW-1H	TP92933	1H	146.2	6.6	yes	1.5-6.5	0.027^{F}	0.08^{F}	-	0.042^{F}	-	0.061^{F}
QYld.FW-2H.1	TP60114	2H	108.6	6.0	no	2.3-7.4	0.05^{F}	0.05F	0.05^{F}	0.05^{F}	0.05^{F}	0.05^{F}
QYld.FW-2H.2	TP34123-TP7819 #	2H	129.8	3.3	yes	6.8	-	-	0.09^{W}	-	-	-
QYld.FW-2H.3	TP78288-TP88727 #	2H	203.3	7.2	yes	1.6-5.2	-	-	0.044^{W}	-	0.069^{F}	0.04^{W}
QYld.FW-4H	TP17370	4H	53.7	5.9	yes	2.1-6.3	-	-	-	0.09^{F}	-	0.04^{F}
QYld.FW-5H	TP100214	5H	162.5	4.0	yes	1.8-4.4	0.04^{F}	-	0.05^{W}	-	-	-
QYld.FW-6H.1	TP65356	6H	8.7	6.9	yes	1.7-9.2	-	0.08^{F}	0.05^{F}	0.05^{F}	-	0.08^{F}
QYld.FW-6H.3	TP77950	6H	37.2	3.2	yes	2.9-4.0	0.03^{F}	0.06^{W}	-	-	0.06^{F}	-
QYld.FW-6H.2	TP35346-TP21790 #	6H	60.6	10.2	yes	18.9	-	0.14^{F}	-	-	-	-

#the actual QTL peak is between the indicated markers. "-" indicates that no significant QTL was detected in that environment, and the superscript letters represent the source of the high value allele (C= Commander, F= Fleet, W= WI4304). LOD = logarithm of the odds. PVE= percentage of variance explained by the QTL. A range of PVE is given when the QTL is significant in more than one environment.

Table S5.4 LOD scores, QTL X E, and Percent of Variance Explained (PVE %) of phenology-adjusted yield QTL in CF, CW and FW populations

QTL	Significant Marker	Chromosome	Position	LOD	QTL x E	Percent of variance explained by the QTL (PVE %)					
						MRC12	MRC13	RAC12	RAC13	SWH12	SWH13
QYld.CF-2H	TP10554	2H	105.9	3.8	yes	-	-	9.2	-	-	3
QYld.CF-6H	TP14684	6H	58.4	10.6	yes	-	5.4	-	22.2	-	-
Total PVE (%) at each environment						**-**	**5.4**	**9.2**	**22.2**	**-**	**3**
QYld.CW-2H-1	TP58367	2H	86.1	9.5	yes	0.9	-	4.1	-	15.8	2.2
QYld.CW-2H-1	TP23323	2H	159.6	6.5	yes	5.2	1.8	-	-	-	-
QYld.CW-3H	TP29580 -TP62354*	3H	58.3	27.0	no	16.5	12.2	7.9	4.4	9.6	8.3
QYld.CW-4H-1	TP61189	4H	69.5	33.6	no	20	14.8	9.5	5.3	11.6	10.1
QYld.CW-4H-2	TP73004	4H	166.6	7.7	no	4.2	3.1	2	1.1	2.4	2.1
QYld.CW-5H	TP68883	5H	171.6	7.4	yes	1.2	10.2	3.4	5.4	3.8	-
QYld.CW-6H	TP77911	6H	83.0	7.5	no	4.1	3	2	1.1	2.4	2.1
QYld.CW-7H	HvCO1 - TP34872**	7H	84.0	15.7	yes	22.4	2.4	3	-	3.9	-
Total PVE (%) at each environment						**74.5**	**47.5**	**31.9**	**17.3**	**49.5**	**24.8**
QYld.FW-2H-1	TP33039	2H	107.1	4.2	no	1	1.1	0.7	0.9	1	0.9
QYld.FW-2H-2	TP34123 - TP6042*	2H	133.7	4	yes	-	-	2.8	-	-	-
QYld.FW-2H-3	TP75824	2H	214	45.4	yes	6.4	10.4	10.6	11.6	4.4	11.3
QYld.FW-5H	TP38042	5H	161.6	3.2	yes	0.4	-	-	-	-	-
QYld.FW-6H-1	TP89984	6H	9.6	3.6	yes	-	1.2	0.9	-	-	1.3
QYld.FW-6H-2	TP2594 - TP5400**	6H	81.6	79.8	yes	57.4	36.8	44.7	38.7	47.5	52.3
QYld.FW-6H-3	TP46163	6H	98.5	5.4	no	3	3.1	2.1	2.5	2.7	2.7
Total PVE (%) at each environment						**68.2**	**52.6**	**61.8**	**53.7**	**55.6**	**68.5**

*The QTL is found at estimated genetic predictor position between the two markers, the two markers representing the lower and the upper boundaries of the QTL interval, **=The QTL is found at estimated genetic predictor position between the two shown markers but the two markers are outside of the calculated QTL interval based on 1.5-LOD interval.

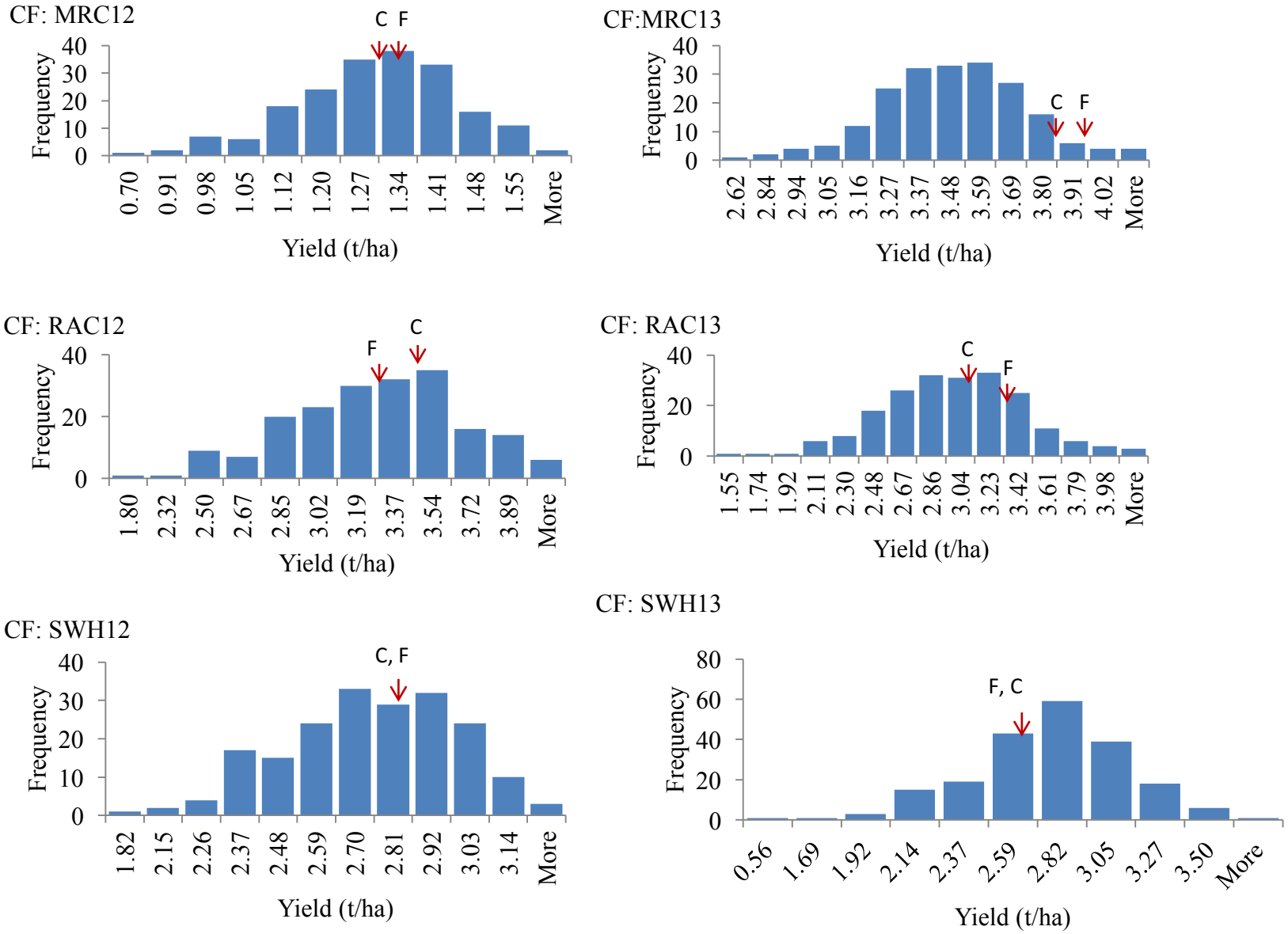


Fig. S5.1 Histogram of yield at six environments in CF population

CF= Commander x Fleet, MRC12= Minnipa 2012, MRC13= Minnipa 2013, RAC12= Roseworthy 2012, RAC13= Roseworthy 2013, SWH12= Swan Hill 2012, SWH13= Swan Hill 2013

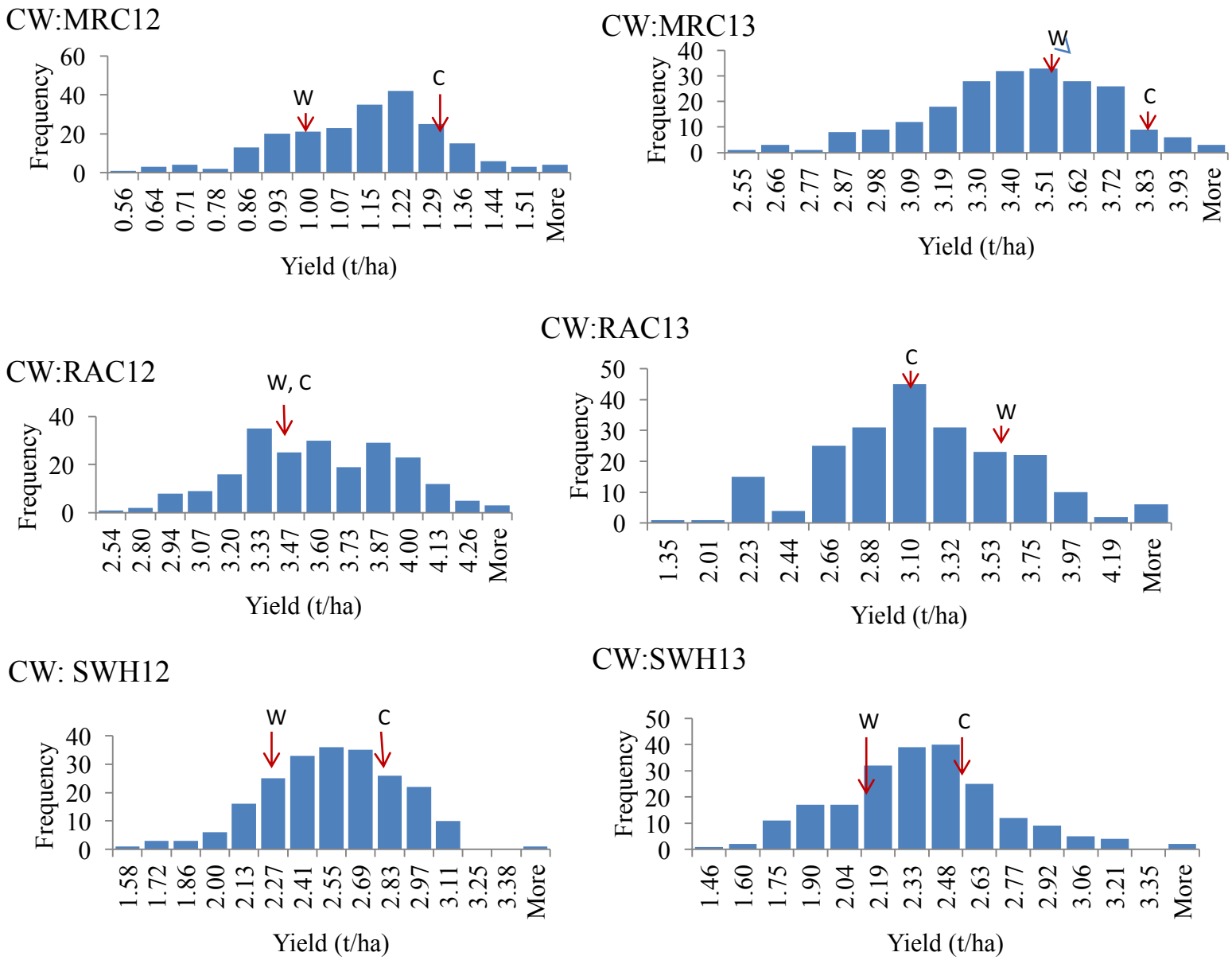


Fig. S5.2 Histogram of yield at six environments in CW population

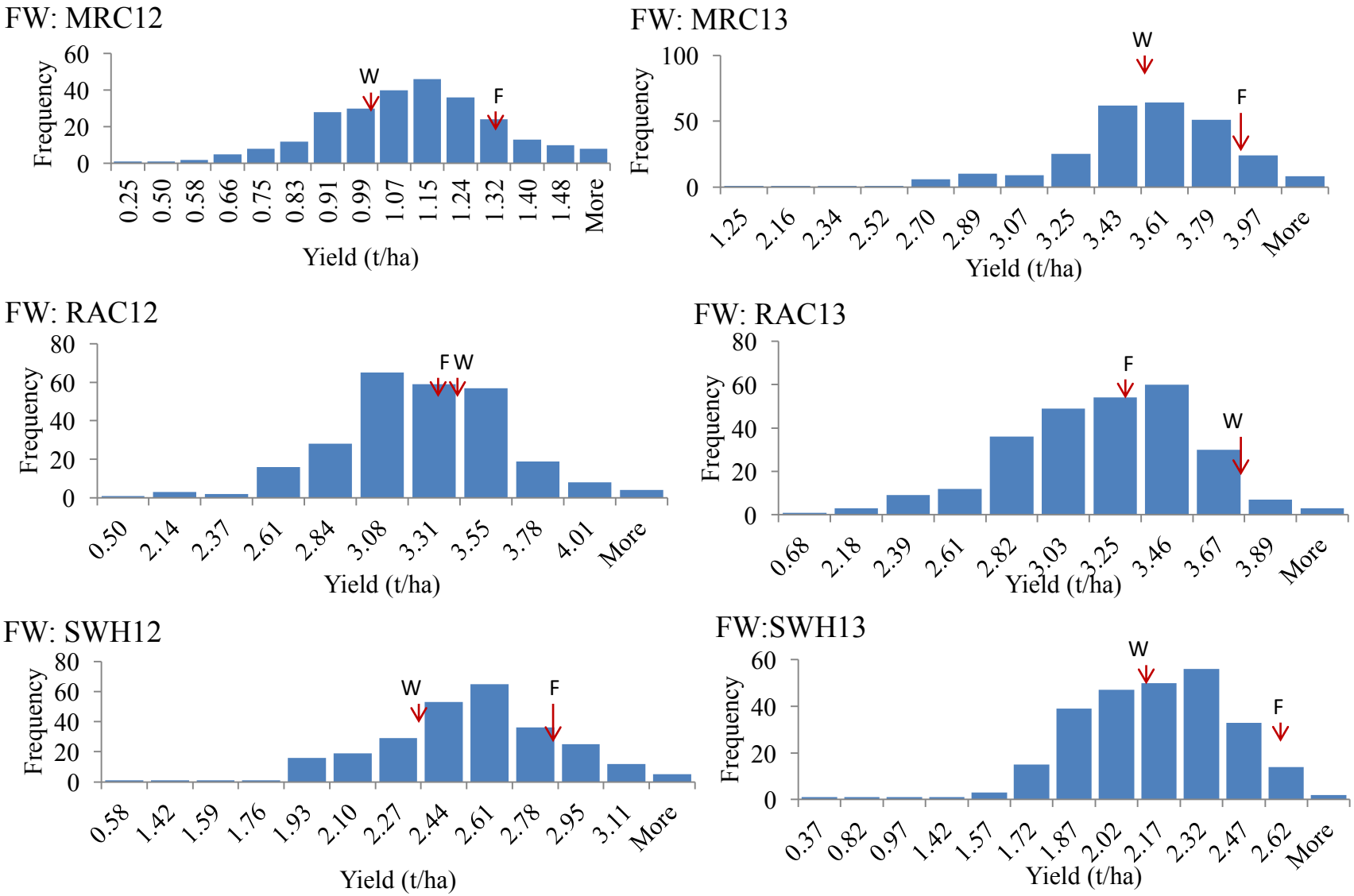
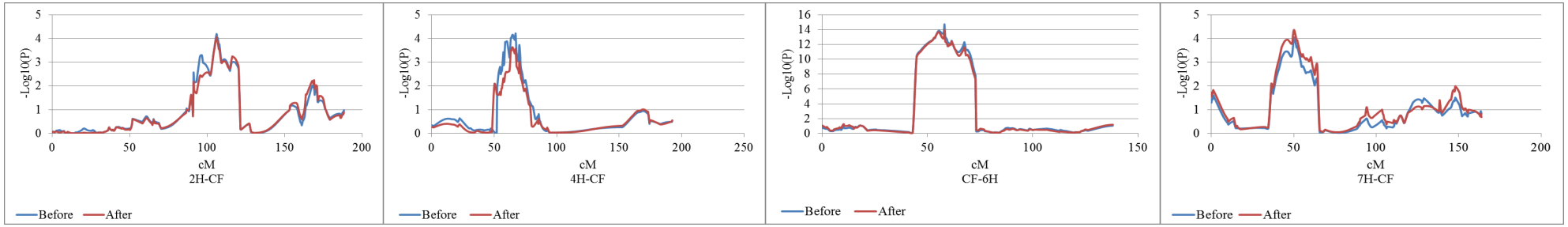
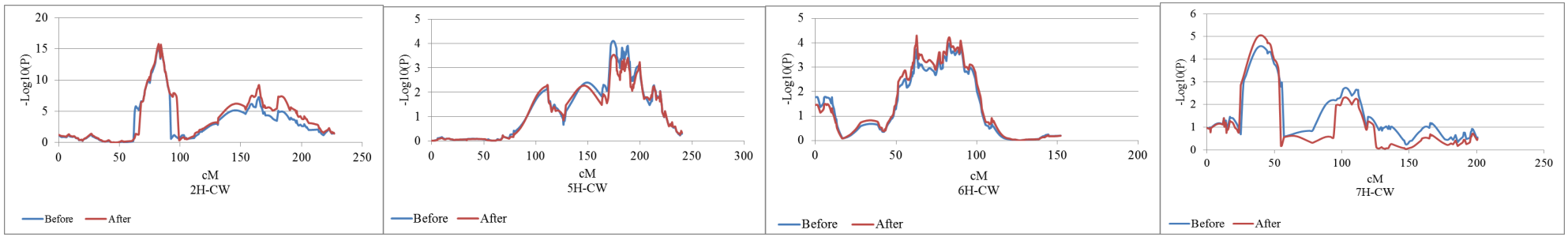


Fig. S5.3 Histogram of yield at six environments in FW population

A. Commander x Fleet



B. Commander x WI4304



C. Fleet x WI4304

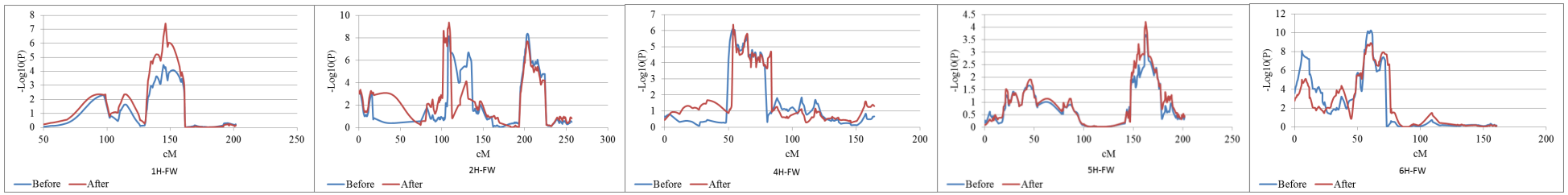


Fig. S5.4 Yield QTL before correction (blue line) and after correction (red line) for maturity score in Commander x Fleet (A), Commander x WI4304 (B), and Fleet x WI4304 (C) populations

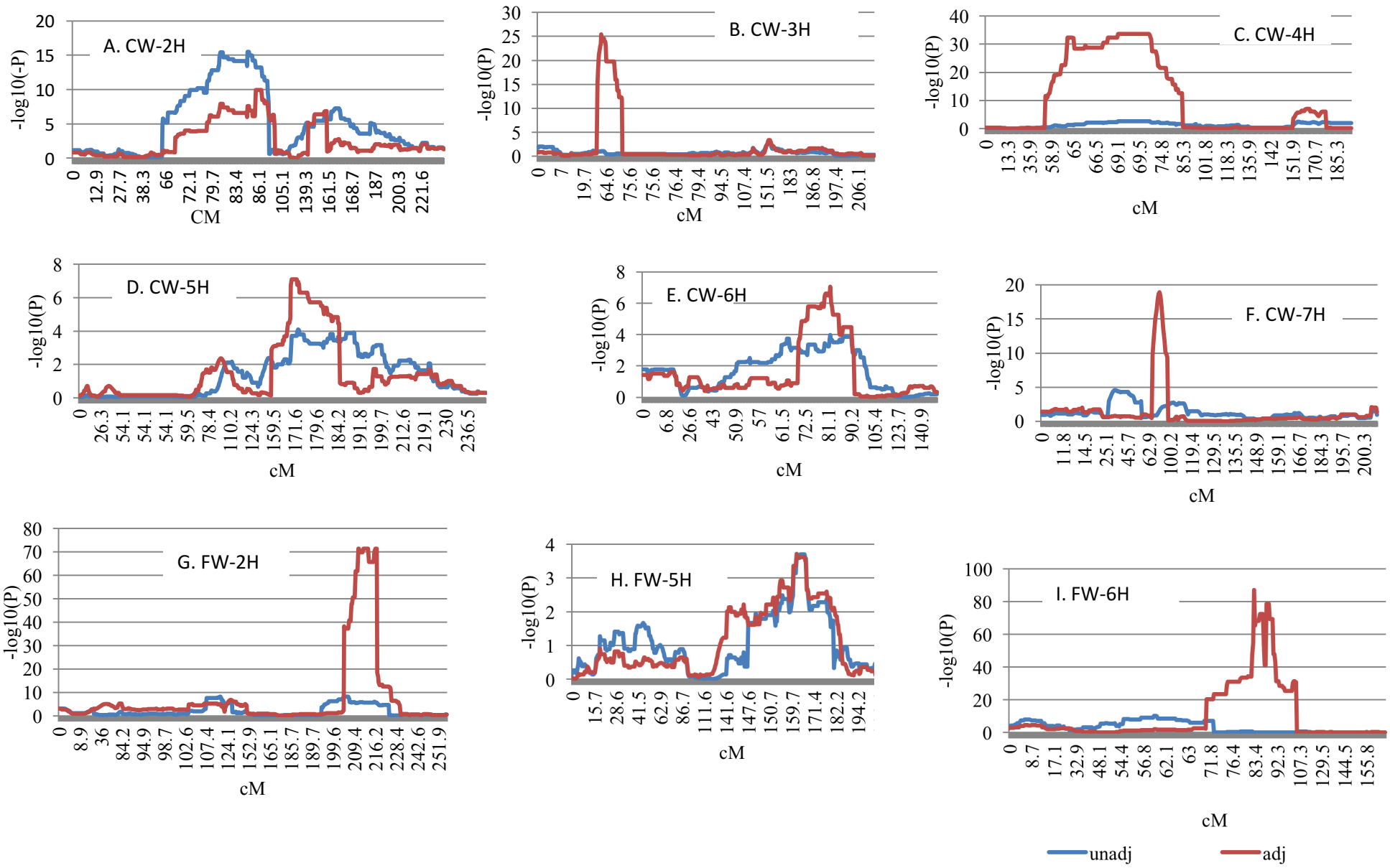


Fig.S5.5 yield QTL profiles with unadjusted (blue) and phenology adjusted (red) data of CW (A-F) and FW (G-I) populations. CF population is not shown, as adjustment for Phenolology had no effect in that population

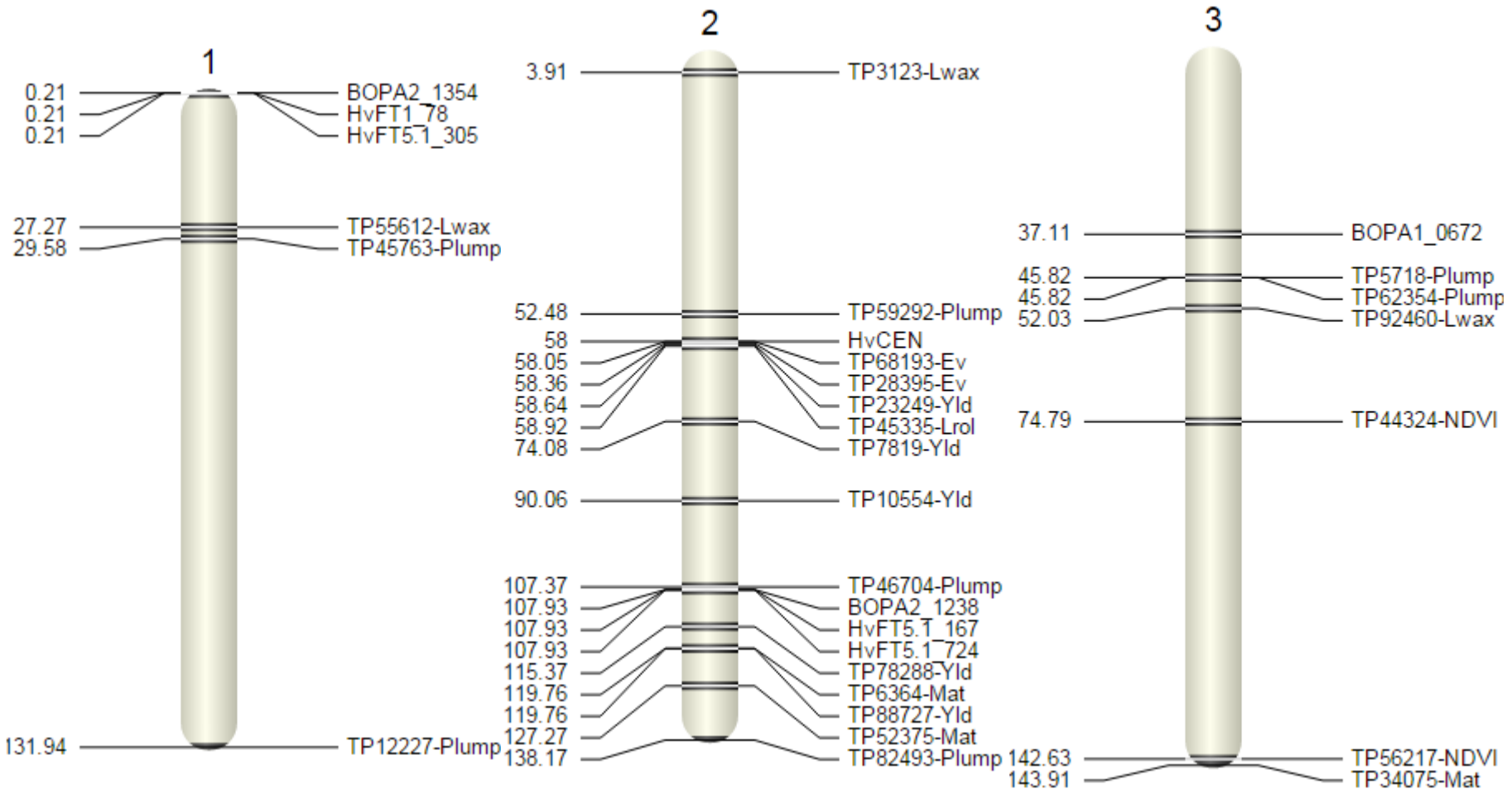


Fig. S5.6 Alignment of significant markers for yield, grain plumpness, maturity and other developmental traits on the physical map of barley, the numbers above the charts represent the barley chromosomes (1= 1H, 2= 2H, 3= 3H, 4= 4H,5= 5H, 6= 6Hand 7= 7H)

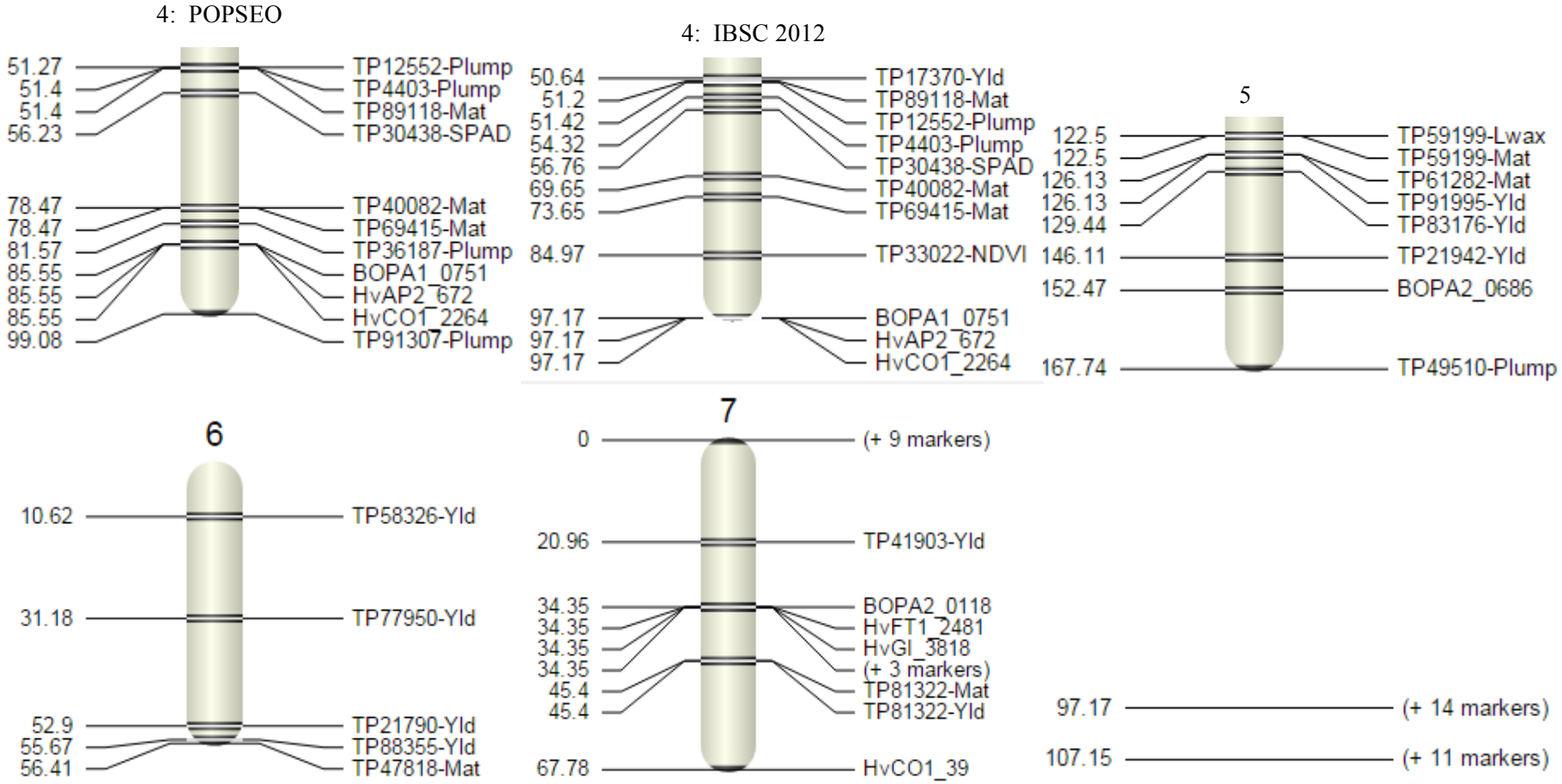


Fig. S5.6 *(continued)*